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In the Light of Evolution
Volume VI: Brain and Behavior

In the Light of Evolution
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GEORG F. STRIEDTER, JOHN C. AVISE, and FRANCISCO J. AYALA,
Editors

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Cover image: Pictured is a diffusion MRI image of a human brain, viewed from above, with the back of the head at the bottom of the image. Each line represents thousands of axons, traveling as a group along a particular axis (green: front to back; red: left to right; blue: top to bottom). This technique represents one of numerous methods used to infer the evolutionary processes that shaped the brain and behavior. Articles in this Arthur M. Sackler Colloquium, "In the Light of Evolution VI: Brain and Behavior," explore research on how and why complex nervous systems evolved, showing the progress that has been made since the dawn of evolutionary neuroscience 150 years ago. Image courtesy of Patric Hagmann (Department of Radiology, University Hospital Center, University of Lausanne, Switzerland).

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Arthur M. Sackler, M.D.
1913–1987

Born in Brooklyn, New York, Arthur M. Sackler was educated in the arts, sciences, and humanities at New York University. These interests remained the focus of his life, as he became widely known as a scientist, art collector, and philanthropist, endowing institutions of learning and culture throughout the world.

He felt that his fundamental role was as a doctor, a vocation he decided upon at the age of four. After completing his internship and service as house physician at Lincoln Hospital in New York City, he became a resident in psychiatry at Creedmoor State Hospital. There, in the 1940s, he started research that resulted in more than 150 papers in neuroendocrinology, psychiatry, and experimental medicine. He considered his scientific research in the metabolic basis of schizophrenia his most significant contribution to science and served as editor of the *Journal of Clinical and Experimental Psychobiology* from 1950 to 1962. In 1960 he started publication of *Medical Tribune*, a weekly medical newspaper that reached over one million readers in 20 countries. He established the Laboratories for Therapeutic Research in 1938, a facility in New York for basic research that he directed until 1983.

As a generous benefactor to the causes of medicine and basic science, Arthur Sackler built and contributed to a wide range of scientific institutions: the Sackler School of Medicine established in 1972 at Tel Aviv University, Tel Aviv, Israel; the Sackler Institute of Graduate Biomedical Science at New York University, founded in 1980; the Arthur M. Sackler Science Center dedicated in 1985 at Clark University, Worcester, Massachusetts; and the Sackler School of Graduate Biomedical Sciences, established in 1980, and the Arthur M. Sackler Center for Health Communications, established in 1986, both at Tufts University, Boston, Massachusetts.

His pre-eminence in the art world is already legendary. According to his wife Jillian, one of his favorite relaxations was to visit museums and art galleries and pick out great pieces others had overlooked. His interest in art is reflected in his philanthropy; he endowed galleries at the Metropolitan Museum of Art and Princeton University, a museum at

Harvard University, and the Arthur M. Sackler Gallery of Asian Art in Washington, D.C. True to his oft-stated determination to create bridges between peoples, he offered to build a teaching museum in China, which Jillian made possible after his death, and in 1993 opened the Arthur M. Sackler Museum of Art and Archaeology at Peking University in Beijing.

In a world that often sees science and art as two separate cultures, Arthur Sackler saw them as inextricably related. In a speech given at the State University of New York at Stony Brook, *Some reflections on the arts, sciences and humanities*, a year before his death, he observed: "Communication is, for me, the *primum movens* of all culture. In the arts . . . I find the emotional component most moving. In science, it is the intellectual content. Both are deeply interlinked in the humanities." The Arthur M. Sackler Colloquia at the National Academy of Sciences pay tribute to this faith in communication as the prime mover of knowledge and culture.

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Preface to the
In the Light of Evolution Series

Biodiversity—the genetic variety of life—is an exuberant product of the evolutionary past, a vast human-supportive resource (aesthetic, intellectual, and material) of the present, and a rich legacy to cherish and preserve for the future. Two urgent challenges, and opportunities, for 21st-century science are to gain deeper insights into the evolutionary processes that foster biotic diversity, and to translate that understanding into workable solutions for the regional and global crises that biodiversity currently faces. A grasp of evolutionary principles and processes is important in other societal arenas as well, such as education, medicine, sociology, and other applied fields including agriculture, pharmacology, and biotechnology. The ramifications of evolutionary thought also extend into learned realms traditionally reserved for philosophy and religion.

In 1973, Theodosius Dobzhansky penned a short commentary entitled "Nothing in biology makes sense except in the light of evolution." Most scientists agree that evolution provides the unifying framework for interpreting biological phenomena that otherwise can often seem unrelated and perhaps unintelligible. Given the central position of evolutionary thought in biology, it is sadly ironic that evolutionary perspectives outside the sciences have often been neglected, misunderstood, or purposefully misrepresented.

The central goal of the *In the Light of Evolution* (*ILE*) series is to promote the evolutionary sciences through state-of-the-art colloquia—in the series of Arthur M. Sackler colloquia sponsored by the National Academy of Sciences—and their published proceedings. Each installment explores

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evolutionary perspectives on a particular biological topic that is scientifically intriguing but also has special relevance to contemporary societal issues or challenges. Individually and collectively, the *ILE* series aims to interpret phenomena in various areas of biology through the lens of evolution, address some of the most intellectually engaging as well as pragmatically important societal issues of our times, and foster a greater appreciation of evolutionary biology as a consolidating foundation for the life sciences.

The organizers and founding editors of this effort (Avise and Ayala) are the academic grandson and son, respectively, of Theodosius Dobzhansky, to whose fond memory this *ILE* series is dedicated. May Dobzhansky's words and insights continue to inspire rational scientific inquiry into nature's marvelous operations.

John C. Avise and Francisco J. Ayala
Department of Ecology and Evolutionary Biology,
University of California, Irvine (January 2007)

Preface to
In the Light of Evolution, Volume VI: Brain and Behavior

T his book is the outgrowth of the Arthur M. Sackler Colloquium "Brain and Behavior," which was sponsored by the National Academy of Sciences on January 20–21, 2012, at the Academy's Arnold and Mabel Beckman Center in Irvine, CA. It is the sixth in a series of Colloquia under the general title "*In the Light of Evolution*." The first five books in this series were titled *Adaptation and Complex Design* (Avise and Ayala, 2007), *Biodiversity and Extinction* (Avise et al., 2008), *Two Centuries of Darwin* (Avise and Ayala, 2009), *The Human Condition* (Avise and Ayala, 2010), and *Cooperation and Conflict* (Strassmann et al., 2011).

In *On the Origin of Species by Means of Natural Selection*, Darwin (1859) barely mentioned the brain. Only in *The Descent of Man, and Selection in Relation to Sex*, published in 1871, did Darwin emphasize that the human nervous system, like any other organ system, must have evolved. Even so, Darwin himself wrote little on the brain. Instead, Darwin asked his good friend T. H. Huxley to write a chapter for the second edition of *The Descent of Man, and Selection in Relation to Sex* that dealt specifically with human brain evolution. In this chapter, Huxley laid to rest Richard Owen's earlier argument that human brains are outliers among mammalian brains. Instead, Huxley argued that our brains resemble the brains of other apes in all fundamental respects. He even downplayed the greater size of human brains, noting that brain size is quite variable among humans. Importantly, Huxley did not deny that our brains must somehow differ from the brains of other apes, for he could see no other way to explain our unique cognitive capacities, most notably language. However, Huxley

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(1863b) postulated that the differences that set our brains apart are not apparent in gross dissections (Cosans, 1994; Desmond, 1994; Gross, 1998; Striedter, 2005).

Of course, in the days of Darwin and Huxley, the only methods available for studying large brains were gross dissections or, for functional analyses, gross brain lesions. It was only in the late 1880s that Ramón y Cajal focused neuroanatomy onto structural details by applying Golgi's famous staining method to the nervous systems of various species (De Carlos and Borrell, 2007). Similarly, techniques for electrical recording of neural activity and brain stimulation were just starting to be developed in the 1870s by Richard Canton, Eduard Hitzig, and many other pioneers (Ferrier, 1886; Young, 1970; Niedermeyer, 2005). Aside from these technical constraints, neurobiological knowledge was limited in Darwin's day to relatively few species. In particular, ape brains were rare in England at the time, because they could only be obtained through research expeditions to Africa. Gorillas, for example, were not even discovered by Western scientists until Richard Owen (1859) described them and their brains in the late 1850s.

Since that dawn of evolutionary neuroscience, the arsenal of methods and panoply of data relevant to brain evolution have expanded tremendously. Intracellular and extracellular chronic recording techniques, immunohistochemistry, axon tracing, and excitotoxic brain lesions are just a few of the many methods that revolutionized our understanding of brain structure and function. Obviously, neuroscience has also been transformed by molecular methods that Darwin could not have envisioned. Researchers can now compare gene sequences and gene expression patterns across species. They can also test causal hypotheses about how genes control neural development, brain function, and, ultimately, behavior. Collectively, these methods make it possible to compare across species not just individual structures, such as genes or brain regions, but molecular interactions, developmental processes, and intriguing behaviors. Finally, the range of species studied by comparative neurobiologists now includes not just a few model species but a broad assemblage of vertebrates and, increasingly, invertebrates (Strausfeld, 2012).

These methodological advances have unleashed a flood of data relevant to brain evolution. Fortunately, conceptual advances in data analysis kept pace. Particularly important have been breakthroughs in phylogenetic systematics, which have yielded more elaborate and detailed phylogenetic trees, or cladograms, and sophisticated statistical methods for evaluating phylogenetic correlations between various traits (Nunn, 2011). Cladists have also developed a rigorous methodology for distinguishing similarities caused by homology from those similarities that resulted from independent evolution (Northcutt, 1984; Nieuwenhuys, 1994a; Pritz,

2005). With these methodologies, comparative biologists can begin to infer the evolutionary processes that created the complex tapestry of neurological systems in extant species.

Because the field of evolutionary neuroscience now includes a vast array of different approaches, data types, and species, how can one select from this diversity a set of 17 chapters that represent the field adequately? The task seems Herculean, if not Sisyphean. Confronted with this challenge, we opted for an eclectic approach. Thus, we here gather 17 chapters that represent a broad assortment of contemporary research in evolutionary neurobiology.

Part I

EVOLUTIONARY ORIGINS OF
NEURONS AND NERVOUS SYSTEMS

The first three chapters address the ancient history of neuron-related molecules and centralized nervous systems. In Chapter 1, Cecilia Conaco and colleagues review earlier findings that many of the molecules found in neuronal synapses, especially within the postsynaptic density, predate the evolution of neurons. The authors then use an analysis of gene coexpression patterns to show that these protosynaptic genes in sponges, which lack proper neurons, form several modules of interacting genes. With the evolution of neurons, these small modules fused into a larger module with a novel function, namely to build synapses. Thus, the research has moved beyond the relatively simple task of homologizing individual genes and begun to trace the evolution of complex and changing gene networks. An interesting, if as yet barely explored, implication of the idea that gene networks can change function is that the homologous gene networks may function in the development or function of nonhomologous structures (Striedter, 1998). This possibility is rarely acknowledged (Tomer et al., 2010).

In Chapter 2, Harold Zakon reviews the evolution of voltage-gated sodium (Na-v) channels. These channels probably descended from voltage-gated calcium channels, which were probably derived from voltage-sensitive potassium channels. Why did Na-v channels become the major driving force behind neuronal action potentials? The answer is probably because Na was plentiful in the ocean, where neurons first evolved, and because Na influx tends not to interfere with intracellular calcium signaling. Once incorporated into neurons, Na-v channels

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were modified in diverse, interesting ways. For example, they evolved regulatory sequences that allowed them to be clustered at the axon initial segment and at Nodes of Ranvier in myelinated axons. Additional modifications evolved after the ancestral Na-v gene was duplicated, once near the origin of vertebrates and then again (repeatedly) in several vertebrate lineages. One of the most interesting Na-v modifications is the evolution of resistance to TTX, which typically blocks Na-v channels, in pufferfishes and other species that use TTX to ward off predators.

Glenn Northcutt analyzes, in Chapter 3, when and in which lineages complex brains evolved. Favoring a cladistic approach, Northcutt concludes that the last common ancestor of all bilaterian animals, living 600–700 Mya, probably had a diffusely organized nervous system. Cephalic neural ganglia apparently evolved soon thereafter and were retained in many lineages. Truly complex brains evolved even later and did so repeatedly, in mollusks, arthropods, and chordates (including vertebrates). This conclusion contrasts sharply with the conclusions of other researchers, who are struck by similarities in developmental gene expression patterns among vertebrate, insect, and annelid nervous systems. To them, these similarities must represent homologies. That is, they argue that similar gene expression patterns must have existed in the last common ancestor of fruit flies, vertebrates, and worms. Northcutt begs to differ, arguing that the expression of these genes in brains is caused by convergent evolution, perhaps by the co-option of gene networks that predate brains. This debate will require more data for a full resolution.

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Functionalization of a Protosynaptic Gene Expression Network

CECILIA CONACO,*† DANIELLE S. BASSETT,‡§ HONGJUN ZHOU,*† MARY LUZ ARCILA,*† SANDIE M. DEGNAN,‖ BERNARD M. DEGNAN,‖ AND KENNETH S. KOSIK*†#

Assembly of a functioning neuronal synapse requires the precisely coordinated synthesis of many proteins. To understand the evolution of this complex cellular machine, we tracked the developmental expression patterns of a core set of conserved synaptic genes across a representative sampling of the animal kingdom. Coregulation, as measured by correlation of gene expression over development, showed a marked increase as functional nervous systems emerged. In the earliest branching animal phyla (Porifera), in which a nearly complete set of synaptic genes exists in the absence of morphological synapses, these "protosynaptic" genes displayed a lack of global coregulation although small modules of coexpressed genes are readily detectable by using network analysis techniques. These findings suggest that functional synapses evolved by exapting preexisting cellular machines, likely through some modification of regulatory circuitry. Evolutionarily ancient modules continue to operate seamlessly within the synapses of modern animals. This work shows that the application of network techniques to emerging genomic and expression data can provide insights into the evolution of complex cellular machines such as the synapse.

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I n the tree of life, sponges (Porifera), generally recognized as the old-est surviving metazoan phyletic lineage (Fig. 1.1*B*), occupy a highly informative position for understanding the evolution of features that uniquely characterize animals (Srivastava et al., 2010). The synapse, a cel-lular machine formed through the dynamic assembly of multiple proteins that together perform a specific biological function, is one such metazoan specialization. The synaptic machinery delivers a chemical signal via vesicle fusion at the presynaptic neuronal membrane to postsynaptic receptors, which convert that signal back to an electrical impulse in the postsynaptic neuronal cell. Surprisingly, the genome of the Poriferan demosponge, *Amphimedon queenslandica*, contains an almost complete set of genes homologous to those found in mammalian synapses (Fig. 1.1*A*), although the organism does not assemble any structure morphologi-cally resembling a synapse (Sakarya et al., 2007; Srivastava et al., 2010). Although limited gene innovation and the invention of new protein inter-action sites can partially explain how preexisting genes came together to form the synaptic complex (Sakarya et al., 2010), the multiple evolutionary steps involved in building a cellular machine through the assembly of an interaction network that can operate as a unit with a discrete biological function remains unknown.

Changes in conserved transcriptional programs arising from modi-fication of instructions encoded in the genome have contributed to our understanding of animal evolution (Barabási and Oltvai, 2004; Oldham et al., 2006, 2008; Brawand et al., 2011). Specific patterns of expression can define discrete tissues, cell types, and even functional protein complexes. Genes with similar expression patterns often have similar function (Eisen et al., 1998). Furthermore, when comparing orthologues across divergent species, highly conserved coexpression is a strong predictor of shared function in similar pathways (Quackenbush, 2003; Stuart et al., 2003; van Noort et al., 2003). These results suggest that functionally related genes might be under similar expression constraints (Carlson et al., 2006). Thus, changes in coexpression relationships for any group of genes may contain information on the assembly and evolution of cellular machines. To understand the evolutionary transition leading to the emergence of a functional synapse, we used network analysis to identify unique pat-terns of synaptic gene coexpression in representative species from diverse phylogenetic positions. We show that "protosynaptic" genes have an inherent modular structure and that the coregulatory links between these modules characterize species with functional synapses. In contrast, ancient eukaryotic cellular machines, such as the proteasome and nuclear pore, already operate in early metazoans, and their associated genes display highly correlated expression patterns over development. These findings
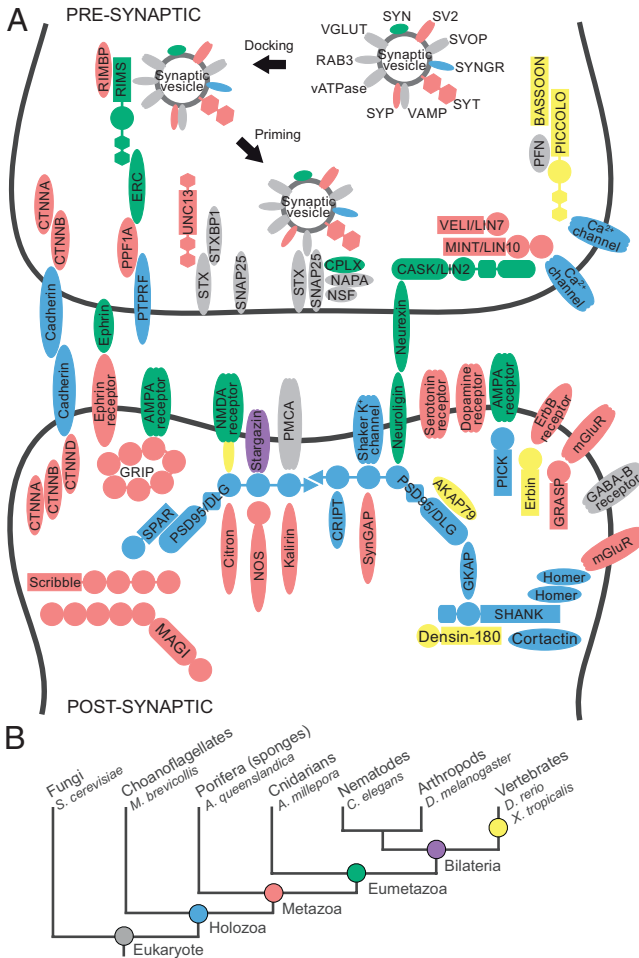
FIGURE 1.1 Origins of synaptic genes. (*A*) Homologues of genes in the human synaptic complex were identified in the genomes of selected organisms representing key phylogenetic steps in animal evolution. Colors indicate the inferred ancestor of origin for each gene, as indicated in *B*. (*B*) Evolutionary relationships among animal phyla. The names of representative species are shown. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

suggest that reorganization of gene expression, most likely through the modification of transcriptional regulation, was a key factor in the evolution of cellular machines such as the synapse.

RESULTS

To study functionalization of the synaptic gene network [Fig. 1.2*A*; Conaco et al. (2012, Fig. S1*A*)], we obtained the expression profiles of sponge synaptic gene homologues by sequencing the *A. queenslandica* transcriptome at four developmental stages from larva to adult. For comparison, expression data were also obtained for the same set of synaptic genes from five representative animals with varying complexities in tissue organization (Fig. 1.1*B*). Animal species included in this study were the cnidarian coral, *Acropora millepora*; invertebrate bilaterians, *Caenorhabditis elegans* (nematode) and *Drosophila melanogaster* (arthropod); and verte-
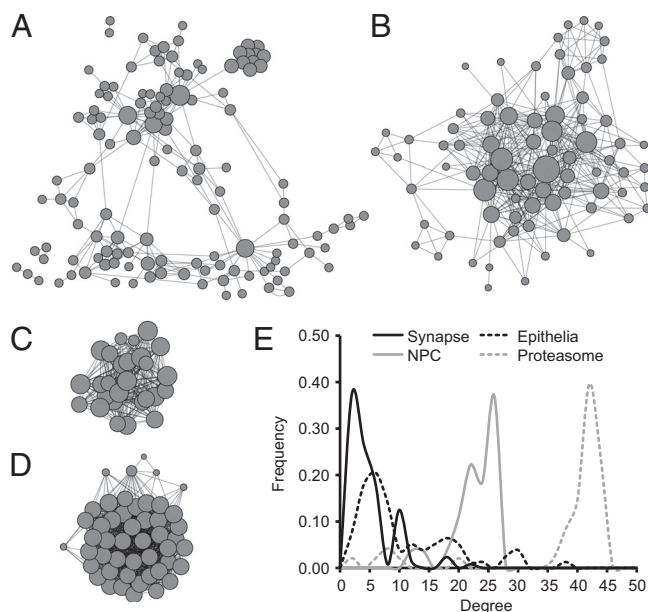
FIGURE 1.2 Structure of protein interactions within the (*A*) synaptic, (*B*) epithelial, (*C*) NPC, and (*D*) 26S proteasome networks. Each node represents a gene. Node size represents the number of interactions formed by a protein, and edge length is proportional to the strength of evidence for a functional link between two proteins. Network structures are based on the human interactome annotated in STRING (Snel et al., 2000) and visualized by using Cytoscape (Shannon et al., 2003). (*E*) Degree distribution patterns of gene networks based on the human interactome. The frequency of nodes that exhibit the indicated number of connections (degree) is shown.

brates, *Danio rerio* (zebrafish) and *Xenopus tropicalis* (frog) (Hillier et al., 2009; Domazet-Lošo and Tautz, 2010; Graveley et al., 2011; Meyer et al., 2011; Yanai et al., 2011). The correlation matrix for synaptic gene homologues from each species was constructed by computing the Pearson correlation coefficient between all pairs of gene expression profiles across development (Fig. 1.3*A*). The correlation matrix represents a network in which the genes are nodes and the correlations between gene expression patterns are edges. We averaged all elements of the correlation matrix to obtain a measure of connectivity or coregulation, *R* (Fig. 1.3*B*, *D*, *F*, and *H*). By using a community detection algorithm (Girvan and Newman, 2002; Blondel et al., 2008; Porter et al., 2009), the modularity, *Q*, of each network was computed by determining the optimal partition of the network into communities whose nodes were more connected to other nodes inside of their own community than expected in a random null model (Fig. 1.3*C*, *E*, *G*, and *I*). The modularity, *Q*, can be interpreted as a measure of the cohesiveness of coregulation: higher *Q* values indicate more segregation between coregulated groups. To determine the statistical significance of our results, we computed the same properties (*R* and *Q*) for various random control models.

The synaptic gene expression profiles were more highly correlated in eumetazoan species than in the sponge (Fig. 1.3*B*). This is apparent in the cnidarian coral, *A. millepora*, which possesses nerve cells organized into a simple diffuse net. The bilaterian synaptic gene networks showed even greater coregulation compared with sponge or coral. Synaptic genes showed significantly increased correlation compared with permuted and random controls in all species [Fig. 1.3*B*; Conaco et al. (2012, Table S1)]. To verify the observed differences in expression coregulation, we performed pairwise comparisons of subsets of synaptic genes common between species. Comparison of genes found in sponge and the other five species showed that the increased correlation in eumetazoans was significant [$P < 1 \times 10^{-5}$, two-tailed t test; Conaco et al. (2012, Table S2)]. Pairwise comparison of average coregulation for genes common between coral and each of the other species further revealed significantly greater correlation in bilaterian organisms ($P < 1 \times 10^{-10}$, two-tailed t test). These pairwise correlation values were significantly greater than coregulation within three separate random control models ($P < 0.05$, two-tailed t test; *Materials and Methods*). However, *Q* values for most of the synaptic gene networks did not show the consistent decrease relative to controls that would be expected in a set of genes that were coherently coregulated. This suggests that the synaptic gene network is composed of subsets of genes with distinguishable differences in their developmental expression patterns, similar to what we would expect from a random collection of genes taken from the transcriptome. These distinct modules may be per-

forming disparate activities that are necessary for the overall function of the synaptic machinery [Fig. 1.3*C*; Conaco et al. (2012, Table S1)].
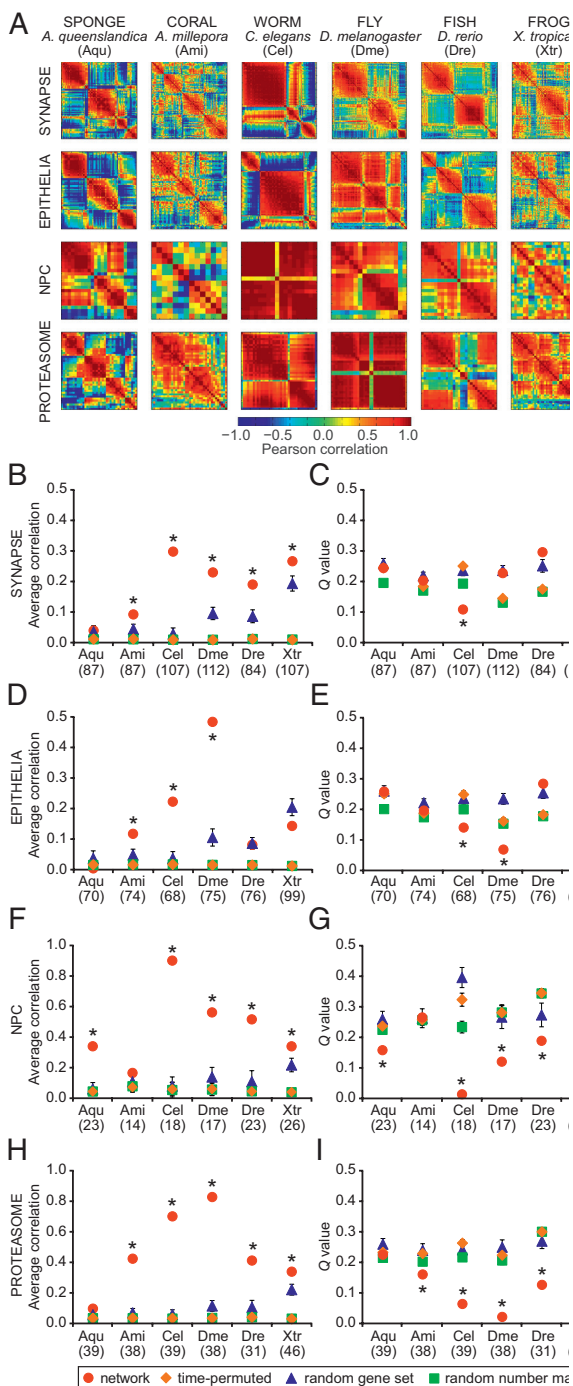
The detection of coregulated gene communities is a data-driven process that is not biased by any prior knowledge of function. We sought to determine whether functionally defined subsets of synaptic proteins corresponded to the gene communities found in the coregulation modules. Nodes in the synaptic protein interaction network of each species were colored according to the coregulation module from which they were derived (Fig. 1.4*A*). Module composition (i.e., node colors) of the three largest functional complexes were tabulated (Fig. 1.4*B*). Those genes that comprise the postsynaptic density tended to fall within a single module for most eumetazoans. This same tendency was also true for the synaptic vesicle genes in most bilaterians. In contrast, sponge synaptic genes in these functional complexes showed a more heterogeneous expression pattern that appeared to follow a different regulatory logic than that of functional synaptic networks, as reflected by the greater diversity in module composition within each biological complex. One striking exception is the vacuolar ATPase complex (vATPase), which is tightly coregulated even in sponge, suggesting a gain of functionality long before animal divergence (Finnigan et al., 2012). It should be noted, however, that, although we did not see similar module enrichment patterns for these functional complexes in the frog, we did observe a strong correlation of synaptic gene expression in this species (Fig. 1.3*A*).

FIGURE 1.3 Correlation and modularity analysis for gene networks in six organisms. (*A*) The strength of genetic coregulation for any two genes in a network was estimated by computing the Pearson correlation coefficient of their expression across developmental stages. Heat maps represent $N{\times}N$ correlation matrices for genes in each network in each species (red, positive correlation; blue, negative correlation). (*B, D, F,* and *H*) Average correlation, R, was computed from the matrices in *A*. (*C, E, G,* and *I*) The presence of distinct coregulated modules was estimated by the Q value (Blondel et al., 2008). The computations for each true network (red circles) were also performed on control datasets: time-permuted (1,000 randomly scrambled versions of the correlation matrix, orange diamonds), random gene set (100 gene sets of size N randomly sampled from the entire transcriptome, blue triangles), and random number matrix (100 matrices generated with the same gene number and developmental stages as the true network, green squares). The number of genes included in the analysis for each network in each species is shown in parentheses. Error bars represent SD of R and Q; some SDs are smaller than the marker size. Asterisks indicate a significant difference from the random gene set control ($P < 0.05$, two-tailed t test). [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

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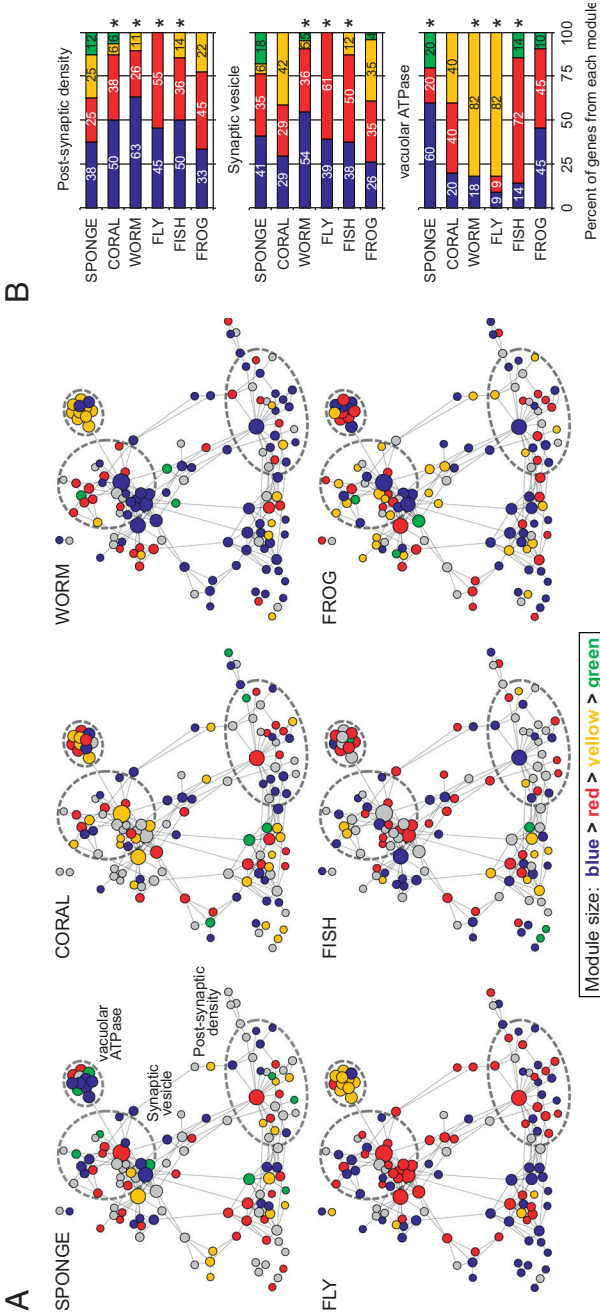
FIGURE 1.4 Functionally defined protein complexes correspond to detected coregulation modules. (*A*) Genes in the synaptic network of each species were assigned to coregulation modules by modularity optimization. Genes were colored according to the module from which they are derived (module size: blue > red > yellow > green). Genes in gray are not represented in the organism or have no available expression data. Dashed circles represent the approximate boundaries of the postsynaptic density, synaptic vesicle, and vATPases. (*B*) Percent of genes in each functional complex that belong to coregulation modules detected by modularity optimization. Colors correspond to the gene modules in *A*. Asterisks indicate complexes for which ≥50% of genes belong to the same coregulation module. Only genes with available expression data in each species were included in the analysis. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

Like the synaptic network, the epithelial network also lacks a morphological correlate in the sponge. In epithelial cells, the adherens junction links to apical-basal polarity genes and Wnt/planar polarity genes [Fig. 1.2*B*; Conaco et al. (2012, Fig. S1*B*)]. Although *A. queenslandica* expresses many orthologues of epithelial genes, the sponge exhibits only rudimentary features of a functional epithelium (Adams et al., 2010; Fahey and Degnan, 2010). As in the synaptic gene set analysis, we extracted the expression patterns of epithelial genes from six species and calculated the average correlation, *R*, and modularity, *Q*, of the coregulation network (Fig. 1.3*D* and *E*). The epithelial network in all species that were tested showed significantly greater *R* when compared pairwise vs sponge [$P < 1 \times 10^{-8}$, two-tailed *t* test; Conaco et al. (2012, Table S2)]. As in the synaptic network, the modularity of epithelial networks was not consistently lower compared with random controls for most of the species tested.

Neurons and epithelial cells and their defining cellular machines appear in eumetazoans after sponges diverged from other animals. We asked whether genes drawn from more ancient machines present in all eukaryotes might show a different pattern of expression characteristic of machines that were functionalized before the origin of animals. We performed a similar modularity optimization on transcriptome data for homologues of genes in the nuclear pore complex (NPC) and the 26S proteasome [Fig. 1.2*C* and *D*; Conaco et al. (2012, Fig. S1*C* and *D*)]. These networks are highly interconnected and exhibit a negatively skewed degree distribution, which differs from the relatively large hubs and positively skewed degree distribution observed in mammalian synaptic and epithelial networks (Fig. 1.2*E*).

The nuclear envelope is a defining feature of eukaryotic cells (Wente and Rout, 2010). Transport of molecules between the nucleus and cytoplasm is mediated by the NPC, which is made up of approximately 30 nucleoporin genes. Coregulation analysis of nucleoporin homologues represented in the transcriptome set revealed higher average correlation and generally lower modularity compared with the synaptic or epithelial networks in the same species (Fig. 1.3*F* and *G*). Most of the NPC networks showed consistently greater *R* and lower *Q* compared with permuted or random size-matched data, suggesting that the components of the NPC act as a single functional unit (Conaco et al., 2012, Table S1). In contrast, greater modularity of the synaptic and epithelial polarity networks suggests a requirement for some modularity in the operation of these machines, perhaps as a result of the presence of ancient submachines, such as the vATPase community.

The 26S proteasome is a well-conserved protein degradation machine composed of products from more than 31 genes (Voges et al., 1999). Coregulation analysis of homologues of proteasomal genes revealed that, like the NPC, the proteasome has higher average correlation and lower modu-

larity compared with the synaptic or epithelial networks within each species (Fig. 1.3*H* and *I*). All eumetazoans showed significantly higher correlation when compared pairwise vs. sponge ($P < 1 \times 10^{-52}$, two-tailed *t* test; Conaco et al., 2012, Table S2). Coregulation and modularity of proteasomal genes differed significantly from permuted or random data, except in the sponge (Conaco et al., 2012, Table S1). Nevertheless, in all species tested, including the sponge, the proteasome gene set emerged as a distinct community when analyzed together with NPC genes (Fig. 1.5) and is therefore likely to represent a functionally significant module.
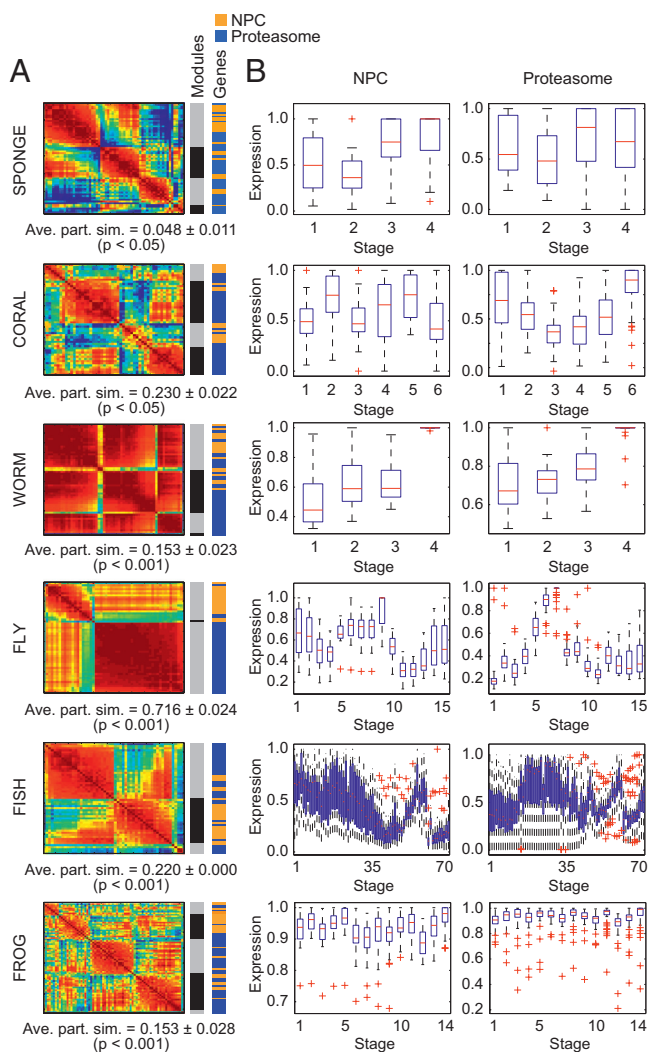
In a unicellular eukaryote, like the yeast, *Saccharomyces cerevisiae*, the NPC and proteasome gene networks exhibit high correlation and low modularity that are quite similar to the average values observed for the metazoans (Conaco et al., 2012, Table S1). These findings further support the hypothesis that gene networks that establish their modern function long before the origin of metazoa exhibit significantly higher correlation and lower modularity, consistent with a greater and more homogeneous connectivity between genes.

These results show that data-driven detection of transcriptional expression patterns can reliably reveal a reorganization of gene networks in association with the emergence of their modern collective function from the unknown functions of these same gene sets in the common animal ancestor. This reorganization appears as increased connectivity and a change in the network structure with functional complexes clustering into coregulated modules. In contrast, more ancient machines, such as the proteasome and the NPC, show a cohesiveness of expression as far back as the eukaryotic ancestor.

DISCUSSION

Synaptic proteins must be available in concentrations that drive self-assembly by mass action according to the affinities among their various interaction domains. Among the core features of synapses are scaffolding proteins that position receptors and ion channels in register with synaptic vesicles across the synaptic cleft and link the pre- and postsynaptic elements to intracellular signaling cascades. Coordinated expression of these proteins, as well as the affinity of the interactions, are among the drivers

FIGURE 1.5 Modularity optimization detects biologically relevant gene communities. (*A*) Heat maps represent the $N \times N$ Pearson correlation matrices for union networks of NPC and proteasome genes (red, positive correlation; blue, negative correlation). Average partition similarities (ave. part. sim.) computed from permutation testing with 1,000 iterations showed that, compared with the randomly scrambled gene set, genes in the union network clustered into communities that more closely recapitulated the true partition between networks ($P < 0.05$). Color bars to the right of the heat maps indicate the boundaries of detected coregulation modules (Modules) and the relative location of NPC (orange) and proteasome (blue) genes within the detected communities (Genes). (*B*) Box plots show the developmental expression patterns of genes within the NPC (*Left*) and proteasome network (*Right*) for each of the six representative species. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

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of synapse assembly. Positive selection at specific sites in PDZ scaffolds appear to have roles in determining the binding partners of these highly connected proteins (Sakarya et al., 2010), an observation consistent with network growth by link dynamics, that is, link detachment and attachment (Berg et al., 2004). Just as mutations in coding sequences can change link dynamics and enable new protein–protein interactions, mutations in cis regulatory sequences can lead to the evolution of new transcriptional linkages and coexpression of gene batteries that were not previously associated. In fact, the sponge already possesses homologues of genes that function in bilaterian neurogenesis, although it is yet to be determined if these factors were responsible for a biological unit originating in the sponge ancestor that was selected for an unknown function and later exapted to assemble the synapse (Richards et al., 2008). These conserved bilaterian developmental and neurogenic genes are associated with spatial patterning of the cnidarian nerve net (Marlow et al., 2009; Layden et al., 2012). Further modification of gene regulatory mechanisms in vertebrates placed many synaptic genes under the control of the transcriptional repressor, REST, thus ensuring exclusive and coordinated expression in neurons (Schoenherr and Anderson, 1995; Bruce et al., 2004; Otto et al., 2007).

The hierarchical structure of gene regulatory signaling networks that control the body plan are thought to evolve by changes in cis regulatory regions resulting in changes in timing, level, and location of gene expression (Peter and Davidson, 2011). In contrast, the network edges of cellular machines represent physical interactions rather than a cascade of signaling events (Spirin and Mirny, 2003). Nevertheless, the resolution of a signaling or interaction network depends on the extent of coregulatory data available to inform the graph edges. Our analysis required that we compare the coregulation and modularity of the same set of genes; however, inclusion of genes linked to the synaptic network that are not shared between the comparison groups would likely improve the coregulation signal as gene innovation and duplication can affect network structure through dynamic interactome rewiring (Arabidopsis Interactome Mapping Consortium, 2011). Although these limitations increase the likelihood of detecting biologically spurious correlations and may contribute to the apparent modularity observed in some random gene sets, the ability of the community detection algorithm to partition genes into their respective cellular machines indicates a functional correlate of the structural communities derived simply from transcriptional coregulation (Fig. 1.5). The generation of more transcriptomes at finer temporal and spatial resolution and the sequencing of genomes from other basal metazoans, as well as improved homologue detection, may strengthen or weaken an alternative explanation that the gene expression patterns in *A. queenslandica* represent a loss of more ancient gene regulatory patterns.

Evolutionary growth of gene interaction networks is a key facet of organismal complexity. Several publications have claimed that gene expression networks are scale-free (Barabási and Oltvai, 2004), and although no rigorous proof of the claim exists, many gene expression networks do display a tail in their degree distributions, indicating the presence of large hubs. Interestingly, one particular model of scale-free network growth suggests that (*i*) networks expand continuously by the addition of new nodes, and (*ii*) new nodes attach preferentially to sites that are already well connected (Udny Yule, 1925). Gene number has not increased by much over the course of metazoan evolution. Thus, the expansion of gene interaction networks, which is required to functionalize metazoan cellular machines, places an exceptionally high premium on enhancing coregulatory patterns between existing genes.

CONCLUSIONS

By using genomewide transcriptome data, we tracked the expression of a common set of synaptic genes in a representative sampling of the animal kingdom. In bilaterians, the expression of synaptic genes is strikingly well coordinated, with smaller coregulation modules detectable within the expression matrix. A particularly prominent module is the vATPase complex found within the presynaptic gene set. Interestingly, synaptic genes in the earliest branching metazoan phyla (Porifera) exhibit a lack of global coregulation compared with eumetazoans with functional nervous systems. Protosynaptic gene expression modules from the sponge, *A. queenslandica*, which lacks synapses and a nervous system, but possesses a nearly complete complement of synaptic genes, are organized into independent communities. These findings suggest that functional synapses evolved through the exaptation of preexisting genes and smaller cellular machines, presumably by modification of regulatory circuitries resulting in coordinated neuronal expression. This work demonstrates that the modularity approach based on network theory provides a very simple and data-driven method for the identification of gene communities, linking this study to a larger array of network diagnostics that could be used in subsequent investigations of the topological organization of gene coexpression networks across species.

MATERIALS AND METHODS

Expression Data

Genes in the synaptic, epithelial, NPC, and 26S proteasome networks were compiled from the literature (Voges et al., 1999; Fahey and Degnan, 2010; Srivastava et al., 2010; Wente and Rout, 2010). Protein interaction

networks were based on the human interactome annotated in STRING (Snel et al., 2000) and visualized by using Cytoscape (Shannon et al., 2003). Homologues for genes in these networks were determined by reciprocal best-hit BLAST alignments of human gene sequences to the genome of each species of interest. Expression data for gene homologues were extracted from transcriptomes obtained by RNA sequencing of four developmental stages in sponge, *A. queenslandica* (Conaco et al., 2012, SI Materials and Methods); six experimental treatments of coral larvae, *A. millepora* (Meyer et al., 2011); four developmental stages in worm, *C. elegans* (Hillier et al., 2009); and 15 developmental stages in fly, *D. melanogaster* (Graveley et al., 2011). Microarray data for 70 developmental stages in zebrafish, *D. rerio* (Domazet-Lošo and Tautz, 2010), and 14 developmental stages in frog, *X. tropicalis* (Yanai et al., 2011), were also included. Microarray expression data for the yeast, *S. cerevisiae*, were obtained from cultures grown to stationary phase (Gasch et al., 2000). To compare expression patterns in transcriptomes obtained by using different methods, the expression for every gene within each dataset was normalized to its maximum value across development (Conaco et al., 2012, Dataset S1).

Coregulation and Modularity Analysis

For each organism, the strength of genetic coregulation of any two genes throughout development was estimated by computing the Pearson correlation coefficient of expression for those two genes over development. By estimating the coregulation strength for all possible pairs of genes, we constructed organism-specific $N \times N$ coregulation networks in which genes were represented by nodes and connections between genes were weighted by the correlation between their expression levels over development. These coregulation networks were characterized by two diagnostic variables: the average correlation, R, and the modularity, Q, as defined in the following paragraphs.

The first diagnostic, the average correlation R, provides a measure of within-network connectivity which can be interpreted as a measure of coregulation. Significant differences in network coregulation between species were identified using pairwise two-tailed t tests of the correlation matrix elements. For these tests, correlation matrices were computed only for the sets of genes that were common between the two species being compared. These union gene sets for pairwise comparisons were constructed without duplicates by using only genes with the best BLAST score to the human protein sequence.

The second diagnostic, the modularity Q, provides a measure of community structure in the coregulation matrix. Importantly, the correlation matrix we used to examine the amount of coregulation (R) can equiva-

lently be viewed as a complex network in which gene–gene edges are signed (i.e., positive or negative correlations) and weighted (correlations range from −1 to 1). In each organism's coregulation network, we tested for the presence of uniquely coregulated groups of genes by using the community detection approach (Porter et al., 2009) of optimizing modularity (Girvan and Newman, 2002) by using the Louvain method (Blondel et al., 2008) [note that a second heuristic, spectral optimization (Newman, 2006), gave nearly identical results: $r = 0.9960$, $P < 0.01$; Conaco et al. (2012, Table S3)]. We define the correlation matrix A and then define w_{ij}^+ to be an $N \times N$ matrix containing the positive elements of A_{ij} and w_{ij}^- to be an $N \times N$ matrix containing only the negative elements of A_{ij}. The quality function to be maximized is then given by the following equation:

$$Q_{\pm} = \frac{1}{2w^+ + 2w^-}\sum_i\sum_j\left[A_{ij} - \left(\gamma^+\frac{w_i^+ w_j^+}{2w^+} - \gamma^-\frac{w_i^- w_j^-}{2w^-}\right)\right]\delta\left(g_i, g_j\right)\tag{1}$$

where g_i is the community to which node i is assigned, g_j is the community to which node j is assigned, γ^+ and γ^- are resolution parameters, and the following equation applies (Traag and Bruggeman, 2009):

$$w_i^+ = \sum_j w_{ij}^+, w_i^- = \sum_j w_{ij}^-.\tag{2}$$

As evident from Eq. (1), two free parameters in the optimization of modularity for such a signed, weighted network exist (Gómez et al., 2009): the resolution parameters γ^+ and γ^- (Fortunato and Barthélemy, 2007). For simplicity in the present analysis, we chose the traditional value of γ^+ of 1.0 and set γ^- as 0.1 to dampen the effect of negative correlations. Particular emphasis was placed on the positive correlations in the coregulation matrix for two reasons. First, we noted that most gene sets had significantly more positive correlations, and in fact some gene sets had no negative correlations at all (e.g., worm NPC). To ensure that our analysis was consistent across both organisms and machines, we dulled the influence of negative correlations by setting γ^- to be an order of magnitude smaller than γ^+. Secondly, we noted that the positive correlations showed considerably more topological organization than the negative correlations (Conaco et al., 2012, Fig. S2). Further details are provided in Conaco et al. (2012, *SI Materials and Methods*).

We further examined the dependence of our results on the choice of γ^+. We varied γ^+ from 0 to 2 in intervals of 0.1. We find that, for values of γ^+ higher than 1, the network disintegrates into a large number of communities (Conaco et al., 2012, Fig. S3). Our results therefore focus on the smallest yet still coherent modular structures present in these systems.

Robustness and Statistical Validity

To examine the robustness and statistical validity of our findings, we assessed the reliability of the group partitions and tested our results against three separate postmodularity-optimization null models as described in the following paragraphs.

The problem of optimizing the modularity quality function is nondeterministic polynomial-time–hard. It is therefore important to demonstrate that the heuristics that we used produce robust results, that is, that the partitions found by iterative optimizations are highly similar. For each organism and each machine, we calculated the partition similarity (Danon et al., 2005) (which is bounded in [0,1]) between 100 separate optimizations. We found that the average partition similarity was >0.8 for most organisms and machines, with the mean over organisms and networks being even higher (Conaco et al., 2012, Table S1).

In addition to quantifying the reliability of our findings, we examined the statistical validity of our results by comparing the diagnostic variables (R' and Q') derived from the true network to those derived from networks constructed from three separate random null models: true random (random number matrix), time-permuted, and random gene set. The true random null-model network is constructed by generating uniformly distributed random numbers for the same number of genes and developmental stages found in the true dataset (100 instantiations). A coregulation matrix is then constructed and R' and Q' are calculated. The time-permuted null-model network is constructed by randomly scrambling the order of expression for each gene within the network (1,000 instantiations), recomputing the coregulation matrix, and calculating R' and Q'. The random gene set null-model network was constructed by extracting the expression data for an identically sized randomly chosen set of genes from the whole transcriptome (100 instantiations). Further details are provided in Conaco et al. (2012, *SI Materials and Methods*). The statistical significance of the true R and Q values was examined by using a one-sample t test in comparison with the R' and Q' values, respectively, for each random null model (Conaco et al., 2012, Table S1). We noted that the level of background correlation and modularity observed within sets of N genes randomly selected from each of the transcriptomes is variable (Conaco et al., 2012, Fig. S4). One possible explanation for these differences is that the transcriptome datasets were obtained by using different methods.

Biological Relevance of Detected Modules

We asked whether the modules detected from the coregulation matrix could represent functional entities. We began by calculating the correla-

tion matrix $R2$ between the combined gene set of the proteasome and NPC for each species. We optimized the modularity quality function to partition this combined matrix into groups in a data-driven manner. We next asked whether this data-driven partition was statistically similar to the true partition of the genes into the two groups of proteasome genes and NPC genes. To answer this question, we computed the partition similarity between the data-driven partition and the true partition and used permutation testing to determine whether this similarity was statistically significant. The permutation test was implemented by randomly reassigning genes to the two groups of "proteasome" and "NPC," recomputing the correlation matrix $R2'$, partitioning the genes in the correlation matrix into modules, and computing the similarity between this partition and the true partition. This process was repeated 1,000 times to construct a distribution of similarity values expected under the null hypothesis that the coregulation patterns between proteasome and NPC genes do not differ. For each species, the P value to reject this null hypothesis was computed as follows: the number of similarity values derived from the permuted data that were greater than the real similarity value, divided by the number of permutations.

2

Adaptive Evolution of Voltage-Gated Sodium Channels: The First 800 Million Years

HAROLD H. ZAKON

Voltage-gated Na^+-permeable (Nav) channels form the basis for electrical excitability in animals. Nav channels evolved from Ca^{2+} channels and were present in the common ancestor of choanoflagellates and animals, although this channel was likely permeable to both Na^+ and Ca^{2+}. Thus, like many other neuronal channels and receptors, Nav channels predated neurons. Invertebrates possess two Nav channels (Nav1 and Nav2), whereas vertebrate Nav channels are of the Nav1 family. Approximately 500 Mya in early chordates Nav channels evolved a motif that allowed them to cluster at axon initial segments; 50 million years later with the evolution of myelin, Nav channels "capitalized" on this property and clustered at nodes of Ranvier. The enhancement of conduction velocity along with the evolution of jaws likely made early gnathostomes fierce predators and the dominant vertebrates in the ocean. Later in vertebrate evolution, the Nav channel gene family expanded in parallel in tetrapods and teleosts (~9 to 10 genes in amniotes, 8 in teleosts). This expansion occurred during or after the late Devonian extinction, when teleosts and tetrapods each diversified in their respective habitats, and coincided with an increase in the number of telencephalic nuclei in both groups. The expansion of Nav channels may have allowed for more sophisticated neural computation and tailoring of Nav channel kinetics with potassium channel kinetics to enhance energy savings. Nav channels show adaptive sequence evolution for increasing diversity in communication signals

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(electric fish), in protection against lethal Nav channel toxins (snakes, newts, pufferfish, insects), and in specialized habitats (naked mole rats).

Multicellular animals evolved >650 million years ago (Love et al., 2009). The nervous system and muscles evolved shortly thereafter. The phylogeny of basal metazoans is poorly resolved, likely because of the rapid radiation of these then-new life-forms (Rokas et al., 2005), so depending on the phylogeny one embraces, the nervous system evolved once with a loss in sponges, or twice independently in ctenophora and bilateria + cnidaria or bilateria and cnidaria + ctenophora (Moroz, 2009; Schierwater et al., 2009). However, in all animals with nervous systems, neurons generate action potentials (APs), release excitatory and inhibitory neurotransmitters, form circuits, receive sensory input, innervate muscle, and direct behavior.

The history of brain evolution and its key neural genes would fill volumes. I will use voltage-dependent Na$^+$ (Nav, *Na*-permeable *v*oltage-dependent = protein; *scn*, *s*odium *cha*nnel = gene) channels as an exemplar to tell this story because all neuronal excitability depends on Nav channels, there is a good understanding of their function and regulation from biophysical, biochemical, and modeling studies, and there are fascinating examples of ecologically relevant adaptations. An additional rationale is that although many proteins, such as immunoglobins, sperm and egg receptors, olfactory receptors, opsins, and surface proteins of pathogens, are routinely studied in the field of molecular evolution, only recently have ion channels begun to receive greater attention (Lopreato et al., 2001; Geffeney et al., 2005; Zakon et al., 2006, 2011; Arnegard et al., 2010; Liu et al., 2011); of these studies, the majority are on Nav channels.

SODIUM CHANNEL GENES ARE LATECOMERS TO THE 6TM FAMILY OF VOLTAGE-DEPENDENT ION CHANNELS

Voltage-gated ion channels are the basis of electrical excitability of all animals and many single-celled eukaryotes. Potassium leak and voltage-dependent K$^+$ (Kv) channels appeared 3 billion years ago in bacteria and occur in all organisms (Anderson and Greenberg, 2001) (Fig. 2.1). They establish resting potentials and repolarize membranes after excitatory events. Kv channels are the "founding members" of the family of ion-permeating channels whose basic structure is a protein of six transmembrane helices (6TM) that associate as tetramers to form a channel. At some point early in eukaryote evolution, the gene for a 6TM channel likely duplicated, giving rise to a protein with two domains. These proteins then dimerized to form a complete channel (Strong et al., 1993). Such a channel still exists in the two-pore channel family of Ca^{2+}-permeable channels
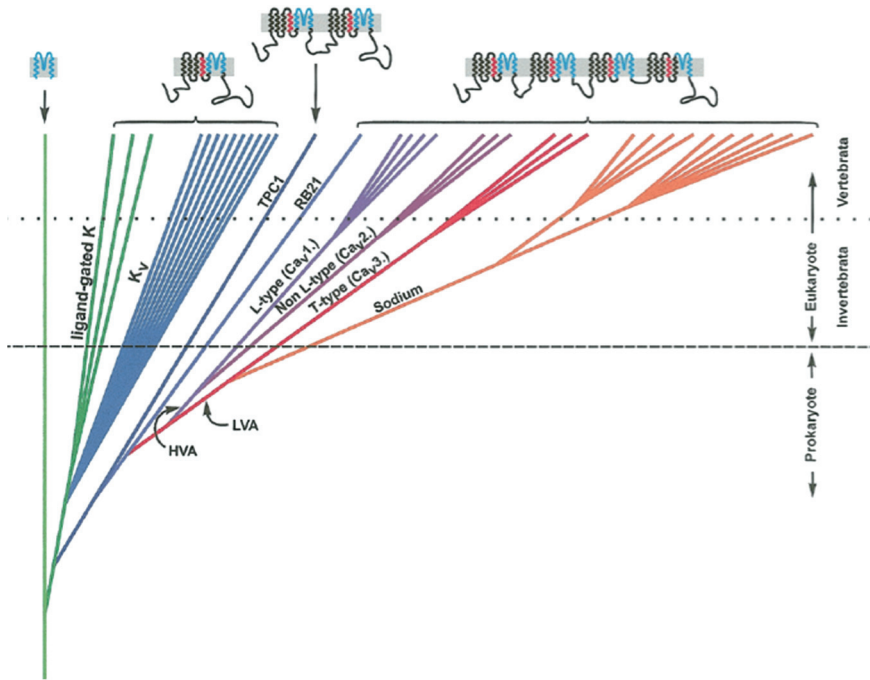
FIGURE 2.1 Schematic diagram of the evolutionary relationships among some key families in the ion channel superfamily. On the top of the figure is the structure of the channels moving from left to right showing a linear leak K^+ channel that is composed of two membrane-spanning helices and a pore (blue), a 6TM channel with a single voltage sensor (red), and four domain 4x6TM channels with four voltage sensors. There is uncertainty about the origin of the 4x6TM family, which more likely evolved in eukaryotes than prokaryotes, as indicated in this figure. A more precise and detailed relationship among Cav and Nav channels in basal metazoans and their sister group, the choanoflagellates, is given in Fig. 2.3. Reprinted from *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 129/1, Peter A. V. Anderson, Robert M. Greenberg, Phylogeny of ion channels: Cues to structure and function, 12-17, Copyright (2001), with permission from Elsevier. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

localized in endosomes and lysozomes (Galione et al., 2009). The gene for a two-domain channel likely duplicated to make a protein with four domains capable of forming a channel on its own (4x6TM). Eventually such a four-domain channel evolved (or retained) permeability to Ca^{2+}, and these handily became involved in intracellular signaling. Other Ca^{2+}-binding proteins and enzymes first appeared in single-celled eukaryotes (Cai, 2008). Additionally, there are single 6TM Na^+-permeable channels in

bacteria (Koishi et al., 2004). Their relationship to eukaryotic Nav channels is unclear, and they will not be discussed in this review.

The three main types of Cav channels are L, N/P/Q/R, and T. Generally speaking, L-type channels are found in muscle and neuronal dendrites, and N/P/Q/R are found in synaptic terminals and regulate transmitter release, whereas T types, which are sensitive to voltages close to resting potential, underlie spontaneous firing and pacemaking. These three subfamilies appear early in animals in a common ancestor of bilateria and cnidaria (Liebeskind et al., 2011) (Fig. 2.2). Choanoflagellates, single-celled protists that are the sister group to metazoans, and sponges have a single Cav channel gene that is ancestral to the L and N/P/Q/R families. The origin of the T-type channels is not clear.
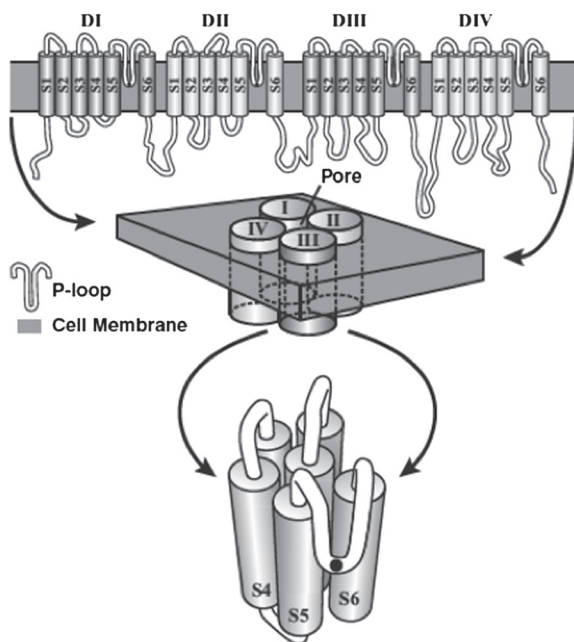
FIGURE 2.2 Hypothetical secondary structure of a Nav channel. *Top*: The Nav channel is composed of four repeating domains (I–IV), each of which has six membrane-spanning segments (S1–S6), and their connecting loops (in white). *Middle*: The four domains cluster around a pore. *Bottom*: The four P loops dip down into the membrane and line the outer mouth of the channel that is evident in an *en face* view of a single domain. The black dot represents the single amino acid at the deepest position of each of the four P loops that determines Na$^+$ ion selectivity. From Liebeskind et al. (2011).

Nav channels share the 4x6TM structure (Figs. 2.1 and 2.2) with Cav channels, and it has been suggested that Nav channels evolved from Cav channels (Hille, 2001). Analysis of putative Cav and Nav channel genes from fungi, choanoflagellates, and metazoans confirm this speculation and show that choanoflagellates have a channel that groups with recognized Nav channels with strong support (Fig. 2.3). The selectivity filter of 4x6TM channels depends on a single amino acid in each of the four domains that come together and face each other, presumably forming the deepest point in the pore. The selectivity filter of the choanoflagellate and other basal metazoans (DEEA) is midway between bona fide Cav (EEEE) and Nav1 (DEKA) channel pores and lives on in metazoans in a Nav channel found only in invertebrates (Nav2) (Zhou et al., 2004) (Fig. 2.3). This pore sequence and studies of the invertebrate Nav2 suggest that the choanoflagellate Nav channel is likely permeable to both Ca^{2+} and Na^+ and may not be a pure Na^+-selective channel. This will be determined when the choanoflagellate Nav channel is expressed and studied in detail.

The presence of a K in domain III of the pore, as in the bilaterian Nav1, increases Na^+ selectivity substantially (Fig. 2.3). There is a K in domain II in the Nav channel pore of motile jellyfish (medusozoa) but not in sedentary anemones (anthozoa). The selectivity filter DKEA enhances Na^+ selectivity less than DEKA but more than DEEA (Schlief et al., 1996; Lipkind and Fozzard, 2008). The nervous system of jellyfish has clusters of neurons approaching a real central nervous system, whereas that of anemones is more of a nerve net. Thus, enhanced Na^+ selectivity occurred in parallel in medusozoan and bilaterian Nav channels along with increasing structural complexity of the nervous system (Liebeskind, 2011).

There is little question as to the adaptive advantage conferred by Na^+-selective channels in early animals. It was not only that, with the advent of multicellularity, they fulfilled the need in a newly evolved nervous system for rapid communication across distant parts of organisms, but that they did so by marshalling an ion that was abundant in the ocean and would minimally perturb intracellular Ca^{2+} levels and, therefore, intracellular signaling (Hille, 2001).

Besides the obvious change from Ca^{2+} to Na^+ permeability, other changes occurred as well. The short intracellular loop between domains III and IV evolved function as the inactivation "ball" (West et al., 1992). In voltage-dependent K^+ channels all four voltage sensors must be "engaged" for the channel to open. In the Na^+ channel, activation is accomplished by the three voltage sensors in domains I–III; the voltage sensor in domain IV initiates inactivation (Chanda and Bezanilla, 2002; Chanda et al., 2004). No Cav channel has been examined in such a way that we do not know whether they also have equivalently acting voltage sensors or whether the voltage sensor in domain IV had already evolved a novel function.
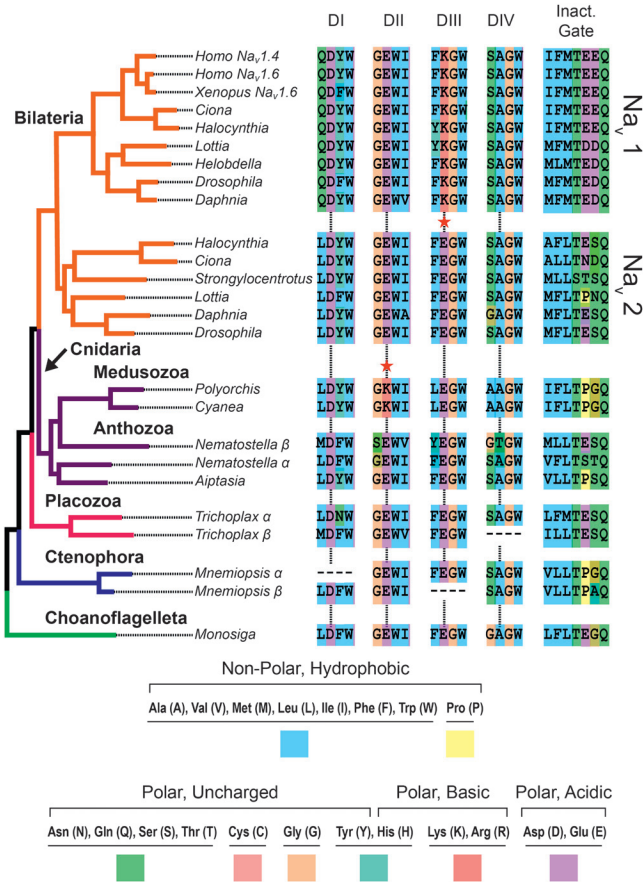
FIGURE 2.3 Maximum likelihood phylogeny of the voltage-gated sodium channel family. The common ancestor of choanoflagellates (represented by *Monosiga* in green) and animals had a Nav channel that was likely permeable to Ca^{2+} and Na^+ (pore motif = DEEA). This motif is present in the Nav channels of anthozoan cnidaria (anemones, coral) and the Nav2 channel of invertebrates. The presence of a lysine (K) in the pore improves Na^+ selectivity (indicated by red star). A lysine is found in the Nav1 channels of bilaterians (DEKA) and Nav channel of medusozoan cnidaria (jellyfish) (DKEA), both of which have more centralized nervous systems than anthozoans and are motile. Additionally, there is strong conservation of a hydrophobic (blue) triplet of amino acids in the "inactivation gate" region. From Liebeskind et al. (2011). [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

EVOLUTION OF NA$^+$ CHANNEL CLUSTERING AT THE AXON INITIAL SEGMENT AND THE NODES OF RANVIER

Myelination and saltatory conduction are key innovations of the vertebrate nervous system that markedly increase axonal conduction velocity [myelination evolved multiple times in some invertebrate lineages as well despite a widespread and persistent belief to the contrary (Hartline and Colman, 2007; Wilson and Hartline, 2011)]. Myelination is not present in agnathans but occurs in all gnathostomes, likely appearing first in a placoderm ancestor (Zalc et al., 2008). Saltatory conduction depends on high densities of Nav channels at the nodes of Ranvier that inject sufficient current into the axon to depolarize the adjacent node to threshold. KCNQ-type K$^+$ channels, which help to repolarize the AP, cluster at nodes as well, both channels tethered to ankyrin and thence to the cytoskeleton.

Remarkably, both Nav and KCNQ K$^+$ channels evolved the same specific nine-amino acid motif for ankyrin binding (Hill et al., 2008). This motif first appears in the Nav channels of ascidians and agnathans and, indeed, Nav channels cluster at axon initial segments (AIS) in the lamprey. In lampreys, and presumably nonvertebrate chordates, the high-density clustering of Nav channels adjacent to the soma ensures sufficient current injection into the high-resistance axon in the face of current shunting by the low-resistance soma (Kole et al., 2008). *Shiverer* mice, which have a mutation that prevents the formation of compact myelin, retain a high density of Nav channels (Nav1.6) at the AIS but not along the axon (Boiko et al., 2003). This emphasizes the distinction between older non–myelin-dependent mechanisms for clustering Nav channels at the AIS and more recent myelin-dependent clustering of Nav channels at nodes. A surprising observation is that the AIS is mobile, moving toward the soma when a neuron's firing rate is low and away from the soma when it is high (Grubb and Burrone, 2010). This is likely different from the nodes of Ranvier, which are smaller and constrained by the myelin sheath. However, this remains to be investigated.

KCNQ channels only occur in gnathostomes. Once KCNQ channels appeared, all of the molecular components for construction of the nodes of Ranvier were in place. By this time the key genes for myelin components had also evolved (Schweigreiter et al., 2006; Li and Richardson, 2008).

MAKING UP FOR LOST TIME: VERTEBRATE NAV CHANNEL GENES DUPLICATED EXTENSIVELY IN TELEOSTS AND TETRAPODS

Invertebrates have two Nav channel genes, Nav1 and Nav2, each in single copy. We have little information on the normal physiological role of

Nav2 channels in invertebrates [knockouts in *Drosophila* are not lethal and produce only a mild phenotype (Stern et al., 1990; Kulkarni et al., 2002)]. It is interesting that both genes have been lost in nematodes (Bargmann, 1998), most of which are small and depend on passive transmission of electrical activity. The predominant Nav channel gene in invertebrates (*para* in *Drosophila*), and the only Nav channel gene in vertebrates, is Nav1. However, Nav1 has duplicated in vertebrates.

In a prescient insight in 1970, Susumu Ohno suggested that vertebrates underwent two rounds of whole-genome duplication (WGD) at their origin (2R hypothesis) and that a subsequent third WGD occurred in teleost fishes (3R) (Ohno, 1970). Ohno believed that these ploidy events provided the raw genetic material from which emerged many of the defining features of vertebrates. Although originally controversial, his view has been empirically confirmed (Meyer and Schartl, 1999; Jaillon et al., 2004). Nav1 channel genes show a perfect read-out of this history. A single Nav1 channel gene is present in tunicates, two in lampreys, four in elasmobranchs and in the common ancestor of teleosts and tetrapods (Lopreato et al., 2001; Novak et al., 2006; Widmark et al., 2011; Zakon et al., 2011). As expected from a teleost-specific WGD, eight Nav channel genes are found in teleosts (Fig. 2.4).

However, further gene duplication/retention occurred in tetrapods above and beyond that predicted by 2R. Two of the four Nav channel genes of our tetrapod ancestors underwent a series of tandem duplications in early amniotes, so that the stem reptilian ancestor of modern-day reptiles, birds, and mammals had nine Nav1 channel genes (Widmark et al., 2011; Zakon et al., 2011). A final duplication occurred early in the mammalian lineage, giving us 10 Nav channel genes.

Was the retention of these duplicate genes in tetrapods adaptive? We can approach this by comparing the fates of Nav channel genes with other genes in tetrapods throughout 2R and beyond. In tetrapods, the genes surrounding the Nav channel genes that would have duplicated along with them in 2R show little or no evidence of further duplication and retention; indeed, some show a loss of one or more 2R duplicates (Fig. 2.4). This pattern of duplication and retention of Nav channel genes is statistically significantly different compared with that of the immediately surrounding genes (Zakon et al., 2011). A similar analysis in teleosts shows that nearby genes, such as members of the TGF-β receptor superfamily, were also more likely to be lost than retained (Widmark et al., 2011). Furthermore, an analysis of Cav, transient receptor potential, and various K^+ channel subfamilies shows that there was no widespread duplication and retention of other ion channel genes in the tetrapod 6TM family since the teleost–tetrapod divergence (Zakon et al., 2011). Thus, we infer that selection acted on the Nav channel duplicates independently in teleosts
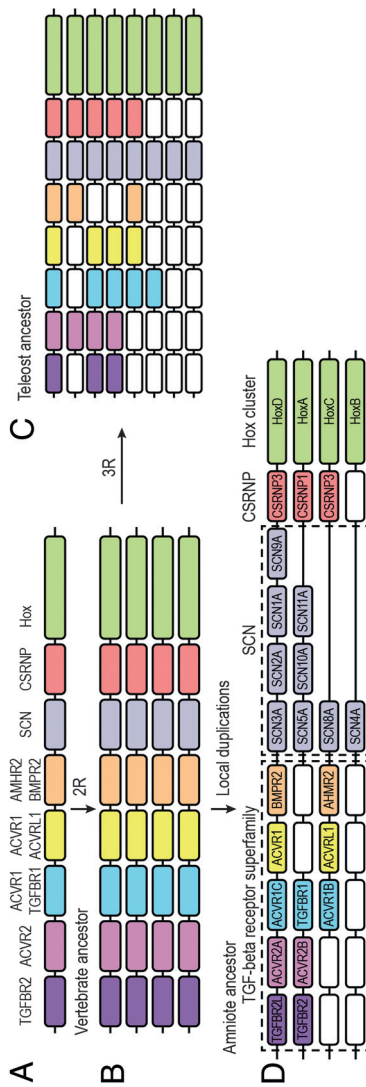
FIGURE 2.4 The Nav channel gene family underwent an expansion in parallel in teleosts and tetrapods. (*A*) A schematic chromosome with Nav channel genes (SCN, sodium channel) surrounded by other genes. (*B*) This chromosome, along with all of the other ancestral chordate chromosomes, duplicated twice at the origin of vertebrates (2R). (*C*) There was an additional round of genome duplication in teleosts (3R) and (*D*) tandem duplications of Nav channel genes in ancestral tetrapods and amniotes. There is no indication of any loss of Nav channel genes despite losses of surrounding genes in both teleosts and tetrapods. Furthermore, although not shown here, no other ion channel gene family duplicated after the teleost and tetrapod divergence. Thus, there was likely to be strong selection for the preservation of Nav channel gene duplicates. Reprinted from Jenny Widmark, Görel Sundström, Daniel Ocampo Daza, Dan Larhammar, Differential evolution of voltage-gated sodium channels in tetrapods and teleost fishes, *Molecular Biology and Evolution*, 2011, by permission of Oxford University Press. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]
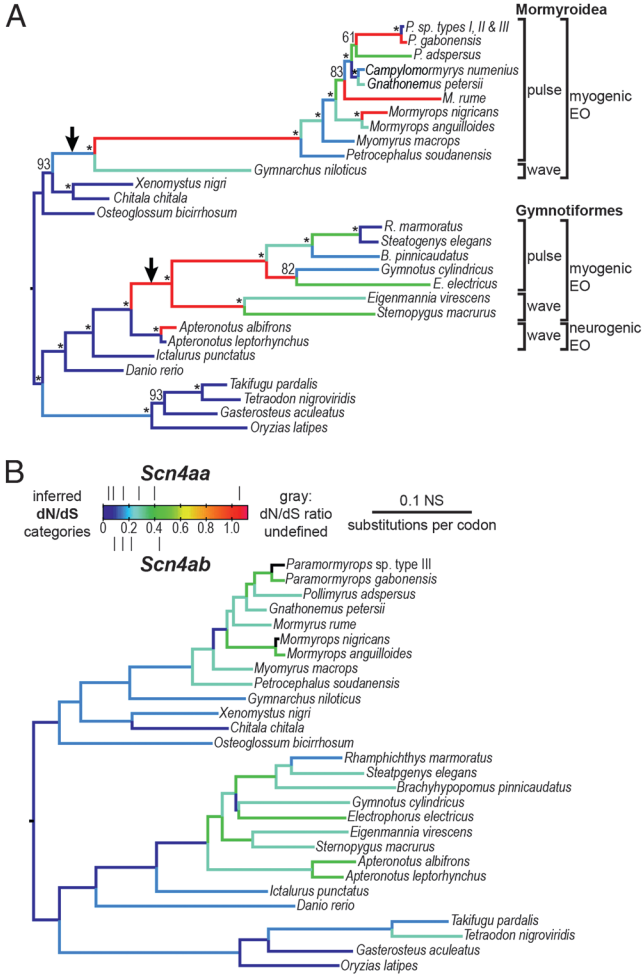
FIGURE 2.5 Nav1.4a is a fast-evolving Nav channel expressed in the electric organs of two independently derived lineages of weakly electric fish. Two paralogous genes, (A) *scn4aa*, which encodes Nav1.4a, and (B) *scn4ab*, which encodes Nav1.4ab, are expressed in the muscles of teleost fish. In the two lineages of weakly electric fishes, the mormyroidea and gymnotiformes, the gene for Nav1.4a (*scn4aa*) lost its expression in muscle and is only expressed in the electric organ. Nav1.4a underwent a burst of accelerated evolution at the origin of each lineage of electric fish. Nav1.4b, which is expressed in muscle and may also be expressed in the electric organ, evolved at a lower rate. The rate of nonsynonymous substitutions/nonsynonymous sites/rate of synonymous substitutions/synonymous site (dN/dS) in each gene is shown by a color scale in which cool colors represent

and tetrapods to preserve them. Future work detailing where Nav channels are expressed and how they behave in ray-finned fish, lungfish, and nonmammalian tetrapods will shed light on this question.

The addition of new Nav channels to the existing repertoire likely realized two benefits: enhanced computational ability and increased energetic efficiency. For example, Nav1.1 is expressed in fast-firing inhibitory cortical interneurons, and its properties allow these neurons to fire at sustained high rates (Ogiwara et al., 2007). In pyramidal neurons, Nav1.6 is found in the distal part of the AIS, whereas Nav1.2, which activates at voltages around 20 mV more positive than Nav1.6, is found more proximally. This will ensure that APs that are first generated in the most distal AIS propagate down the axon and these are followed by APs generated in the proximal AIS that backpropagate into the soma (Hu et al., 2009).

The extent to which Na$^+$ channels inactivate before K$^+$ currents commence influences energy consumption; optimally Nav channels should completely inactivate before the K$^+$ channels open to minimize use of the ATP-dependent Na$^+$/K$^+$ pump (Hasenstaub et al., 2010; Schmidt-Hieber and Bischofberger, 2010; Sengupta et al., 2010). It is possible that variation in the properties of Nav channels allows more precision in matching their inactivation with Kv channels to save energy.

ADAPTIVE EVOLUTION OF NAV CHANNELS: WEAKLY ELECTRIC FISH

In most organisms ion channels cause behavior indirectly by triggering muscle movements. Weakly electric fish, however, emit electric signals directly into the water, and these are shaped by the biophysical properties of Nav and Kv channels in their electric organs. In nonteleost vertebrates the Nav channel Nav1.4 is expressed in muscle; because of the teleost-specific WGD, teleosts have two paralogs, Nav1.4a and Nav1.4b, in their muscles (Zakon et al., 2006; Arnegard et al., 2010) (Fig. 2.5). There must

low rates of sequence evolution and hot colors represent high rates. The arrows indicate where Nav1.4a gene expression was lost from muscle in both lineages. The production of either a highly regular wave type or an irregular pulse type of electric organ discharge is indicated in both groups. In both lineages of electric fishes, the electric organ develops from muscle (myogenic), except for one group (Apteronotidae) in which it is derived from the axons of motorneurons. From Arnegard et al. (2010). [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

be strong selection for the retention of the expression of both paralogs in muscle because they are both expressed in muscles of most teleosts examined. In other words, the expression of both genes in fish muscle remains after 250 million years of teleost history. The only exceptions are two lineages of weakly electric fishes. These two groups—the South American gymnotiforms and African mormyriforms—evolved electric organs independently. In both lineages the gene for Nav1.4a (*scn4aa*) lost its expression in muscle and became compartmentalized in the electric organ. Nav1.4 in mammals is under strong purifying selection because mutations in the gene for this channel often cause muscle paralysis or myotonia. Freed from its constraints, Nav1.4a underwent a burst of evolutionary change at the origin of both groups of electric fishes, with numerous substitutions in key regions of the channel, many involved in inactivation (Zakon et al., 2006; Arnegard et al., 2010). The pace of evolutionary change quickened in similar regions of the channel in both groups; in some cases the same or neighboring amino acids changed in both groups. Although these substitutions have not yet been introduced into a channel and their effects tested, the implication is that these substitutions have facilitated the diversity of species-specific signals in these fish. An unanswered question is this: if nonelectric teleosts need two Nav channel paralogs, how do electric fish cope with only a single channel?

Muscles have diversified in other lineages of fishes. For example, rapidly contracting sound-producing muscles evolved independently in at least three lineages of fishes (Bass and Ladich, 2008), and heater muscles that no longer contract but that engage in futile Ca^{2+} cycling to generate heat, in two lineages (Block et al., 1993). It would be intriguing to know whether Nav channels show a similar pattern of compartmentalized expression and rapid evolutionary change in specialized muscles and muscle-derived organs of these lineages. Has the duplication of a muscle-expressing Nav channel gene facilitated the evolution of multiple novel muscle-derived structures in teleosts?

ADAPTIVE EVOLUTION OF NAV CHANNELS: TETRODOTOXIN RESISTANCE

The best-studied cases of adaptive evolution of Nav channel genes involve the evolution of resistance to the various neurotoxins that act on Nav channels. A number of animals use the neurotoxin tetrodotoxin (TTX), mainly for protection against predators (Gladstone, 1987) but in a few cases as a weapon to subdue prey (Ritson-Williams et al., 2006). Animals associated with TTX span the animal kingdom. This is because TTX is likely produced by bacteria symbiotically associated with their hosts, or else taken up from the food chain by animals that prey on TTX-accumulating

organisms (Lee et al., 2000). In any event, unlike peptide toxins that are sequestered within a gland, TTX passes through cell membranes so that although it may be concentrated in certain tissues, all tissues are more or less exposed to it (Williams and Caldwell, 2009). Thus, those animals that sequester high concentrations of TTX have evolved mechanisms to protect themselves from its effects (Kidokoro et al., 1974; Flachsenberger and Kerr, 1985). Because invertebrates possess only a single Nav channel gene, TTX resistance could occur easily enough with a single amino acid substitution. However, TTX resistance in vertebrates is more complex because vertebrates have multiple Nav channel genes. Evolution of TTX resistance in vertebrates offers an interesting case of parallel molecular evolution.

Pufferfishes, the most famous being the culinary delicacy *Fugu* of Japan, sequester TTX. This is a general trait of tetraodontiform fishes of which there are more than 120 species. Sequencing of Nav channel genes from *Fugu* and other pufferfishes shows that many of the same TTX-resistant amino acid substitutions have occurred multiple times in various Nav channels and lineages of pufferfishes (Yotsu-Yamashita et al., 2000; Venkatesh et al., 2005; Jost et al., 2008). We still do not know how pufferfish were able to survive with only one or a few TTX-resistant Nav channels. The most likely scenario is that TTX-resistant mutations accumulated gradually in the Nav channel genes as fish were initially exposed to a light load of TTX. Gradually, as more channels gained resistance, they were able to carry a greater toxic load. This is suggested by the fact that certain substitutions were present in ancestral tetraodontids, with other substitutions appearing in different lineages of pufferfish and in different Nav channels (Jost et al., 2008).

Some of the most remarkable work in this field concerns the rich and extensively studied garter snake–newt system. Newts such as the California newt (*Taricha torosa*) sequester high levels of TTX for protection against predators. However, in some regions in the Pacific Northwest and northern California, the common garter snake (*Thamnopis sirtalis*) overlaps with some populations of the newt. Garter snakes that do not overlap with the newts are severely affected by ingesting newts and will vomit up the newt if they are lucky and die if they are not. However, populations of garter snakes sympatric with the newts are resistant to TTX and handily take newts. Variation in the extent of TTX resistance in different garter snake populations suggests that each population has evolved resistance independently. Even more striking, TTX resistance has evolved multiple times in populations of other species of garter snakes that are also sympatric with *Taricha* in the Pacific Northwest and California, as well as other snake species sympatric with other newts or frogs that use TTX in South America and Asia (Feldman et al., 2009, 2012). Finally, sequencing and testing of expressed Nav channels (Nav1.4, a muscle-expressing Nav

channel encoded by the *scn4a* gene) have highlighted that these channels show amino acid substitutions in the pore where TTX binds (Geffeney et al., 2005; Feldman et al., 2012). Not surprisingly, the Nav channels of the newts also have evolved TTX resistance to keep the newts from poisoning themselves (Kaneko et al., 1997).

However, this story is richer still. Newts lay their eggs in streams and ponds, and these eggs hatch into gill-bearing larvae. The larvae do not produce much TTX. Adults, however, do. The adults are carnivorous and may be cannibalistic. Larval newts that are "downwind" of adults will flee if they smell TTX wafting toward them in the water (Zimmer and Ferrer, 2007). Thus, TTX is used as a chemical signal [it is similarly used as an attractive pheromone in pufferfish, in which males can detect nanomolar levels of TTX that diffuse into the water from the TTX that females place in their eggs (Matsumura, 1995)]. It is not known yet what receptor detects the TTX in either newts or pufferfish. One possibility is that it is a Nav channel that has evolved to open, rather than close, upon TTX binding.

Newt eggs are protected from most vertebrate predators because of their high titer of TTX. Nevertheless, caddis fly (*Limnephilus flavastellus*) larvae have evolved TTX resistance and will eat newt eggs (Gall and Brodie, 2011). It is not yet known whether this is due to a substitution in the pore of the Nav channel. Given that invertebrates have only a single Nav channel gene, this seems likely, and it will be interesting to see whether other invertebrate egg-predators are resistant to TTX.

ADAPTIVE EVOLUTION OF NAV CHANNELS: PROTON INSENSITIVITY

Naked mole rats (*Heterocephalus glaber*) live at high density in subterranean tunnels and seldom emerge into the light. They have evolved a number of adaptations for this life history, among them insensitivity to acid (Park et al., 2008). The levels of CO_2 that build up in their tunnels make carbonic acid; humans exposed to these levels of CO_2 report stinging pain. However, naked mole rats show no pain-related behaviors and their C-fiber nociceptors are not activated by acid. Molecular and physiological examination of the naked mole rat's acid-sensing (ASIC) and transient receptor V1 (TRPV1) channels, the channels in vertebrates that subserve acid sensitivity, showed no unusual behavior in these animals. Insofar as protons are also small monovalently positively charged molecules, these interact with and block Na^+ channels. The Nav channel Nav1.7 sets the threshold for firing of C-fiber nociceptors. Naked mole rat Nav1.7, indeed, is extremely sensitive to proton block, ensuring that, at low pH, Nav1.7 will be blocked and the C-fiber nociceptors are not activated (Smith et al., 2011).

ADAPTIVE EVOLUTION OF NAV CHANNELS IN REAL TIME: INSECTICIDE RESISTANCE

One unintended consequence of the liberal and worldwide use of dichlorodiphenyltrichloroethane, pyrethrin, and pyrethroid insecticides has been the rapid, massively parallel evolution of resistance to these pesticides in insects (Taylor et al., 1993; Liu et al., 2000; Davies et al., 2007; Jones et al., 2012). Starting with their use in the 1940s, the first indications of resistance, so-called knockdown resistance because insects were no longer knocked down by normal concentrations of the insecticide, were evident in the early 1950s. These insecticides target the Nav1 channels of insects. They cross the cell membrane and lodge in a hydrophobic pocket in the inner mouth of the channel, where they are believed to prevent the inactivation gate (domain III–IV linker) from occluding the inner mouth of the channel. This allows Na^+ ions to continue flowing into the cell, causing hyperexcitabiity. Amino acid substitutions have been discovered in a variety of insects at a number of sites in the inner mouth of the insect Nav channel (*para* in *Drosophila*) that either reduce pesticide binding or alter the channel properties to counteract the effects of insecticides. An example of the latter is a substitution that causes the channel to open at more positive potentials and to enhance the rate at which Nav channels enter closed-state inactivation. This minimizes the number of open channels counteracting the prolonged channel opening caused by insecticides.

The rapid evolution of Nav channels in insects exposed to insecticides is one of many warnings we have about the robust abilities of insect pests to overcome our best attempts to wipe them out.

CONCLUSIONS AND FUTURE DIRECTIONS

Like many key components of the nervous system, Nav channels existed before neurons. It is likely that the Nav channels of choanoflagellates and early metazoans were permeable to both Na^+ and Ca^{2+} and evolved enhanced selectivity to Na^+ in parallel in early bilaterians and jellyfish. Although it is convenient to think that invertebrates possess only a single Nav1 channel gene, it is worth scouring the wealth of new genomes to determine whether there are any lineage-specific duplications, and if so, what this might mean. Further, we have little information on the Nav2 channels of invertebrates.

The parallel expansion of Nav channel genes in tetrapods and teleosts occurred along with an increase in the number of telencephalic nuclei in both groups. This was coincident with or just after the great Devonian extinction, during which teleosts began their domination of the aquatic and tetrapods of the terrestrial habitats. More types of Nav channels may allow for more sophisticated computational possibilities and energy sav-

ings. It will be intriguing to study the locations and types of Nav channels in lungfish, basal ray-fin fishes (e.g., bichirs, gars), a variety of tetrapods, and teleosts to know whether there is parallel evolution of different channel "types" in teleosts and tetrapods. For example, fast-firing inhibitory neurons in mammals express different Nav channels than more slowly firing pyramidal neurons. Do we see a similar functional partitioning of Nav channel types in teleosts? Are those groups with only four Nav channel genes (elasmobranchs, basal actinpoterygian fishes, basal sarcopterygian fishes) hampered in the complexity of their neural processing?

Finally, on a microevolutionary level, we see that Nav channels can be targets of adaptive changes for increasing diversity in signaling (electric fish), in the arms race against lethal naturally occurring or synthetic toxins (snakes, newts, pufferfish, insects), and in specialized habitats (naked mole rats). There are likely to be more examples of this, especially in animals with unique life histories, and we should keep an eye out for potentially interesting subjects.

ACKNOWLEDGMENTS

3

Evolution of Centralized Nervous Systems: Two Schools of Evolutionary Thought

R. GLENN NORTHCUTT

Understanding the evolution of centralized nervous systems requires an understanding of metazoan phylogenetic interrelationships, their fossil record, the variation in their cephalic neural characters, and the development of these characters. Each of these topics involves comparative approaches, and both cladistic and phenetic methodologies have been applied. Our understanding of metazoan phylogeny has increased greatly with the cladistic analysis of molecular data, and relaxed molecular clocks generally date the origin of bilaterians at 600–700 Mya (during the Ediacaran). Although the taxonomic affinities of the Ediacaran biota remain uncertain, a conservative interpretation suggests that a number of these taxa form clades that are closely related, if not stem clades of bilaterian crown clades. Analysis of brain–body complexity among extant bilaterians indicates that diffuse nerve nets and, possibly, ganglionated cephalic neural systems existed in Ediacaran organisms. An outgroup analysis of cephalic neural characters among extant metazoans also indicates that the last common bilaterian ancestor possessed a diffuse nerve plexus and that brains evolved independently at least four times. In contrast, the hypothesis of a tripartite brain, based primarily on phenetic analysis of developmental genetic data, indicates that the brain arose in the last common bilaterian ancestor. Hopefully, this debate will be resolved by

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cladistic analysis of the genomes of additional taxa and an increased understanding of character identity genetic networks.

The fact that some of these building stones are universal does not, of course, mean that the organs to which they contribute are as old as these molecules or their precursors.

von Salvini-Plawen and Mayr (1977)

Any consideration of the evolution of centralized nervous systems is inextricably linked to an understanding of the phylogeny of living metazoans, their fossil history, the vast range of complexity in their nervous systems, and the development of these nervous systems. For this reason, any attempt to reconstruct the phylogeny of metazoan CNSs must be based on all lines of evidence available. The molecular phylogenetic studies of the last 20 years are particularly important in understanding metazoan interrelationships as well as the time frame in which these animals arose and radiated, and we now have increased insights into the genetics underlying the development of CNSs.

First, I will review the fossil history of the earliest putative metazoans, and then, I will discuss different comparative approaches to analyzing both molecular and morphological data: the molecular clock hypothesis, which has yielded a range of possible dates for the origin and divergence of metazoans; developmental genetics and its contribution to our understanding of the patterning of metazoan bodies, particularly patterning of the CNS; and conclusions based on the first outgroup analysis of metazoan central neural characters. Finally, I will review two hypotheses concerning the morphological complexity of the last common bilaterian ancestor.

FOSSIL RECORD

The fossil record is notoriously incomplete. Fossils essentially exist as snapshots in time, and these snapshots are of varying quality. Some are grainy, providing only a glimpse of organisms and their ecology; others are fine-grained photographs of individual taxa and their ecology (Lagerstätten). Regardless, each snapshot provides unique and critical insights into the minimal age of a radiation. Each snapshot helps calibrate molecular clocks, establish ecological settings of evolutionary events, and reveal unsuspected morphological characters that challenge current conclusions regarding character transformation (Donoghue and Purnell, 2009).

Ediacaran Biota

The earliest reported fossils of possible metazoan embryos and adults are in the Ediacaran Doushantuo Formation (~570 Mya) in southern China (Xiao et al., 1998; Chen et al., 2000, 2009). Small globular fossils, ~200 µm in diameter, show remarkable cellular details and have been interpreted as cnidarian gastrulae and planulae as well as bilaterian gastrulae comparable with living molluscans and echinoderms (Chen et al., 2000). However, the interpretation of these fossils as bilaterian metazoans has been questioned, and they have been reinterpreted as encysted holozoan protists (Huldtgren et al., 2011). Similar problems plague the earliest reported adult bilaterian, *Vernanimalcula*, which is also from the Doushantuo Formation of southern China (Chen et al., 2004a,b). Fossils of *Vernanimalcula* (~200 µm in diameter) have been described as broadly oval and triploblastic with a mouth, a differentiated gut surrounded by paired coeloms, and an anus. The rostral end of these "small spring animals" is also reported to have three pairs of external pits that have been interpreted as sensory organs (Chen et al., 2004a). This interpretation has been questioned, however, and these fossils have been claimed to be taphonomic artifacts in which phosphates were deposited within a spherical object, such as the cysts of algal acritarchs (Bengtson and Budd, 2004).

The earliest fossils of macroscopic organisms interpreted as metazoans, including bilaterians, are in the Ediacaran strata above the Doushantuo Formation (Fedonkin et al., 2007). They average 10 cm but reach an extreme of 1 m in length, and they include forms that are frond-, disk-, and worm-like (Fig. 3.1*A*); their interpretation has had a tumultuous history. Many of these fossils were discovered in the late 1940s and were interpreted as representatives of living metazoan phyla. Forms like *Eoporpita* (Fig. 3.1*A*, 1) were interpreted as cnidarian pelagic medusa (Glaessner, 1984), and frond-like forms, such as *Charniodiscus* (Fig. 3.1*A*, 2), were interpreted as possible cnidarian sea pins (Glaessner, 1962). Still other forms of these fossils were interpreted as stem bilaterians. For example, *Dickinsonia* (Fig. 3.1*A*, 3) was interpreted as a flatworm (Glaessner and Wade, 1966), *Arkarua* (Fig. 3.1*A*, 4) was interpreted as an echinoderm (Gehling, 1987), *Spriggina* (Fig. 3.1*A*, 5) was interpreted as an annelid capable of active swimming (Birket-Smith, 1981), and *Praecambridium* (Fig. 3.1*A*, 6) and a soft-bodied "trilobite" not formally described (Fig. 3.1*A*, 7) were interpreted as stem arthropods (Glaessner and Wade, 1971; Gehling, 1991). After this burst of descriptions, Ediacaran anatomy was reevaluated; claims were made that all Ediacarans were organized on a quilt-like pattern and represented an independent experiment of nonmetazoan animals, termed the Vendobionta, that failed with the evolution of macrophagous bilaterian metazoans (Seilacher, 1989; Buss and Seilacher, 1994; McMenamin, 1998). The concept of the Ediacaran biota as Vendo-
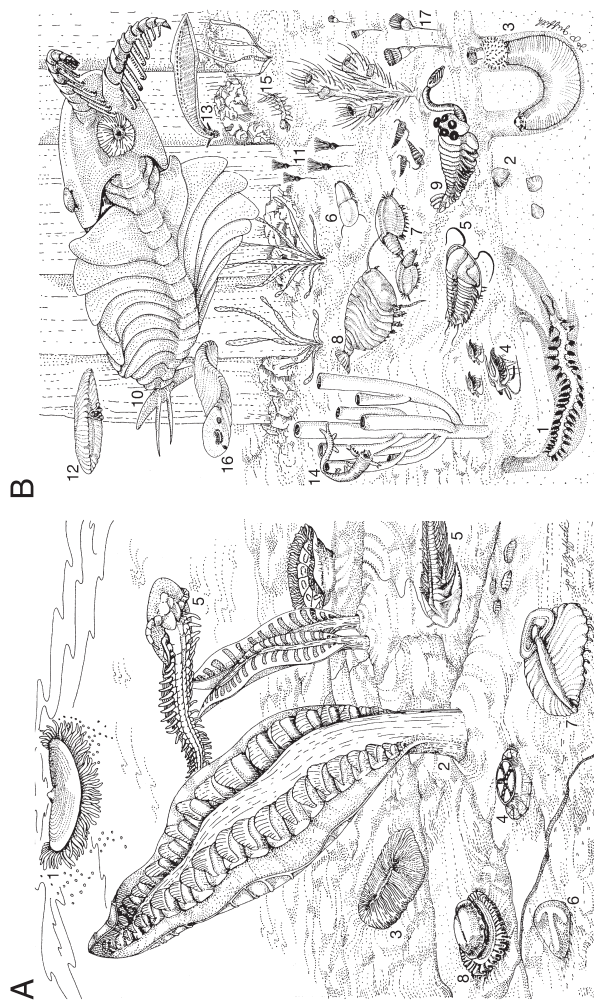
FIGURE 3.1 Reconstruction of the Ediacaran (*A*) and Burgess Shale (*B*) biotas. The Ediacaran biota is reconstructed to convey maximal morphological complexity. (*A*) 1, *Eoporpita;* 2, *Charniodiscus;* 3, *Dickinsonia;* 4, *Arkarua;* 5, *Spriggina;* 6, *Praecambridium;* 7, *soft-bodied "trilobite";* 8, *Kimberella.* (*B*) 1, *Burgessochaeta;* 2, *Lingulella;* 3, *Ottoia;* 4, *Marrella;* 5, *Olenoides;* 6, *Naraoia;* 7, *Canadaspis;* 8, *Sidneyia,* 9, *Opabinia;* 10, *Anomalocaris;* 11, *Gogia;* 12, *Eldonia;* 13, *Pikaia;* 14, *Aysheaia;* 15, *Hallucigenia;* 16, *Odontogriphus;* 17, *Dino-mischus.*

bionta was generally abandoned, because paleontologists came to realize that the Ediacaran biota represents a wide range of morphological forms (Fedonkin et al., 2007; Erwin et al., 2011).

To date, the Ediacaran biota includes some 160 taxa (Fedonkin et al., 2007) found in 40 separate locations representing all parts of the globe except Antarctica. These biotas are dispersed among three stratigraphic zones in named assemblages based on a cladistic analysis of their spatial and temporal distribution (Waggoner, 2003): an Avalon assemblage (579 to ~560 Mya), a White Sea assemblage (~560 to ~550 Mya), and a Nama assemblage (~550–541 Mya).

The Avalon assemblage is dominated by simple, frond-like taxa, such as *Charnia*, grouped into a clade termed the Rangeomorpha (Erwin et al., 2011). Macroscopic bilaterians are absent from the Avalon assemblage as well as trace fossils, such as surface tracks or shallow horizontal burrows, that would indicate the presence of small bilaterians that had developed muscles and coeloms to hydraulically locomote. One could infer from the Avalon assemblage that small bilaterians had not yet evolved, but this assemblage is the only Ediacaran assemblage from deep water; therefore, it is possible that small locomotory bilaterians existed at this time but were restricted to shallow ecological zones (Bottjer and Clapham, 2006).

The White Sea assemblage represents the peak diversity of Ediacaran biota, including all of the taxa in Fig. 3.1*A*, which are grouped into nine clades (Erwin et al., 2011). The clade Kimberellomorpha is of particular interest, because it includes *Kimberella* (Fig. 3.1*A*, 8), a small, oval-shaped animal that seems to have glided on a muscular foot and have an anterior end that houses a retractable arrow-shaped organ that was used to scratch the upper surface of the microbial mats on which it moved (Fedonkin and Waggoner, 1997; Jensen et al., 2006). Trace fossils in the White Sea assemblage are diverse and suggest the presence of small bilaterians (Erwin et al., 2011).

The Nama assemblage has less diversity than either the Avalon or White Sea assemblages, and it is dominated by frond-like taxa, called arboreomorphs, and simple cylindrical, sessile taxa, called erniettomorphs (Waggoner, 2003; Erwin et al., 2011). Bilaterian body fossils are absent, but small calcified shells of *Cloudina* and *Namacalathus* and the earliest evidence of predation in the form of holes bored into these calcified shells do occur (Bengtson and Zhao, 1992).

Our understanding of body organization and phylogeny of Ediacarans is incomplete, but a conservative interpretation of the paleontological data indicates that most animals existed primarily on microbial mats; it was likely a 2D world, with sessile frond-like forms and vagile, small organisms that trophically were suspension feeders and grazers. There is little to no evidence that pelagic medusae existed (Fig. 3.1*A*, 1),

but there is considerable evidence that sponges and sessile cnidarians were scattered across the microbial mats as were a number of bag-, frond-, and spindle-shaped taxa, forming clades that may be unrelated to any living metazoans. One or more of the three radiations of the small vagile organisms may be close relatives, or even stem members, of three clades of extant metazoans.

Cambrian Explosion

The close of the Ediacaran was marked by a massive reduction in the Ediacaran biota, with only a small number of Ediacaran taxa continuing into the Early Cambrian (Conway Morris, 1993). The small, calcified shells first seen in the late Ediacaran continue to diversify, however, in the early Cambrian to include a wide variety of plates, spines, and small shelly fauna, which seem to be the skeletal elements of bilaterians that ranged from a few millimeters to several centimeters in length (Matthews and Missarzhevsky, 1975). Clearly, bilaterians became armored in the Ediacaran–Cambrian transition. This finding suggests that the development of hard mouth parts may have been a key innovation to allow for additional expansion of macrophagous predators, giving rise to the first arms race (Bengtson and Zhao, 1992). The small shelly taxa diversified over the next 14 Myr, which culminated in the Cambrian explosion of bilaterian diversity; this explosion seems to have occurred over a relatively short 10 Myr (Conway Morris, 2000b). Despite the rapidity of the Cambrian explosion, we have two fine-grained snapshots of the event captured in the exceptionally well-preserved, soft-bodied Lagerstätten of the Chengjiang biota of the early Cambrian (~525 Mya) from the Yunan Province in South China and the Burgess Shale biota of the Middle Cambrian (~505 Mya) from British Columbia. Each of these biotas has been extensively described (Whittington, 1985; Briggs et al., 1994; Chen et al., 1997; Conway Morris, 1998; Xian-Guang et al., 2004) and analyzed for community composition and structure (Conway Morris, 1986; Zhao et al., 2010).

Despite the Chengjiang and Burgess Shale biotas existing in distinctly different environments—the Chengjiang community is thought to have existed in a relatively shallow marine environment, possibly a partially enclosed embayment subject to periodic, storm-generated turbidity (Chen et al., 1997; Zhao et al., 2010), whereas the Burgess Shale biota is thought to have existed as a deep-water community on muddy sediments banked against the front of a stromatolite reef (Fig. 3.1*B*), where it was thus unstable and subject to periodic slumps, carrying parts of the community into deeper, anaerobic waters (Whittington, 1985; Briggs et al., 1994)—both communities share numerous similarities. Both were dominated by arthropods, brachiopods, and priapulid worms (Conway Morris, 1986;

Zhao et al., 2010). Approximately 20% of each fauna consisted of sessile or burrowing infaunal species, and each fauna was dominated by epifaunal species, only 4% of which were pelagic. Feeding strategies included suspension feeding and hunting/scavenging, forming complex food chains comparable with those food chains in many modern benthic marine ecosystems (Castro and Huber, 1992). Members of stem and/or crown groups at the bilaterian phylum level are in these Cambrian communities and occupy niches similar to those niches in modern benthic marine ecosystems, suggesting that competition among taxa was as high then as it is now. For example, in the Burgess Shale biota, infaunal species included polychaete annelids such as *Burgessochaeta* (Fig. 3.1*B*, 1), brachiopods such as *Lingulella* (Fig. 3.1*B*, 2), and priapulid worms such as *Ottoia* (Fig. 3.1*B*, 3), which seems to have been an aggressive predator (Briggs et al., 1994). Epifaunal taxa included suspension- and detritus-feeding arthropods such as *Marrella* (Fig. 3.1*B*, 4), the trilobites *Olenoides* (Fig. 3.1*B*, 5) and *Naraoia* (Fig. 3.1*B*, 6), and a possible crustacean, *Canadaspis* (Fig. 3.1*B*, 7), as well as predatory arthropods such as *Sidneyia* (Fig. 3.1*B*, 8), *Opabinia* (Fig. 3.1*B*, 9), and *Anomalocaris* (Fig. 3.1*B*, 10), which grew to over 1 m in length and were clearly apex predators. Deuterostomes are also represented in the Cambrian biota of the Burgess Shale: the eocrinoid echinoderm *Gogia* (Fig. 3.1*B*, 11) and a possible pelagic holothurian, *Eldonia* (Fig. 3.1*B*, 12), as well as a possible cephalochordate, *Pikaia* (Fig. 3.1*B*, 13).

Unlike the Burgess Shale biota, the Chengjiang biota contains a rich variety of chordates. Two sessile, putative urochordates, *Cheungkongella* and *Shankouclava*, have been described (Shu et al., 2001; Chen et al., 2003) as well as another Pikaia-like chordate, *Yunnanozoon*, which was initially described as a possible cephalochordate with a notochord, segmented trunk muscles, and an expanded pharynx with an endostyle (Chen et al., 1995). This taxon was subsequently reinterpreted as an early vertebrate (Dzik, 1995), and it was then reinterpreted again as the earliest known enteropneust hemichordate (Shu et al., 1996b). Subsequently, a third Pikaia-like taxon, *Cathaymyrus*, was described in the Chengjiang deposits and interpreted as a cephalochordate (Shu et al., 1996a).

Early craniates (hagfishes and vertebrates) may also occur in the Chenjiang biota. *Haikouella* has been interpreted as a craniate-like chordate with a well-developed brain, lateral eyes, a pharynx with gills, and a ventral heart (Chen et al., 1999; Mallatt et al., 2003). A subsequent interpretation of the *Haikouella* material suggests that the head consisted of separate dorsal and ventral movable units connected by external gills (Shu et al., 2003) and that *Yunnanozoon* and *Haikouella* are stem group deuterostomes that are allied to vetulicolians, another problematic group in the Chengjiang biota (Shu et al., 2003, 2010). Thus, the yunnanozoans (*Yunnanozoon*, *Cathaymyrus*, and *Haikouella*) may be stem cephalochor-

dates, or they may be closely allied to vetulicolians and may possibly be stem deuterostomes.

The situation is somewhat clearer regarding the first vertebrates from the Chengjiang Lagerstätte. Two genera, *Haikouichthys* and *Myllokun-mingia*, have been described as agnathan vertebrates, with *Haikouichthys* said to be closely allied to living lampreys and *Myllokunmingia* said to be closely allied to living hagfishes (Shu et al., 1999). However, it has been claimed that this interpretation is based on tenuous characters and that both taxa may form a clade (myllokunmingids) that is basal to living craniates (Janvier, 2003, 2008). Subsequently, another described genus, *Zhongjianichthys*, seems to be a myllokunmingid (Shu, 2003). In any case, these taxa seem to have had paired nasal capsules, large lateral eyes, and, possibly, paired otic capsules, all of which suggest that they may have possessed brains comparable with living agnathan vertebrates (Shu, 2003).

Similar taxonomic problems plague a number of the Burgess Shale taxa. *Aysheaia* (Fig. 3.1*B*, 14) and *Hallucigenia* (Fig. 3.1*B*, 15) have been considered to be primitive onychophoran worms (Briggs et al., 1994) but are more probably an extinct clade (the lobopods, which were possibly a stem group of arthropods) (Budd and Telford, 2009). This finding is also true of *Opabinia* (Fig. 3.1*B*, 9) and *Anomalocaris* (Fig. 3.1*B*, 10), which may be members of a clade of stem arthropods, although their exact relationship to other arthropods is still unclear (Budd and Telford, 2009). *Odontogriphus* (Fig. 3.1*B*, 16), a pelagic, flattened, 12-cm-long animal with tooth-like elements surrounding the mouth, remains an enigma but may be a basal lophotrochozoan related to annelids, brachiopods, or molluscs (Briggs et al., 1994). *Dinomischus* (Fig. 3.1*B*, 17) poses similar problems. The bodies of these 10-cm-long animals consisted of a calyx, which housed the mouth and anus opening onto the upper surface of the calyx, and a stem, which was anchored in the sediment (Briggs et al., 1994). These animals have been compared with both echinoderms and entoprocts, but their taxonomic affinities are presently unclear. Continued study and the discovery of new fossils will likely resolve their positions.

COMPARATIVE APPROACHES

Comparative biologists use two very different approaches in formulating evolutionary statements: cladistics (or phylogenetics) (Hennig, 1966; Wiley and Lieberman, 2011) and phenetics (Sokal and Sneath, 1963; Sneath and Sokal, 1973). Both involve comparing traits or characters (any definable attribute of an organism) among multiple species, but each treats similar characters differently. Cladists, following Hennig (1966), divide similar characters among organisms into three categories: shared primitive characters, shared derived characters, and uniquely derived

characters. Furthermore, cladists hold that only shared derived characters, which they define as phylogenetic or taxic homologs (Patterson, 1982), can form the basis for establishing genealogical relationships. Such relationships are usually illustrated as a dendrogram or a sequence of branches (a cladogram). In contrast, phenetists say that overall similarities define homologies, which can be recognized by structural and compositional correspondence and are said to be phenetic or operational homologies (Sneath and Sokal, 1973). Phenetists also believe that genealogical relatedness depends on the degree of similarity (i.e., the number of operational homologies) shared by a group of organisms.

In most groups of organisms, multiple hypotheses of genealogical relationships can be proposed. Hennig (1966) was the first to discover that these hypotheses can be evaluated objectively by grouping organisms based on shared derived characters. For example, given three taxa—A, B, and C—there are only three possible hypotheses regarding their relatedness: A is more closely related to B; A is more closely related to C; or A, B, and C are equally related. Because phylogeny is a historical process that has occurred only one time, only one of these hypotheses can be valid. The distribution of postulated shared derived characters will indicate the valid hypothesis. That is, the genealogical hypothesis that reveals the largest number of shared derived characters and thus requires the fewest independent origins is the one that is supported. (This conclusion was initially based on simple parsimony but has been recently supported by sophisticated algorithms, such as Bayesian inference.) In rejecting the alternate genealogical hypotheses, their "shared derived characters" are revealed to be shared primitive characters or homoplasies (Wiley and Lieberman, 2011). This finding does not mean that such characters are of no phylogenetic interest. Many shared primitive characters are, in fact, shared derived characters at some lower level of the tree of life and thus linked as transformational homologs to shared derived characters at a higher level. In addition, analysis of homoplasious characters can reveal structural and functional constraints in phylogeny. Although transformational homologies do not specifically define taxonomic groups, they become critical in evaluating the phylogenetic history of characters across clades. Hennig (1966) also discovered that character polarity (i.e., primitive or derived) could be determined by an outgroup rule, which proposes that, when two or more homologous characters occur within a group, the character outside the group is the primitive character, whereas the character found only within the group is the derived character. Realization of the predictive power of the outgroup rule in the work by Hennig (1966) has given rise to a wide range of evolutionary studies that have attempted to reconstruct the phylogenetic history of molecular characters (Halanych and Passamaneck, 2001), morphological characters (Northcutt, 1984; Butler,

1994; Striedter, 1997), behavioral and ecological characters (Krubitzer et al., 2011), and biogeographical events (Grande and Bemis, 1998). Such studies are usually called cladistic studies, because they rely on the outgroup rule in the work by Hennig (1966), although they deal with the phylogeny of a character rather than reconstructing the phylogenetic history of taxa. It has been claimed that studies involving the outgroup rule in the work by Hennig (1966) in this way are not truly cladistic analyses (Strausfeld, 2012), presumably because they rely on cladograms generated in other studies; however, there is a general consensus that they do fall under the rubric of cladistic methodology (Nieuwenhuys et al., 1998; Striedter, 2005).

Because of its logic and methodological transparency, cladistics has largely replaced phenetics in zoological systematics, except at the species level, and much of its methodology is widely used to analyze the phylogenetic history of both genotypic and phenotypic characters. The phenetic approach is still widely used, however, in developmental genetic studies, in which evolutionary statements are based on a two-taxon approach, possibly because until recently, it has been difficult to explore the genetic basis of phenotypic characters widely among different taxa. The roles of both cladistic and phenetic approaches are examined in the next three sections dealing with the molecular clock hypothesis, the genetic basis of bilaterian body plans, and an outgroup analysis of metazoan neural characters.

Molecular Clock Hypothesis

Evolutionary biologists seek to date the origin of metazoan clades and determine the rate at which they evolve. Initially, clade origins were based solely on the earliest occurrence in the fossil record of that clade, and the temporal rate was established by current rates of sedimentation and, subsequently, radiometric dating (Benton et al., 2009). Given the incompleteness of the fossil record, however, the accuracy of these estimates of origin and tempo were open to question. In the early 1960s, it was discovered that differences between lineages in the number of amino acids in several proteins seemed to be roughly linear in time and that evolutionary changes in these proteins, as well as in genes, could be used to infer the separation in time of different lineages (Zuckerkandl and Pauling, 1962; Margoliash, 1963; Kumar, 2005). This discovery led to the neutral theory of molecular evolution, which claimed that most changes in proteins and genes would be neutral and that fixation of these molecules would accumulate at a clock-like rate (Kimura, 1968). It has become clear, however, that the rate of change in different molecules in different clades varies tremendously (Ayala, 1986, 1997; Rodríguez-Trelles et al., 2004). This problem has been addressed by modeling relaxed molecular clocks, in which mean rates of sequence divergence for each molecule have been

calculated (Wray et al., 1996) or the molecular clock is calibrated by one or more points based on fossil dates (Benton et al., 2009). Relaxed molecular clocks are frequently generated in a two-step process: the most supported cladogram is generated by cladistic analysis of the molecules of interest, and then, a clock is calibrated by using the time of origin of several clades based on the fossil record.

Conclusions based on mean rates of sequence divergence differ greatly from those conclusions based on multiple fossil calibration points in regard to the time of origin for metazoan clades. Using mean rates of sequence divergence, the origin and divergence of bilaterians has been placed at ~1.0–1.2 billion years ago (Wray et al., 1996). In contrast, fossil-calibrated molecular clocks place the origin and divergence of bilaterians at ~700–600 Mya (Bromham et al., 1998; Douzery et al., 2004; Peterson et al., 2004; Peterson and Butterfield, 2005; Erwin et al., 2011). These later dates suggest that, although animals arose during the Cryogenian Period (~850–635 Mya), bilaterians arose and began to radiate during the Ediacaran Period.

Genetic Basis of Bilaterian Body Plan

In the last 20 years, developmental biologists have made spectacu-lar strides in revealing the genetic basis of the regulatory networks that underlie anterior–posterior and dorsoventral patterning of body organi-zation in many bilaterian metazoans (Lewis, 1978; Nüsslein-Volhard and Wieschaus, 1980; Bopp et al., 1986; Cohen and Jürgens, 1990; McGinnis and Krumlauf, 1992; Holley et al., 1995). Anterior–posterior patterning involves a homeobox gene superfamily in which *orthodenticle* and its paralogue (*Otx*) are expressed in the rostral head, followed more caudally by the expression of paired-box (*Pax*) genes and most caudally by *Hox* genes, which continue to be expressed in the trunk. Much early work on body patterning was based on two taxon comparisons involving fruit flies and mice, but this research has now included extensive outgroup analyses (Noll, 1993; Finnerty and Martindale, 1998; Nederbragt et al., 2002; Holland and Takahashi, 2005).

One consequence of the discovery of the genetic basis of anterior–posterior body patterning in bilaterian metazoans was the realization that *Otx*, *Pax*, and *Hox* genes are also expressed in a rostral to caudal sequence in those bilaterians that possess brains (Reichert and Simeone, 2001; Hirth et al., 2003; Denes et al., 2007; Arendt et al., 2008; Hirth, 2010). This finding gave rise to the tripartite brain hypothesis, which proposes that there is a monophyletic origin of the brain in bilaterians. The origi-nal hypothesis (Hirth et al., 2003) was based on a two-taxon comparison (between an arthropod and chordates), but more recently, this hypothesis

was extended to a rudimentary three-taxon outgroup analysis (Arendt et al., 2008) involving an annelid (a spiralian protostome), an arthropod (an ecdysozoan protostome), and a mammal (a deuterostome). To date, however, there has been no attempt to polarize expression of these homeobox genes in ecdysozoan or spiralian protostomes, which possess less complex CNSs, although a tripartite brain in deuterostomes has traditionally been interpreted as a derived character (Nieuwenhuys et al., 1998; Striedter, 2005). Hopefully, continued study of the genetic regulatory networks underlying anterior–posterior patterning in ecdysozoan and spiralian protostomes will provide additional insights into the phylogeny of these networks and brain evolution in bilaterians.

The discovery of the genetic processes involved in the dorsoventral patterning of the body in several bilaterian metazoans (Holley et al., 1995; De Robertis and Sasai, 1996; Arendt and Nübler-Jung, 1997) may also provide support for the tripartite brain hypothesis. It has been shown that the gene *short gastrulation* (*sog*) in *Drosophila* is functionally homologous to the gene *chordin* (*chd*) in *Xenopus*; both promote dorsal development, whereas the gene *decapentapelgic* (*dpp*) in *Drosophila* and its homolog *bmp-4* in vertebrates promote ventral development (Holley et al., 1995). Both *sog* and *chd*, in conjunction with other genes, also promote formation of neurogenic ectoderm. However, the expression of *sog* and *chd* are inverted in the blastula of *Xenopus* relative to their expression in stage 12 embryos of *Drosophila*. This finding leads to the suggestion that vertebrates evolved from protostomes by a dorsoventral inversion (De Robertis and Sasai, 1996; Arendt and Nübler-Jung, 1997), resurrecting an earlier inversion hypothesis proposed in the work by Geoffroy Saint-Hilaire (1830). If a dorsoventral inversion of the body axis occurred with the origin of chordates, then their brains could be considered homologous (De Robertis and Sasai, 1996; Arendt and Nübler-Jung, 1997; Reichert and Simeone, 2001). Once again, this claim is based on a two-taxon phenetic comparison and not a cladistic one. Work on body patterning in an enteropneust hemichordate *Saccoglossus*, which has a diffuse nerve net, reveals the same expression of homeobox genes in the anterior–posterior body axis as in other bilaterians, but the antagonistic actions of *sog* and *dpp* do not restrict neural development to the dorsal body surface of this bilaterian (Lowe et al., 2003; Lowe, 2008). This finding suggests that, although the genetic signaling network is homologous between protostomes and deuterostomes, this network can be deployed to regulate the development of fundamentally different nervous systems. It is possible that the ancestral roles of the regulatory networks involved in anterior–posterior as well as dorsoventral patterning did not extend to patterning CNSs and that elements of these networks were subsequently co-opted in neural development.

Outgroup Analysis of Metazoan Central Neural Characters

Clearly, it is difficult to discern the connection between genetic networks and phenotypic characters (Conway Morris, 2000a; Wagner, 2007). This discernment will become easier as the phylogeny of particular genetic networks is mapped cladistically, with particular attention paid to taxa that occupy critical positions in the metazoan cladogram. Meanwhile, it is useful to conduct an outgroup analysis of the distribution of central neural characters in extant bilaterians. Although we recognize ~40 metazoan phyla, comprising some 1.3 million described species (Edgecombe et al., 2011), only 8 of these phyla (cnidarians, platyhelminthes, annelids, molluscs, nematodes, arthropods, echinoderms, and chordates) comprise 99% of extant metazoan species, and 4 of these phyla (annelids, some molluscs, arthropods, and chordates) have brains; thus, bilaterian metazoans with brains comprise 90% of the extant metazoan species. Clearly, the evolution of a brain as part of an adaptive suite has been under heavy selective pressure. If an outgroup analysis of central neural characters reveals that a brain is a shared primitive character for bilaterians, then the tripartite brain hypothesis might be supported. This finding would be the case, however, only if a brain divided into three parts is a shared primitive character. If tripartite brains are revealed to be a derived neural character, then this finding would be evidence again for the tripartite brain hypothesis. If brains are revealed to be a derived character, then brains in bilaterians must have evolved a number of times independently, which suggests that elements of the genetic network underlying anterior–posterior patterning have also been co-opted for brain patterning a number of times independently.

An outgroup analysis of central neural characters in metazoans is complicated by the lack of a consensus regarding a single metazoan cladogram (Adoutte et al., 2000; Glenner et al., 2004; Hejnol et al., 2009; Edgecombe et al., 2011; Erwin et al., 2011) and difficulties in defining distinct central neural characters. Despite these difficulties, comparative molecular studies have clarified much of the phylogeny. All molecular studies recognize bilaterians as a monophyletic taxon divided into two major clades: the protostomes and the deuterostomes (Fig. 3.2). Furthermore, the protostomes can be divided into two superphyla or clades termed the ecdysozoans and the spiralians (lophotrochozoans). Conflicts regarding metazoan phylogeny currently center on the contentious relationships of acoelomorph flatworms (Acoela and Nemertodermatida), the genus *Xenoturbella* (a small ciliated marine worm), and the basal metazoan clades (cnidarians, ctenophores, placozoans, and poriferans). The cladogram generated in the work by Glenner et al. (2004) was chosen for the present outgroup analysis (with some modifications), because it is the only Bayesian phylogenetic analysis that includes both molecular and morphological data. The Acoela group has been interpreted as the sister group
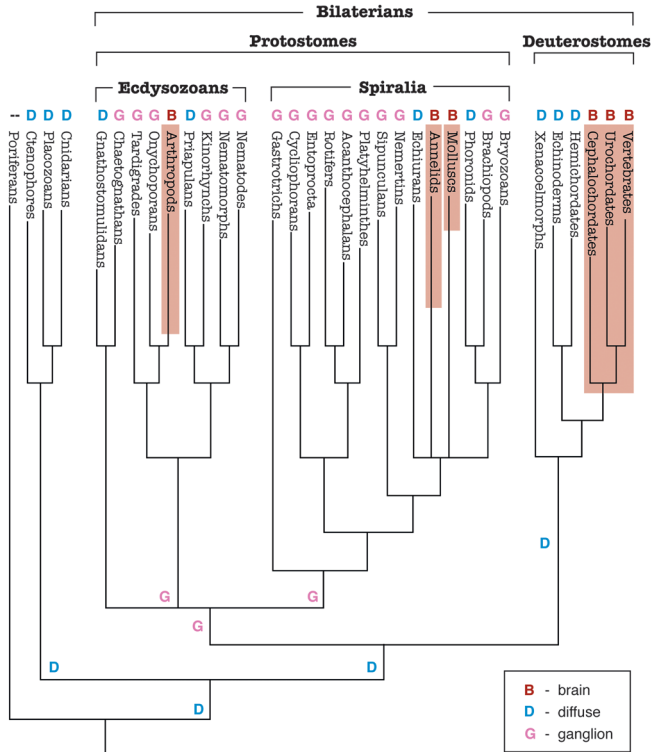
FIGURE 3.2 Outgroup analysis of cephalic neural characters across extant meta-
zoans. The cladogram is modified from the work by Glenner et al. (2004), with the
inclusion of xenacoelmorphs as the sister taxon to other deuterostomes (Philippe
et al., 2011). The analysis indicates that the last bilaterian common ancestor pos-
sessed a diffuse nerve plexus and that brains independently evolved at least four
times among bilaterians.

to all other bilaterians (Glenner et al., 2004) or a deuterostome clade, the
xenocoelmorphs (Hejnol et al., 2009; Philippe et al., 2011), which includes
acoelomorph flatworms, nemertodermatids, and *Xenoturbella*. When xena-
coelmorphs are interpreted as deuterostomes, they are considered either
as the sister group to Ambulacraria (Echinodermata and Hemichordata)
or the sister group to all other deuterostomes (Perseke et al., 2007). The
latter interpretation is accepted in the present outgroup analysis. The fol-
lowing characters—or levels of increasing morphological and functional
complexity in the cephalic CNSs of extant metazoans—are recognized
in the present outgroup analysis: (*i*) diffuse nerve nets or subepidermal

nerve plexuses; (*ii*) simple cerebral ganglia; and (*iii*) brains, defined as a central collection of neural centers, with distributed and hierarchical functions. A considerable range of morphological complexity occurs within each of these cephalic neural characters (Bullock and Horridge, 1965). Diffuse nerve nets range from those nets of cnidarians and ctenophores to those nets in enteropneust hemichordates, in which neural cell bodies occupy a subepidermal nerve plexus with centralized bundles of fast-conducting axons forming dorsal and ventral nerve cords. Cerebral ganglia range from simple, bilobed ganglia in polyclade flatworms to more complex multiple cephalic ganglia in many gastropod molluscs (Bullock and Horridge, 1965). In most annelids, arthropods, and some cephalopod molluscs, brains form by elaboration of one or more cephalic ganglia, whereas in vertebrates, they form by elaboration of their dorsal hollow neural tube. Categorizing cephalic nervous systems in protostomal bilaterians as simple cerebral ganglia or brains is somewhat arbitrary, because the criteria are based on the relative size and functional complexity of the cephalic structure in question. Simple cerebral ganglia and brains in protostome bilaterians thus represent grades of increasing morphological and functional complexity. There is a similar problem in defining a "brain" among chordates: cephalochordates possess a brain that is only slightly more complex than their spinal cord; they do not seem to have a homolog of the cerebrum in vertebrates; and separation of a thalamus and midbrain does not appear to exist, nor does a cerebellum (Nieuwenhuys et al., 1998; Northcutt, 2003). It is possible that future analyses will reveal additional morphological categories among cephalic neural characteristics in metazoans; if so, this information may help to resolve the definition of a brain and thus contribute to our understanding of the evolution of centralized nervous systems.

Distribution of the three cephalic neural characters is plotted on the cladogram in Fig. 3.2. Polarization of these characters among deuterostomes suggests that a diffuse nerve plexus is the primitive character, and a brain is the derived character. In both ecdysozoan and spiralian clades, simple cerebral ganglia seem to be the primitive character, whereas a brain is the derived character. If so, brains have evolved three times independently among protostome bilaterians (Fig. 3.2). The condition in the last bilaterian common ancestor could be either a diffuse nerve plexus or a simple cerebral ganglion, but examination of the metazoan outgroups suggests that the last bilaterian common ancestor possessed a diffuse nerve plexus like the last common ancestor of all metazoans.

Different conclusions might be reached, however, if xenocoelmorphs were determined not to be deuterostomes (Philippe et al., 2011) but the sister group to all other bilaterians (Adoutte et al., 2000). If this finding was the case, it would be impossible to polarize the characters diffuse

nerve plexus and brain in deuterostomes. Because the third neural character, simple cerebral ganglion, is the primitive condition in protostome bilaterians, additional examination of the outgroups, xenocoelmorphs and the basal metazoan clades, would still indicate that the last common bilaterian ancestor and the last common metazoan ancestor possessed a diffuse nerve plexus.

Outgroup analysis of intracladal variation in central cephalic neural characters also indicates that brains have evolved numerous times independently. For example, analysis of cephalic neural characters within the molluscan clade reveals that basal molluscans (monoplacophorians and polyplacophorians) have simple cerebral, pleural, and pedal ganglia interconnected by ventral and lateral medullar cords (Bullock and Horridge, 1965), which are retained in some basal gastropods. Hypertrophy of the various ganglia seems to have occurred independently in several gastropod groups as well as independently in octopod cephalopods (Moroz, 2009), which have evolved the most complex brains among invertebrates.

It could be said that the present outgroup analysis may be flawed by mistaking secondary character reductions (degenerative events) for primitive characters (Reichert and Simeone, 2001; Jenner, 2004; Hirth, 2010). If this flaw was the case, 23 of ~30 phyla would have to possess secondarily degenerated cephalic neural characters, which in the context of this cladogram, would have to have occurred at least 11 times independently. Needless to say, this interpretation would not be parsimonious, but it does raise the question of how secondarily simplified characters can be recognized from primitive simple characters.

In addition to the outgroup rule, there are at least four auxiliary criteria that suggest to zoologists that primitive characters are actually secondarily simplified characters: (*i*) when the characters are in sessile taxa, (*ii*) when the characters are in parasitic taxa, (*iii*) when the characters are in paedomorphic taxa, and (*iv*) when the characters are in taxa with secondary loss of microRNAs. Three of these criteria have been recognized by zoologists for almost 50 years (Bullock and Horridge, 1965). Secondarily simplified characters have long been suspected in sessile tunicate urochordates, bryozoans, phoronids, entoprocts, and parasitic cestode and trematode flatworms and rhombozoans, which are obligate symbiotes in the nephridia of cephalopod molluscs. Similar secondary simplification also frequently occurs when ancestral ontogenies are truncated (paedomorphosis), leading to reduction in body size and morphological complexity, which is widely documented in salamanders (Duellman and Trueb, 1986). The fourth criterion, taxa with secondary loss of microRNAs, may offer a molecular explanation for the first three criteria, and it may also identify additional taxa characterized by multiple character reductions or losses. Acoela, Platyhelminthes, and *Xenoturbella* each seem to have secondarily

lost microRNAs (Erwin et al., 2011), which suggests that many of their morphological characters are secondarily simplified. It should be noted that, in most if not all of the taxa said to possess secondarily simplified characters, these characters are widespread throughout most organ systems in that taxon rather than confined to a single system. Furthermore, the fossil record can be of immense value in polarizing life histories when the earliest members of a clade are vagile and shift to a sessile existence or when there are clear trends in body size.

LAST COMMON BILATERIAN ANCESTOR

There are two very different reconstructions of the morphology of the last common bilaterian ancestor (LCBA) or urbilaterian (De Robertis and Sasai, 1996). Many developmental biologists, relying on the roles of numerous genes and gene networks in the development of arthropods and vertebrates [summarized in Erwin (2006)], suggest that the LCBA was a morphologically complex organism with anterior–posterior differentiation of a head that possessed paired eyes, a tripartite brain, and a segmented trunk with a differentiated gut, heart, and appendages. In contrast, many paleontologists and zoologists would suggest that the LCBA was far simpler morphologically, perhaps a small vernanimalcular-like organism that was patterned in both the anterior–posterior and dorsoventral axes but not segmented. This ancestor would have possessed a mouth and anus connecting a differentiated gut surrounded by coelomic cavities. The nervous system would have been a diffuse nerve plexus, and the apical pole of the organism would have had simple ocelli composed of both ciliary and rhapdomeric photoreceptors. The trunk may have contained contractile muscle cells but no heart, segmented muscles, or appendages, and locomotion would primarily have involved ciliary gliding.

Both molecular clock and paleontological data indicate that bilaterian metazoans arose ~600–700 Mya during the Ediacaran, and they radiated rapidly into most bilaterian crown clades by the end of the Cambrian (Erwin et al., 2011). It is also clear that most genes involved in developmental genetic networks determining anterior–posterior and dorsoventral patterning must already have been in place in the LCBA (Davidson, 2006; Erwin et al., 2011). If the fossils of the Doushantuo *Vernanimalcula* and some of the macroscopic fossils of the Ediacaran biota, such as *Dickinsonia* and *Kimberella*, are interpreted as stem bilaterians, then the body plans of the earliest bilaterians must have been relatively simple and comparable with the body plans of living placozoans, platyhelminthines, and aplacophoran molluscs. Although neural structures are rarely fossilized, it is possible to relate neural complexity to specific grades of body complexity (Bullock and Horridge, 1965). A conservative interpretation of body com-

plexity of the macroscopic Ediacaran biota suggests that these organisms were characterized by diffuse nerve plexuses. A more heterodox interpretation of organisms, such as *Spriggina*, *Praecambridium*, and so-called soft-bodied "trilobites," is that they are members of clades closely related to annelids and arthropods, which would suggest that some Ediacaran organisms may have already evolved cerebral ganglia sufficiently complex to be termed brains. Given the body complexity of Cambrian annelids, arthropods, and chordates, it is reasonable to assume that the CNSs in these clades were characterized by brains. Interestingly, this level of neural complexity may not have been reached by cephalopod molluscans until the Devonian some 70 Myr later, with the origin of octopod cephalopods (Kluessendorf and Doyle, 2000).

Outgroup analysis of inter- and intraclade variations in cephalic neural characters (Fig. 3.2) supports an LCBA model with a diffuse nerve plexus, which subsequently coalesced into a number of cephalic ganglia and nerve cords or a dorsal hollow neural tube. Hypertrophy and increase in cellular differentiation of cephalic ganglionated and dorsal neural tube systems independently reached levels of neural complexity that are defined as brains in arthropods, annelids, and some molluscs and chordates.

Conservation of genetic regulatory networks, which has been termed deep homology (Shubin et al., 2009; Scotland, 2010), has been invoked to claim that all bilaterian brains are homologous (a shared derived character of all bilaterian metazoans) and consist of three anterior–posterior divisions (tripartite brain hypothesis). A basic assumption of this claim is that conserved genetic regulatory networks also have a conserved role in the development of phenotypes. As developmental biologists dissect the genetic mechanisms that control processes underlying the development of phenotypic characters, it seems that some genetic networks determine character identity, whereas others determine character state (Wagner, 2007). Only as the genomes of additional taxa are probed and analyzed cladistically will it be possible to determine if homologous character identity networks underlie phenotypically recognized brain divisions across all bilaterian metazoans. Meanwhile, metazoan interrelationships and the evolution of their nervous systems will continue to be debated, hopefully, with the reminder by the late renowned invertebrate zoologist, Donald P. Abbott, to "[c]ultivate a suspicious attitude toward people who do phylogeny" (Brusca and Brusca, 2003).

ACKNOWLEDGMENTS

Part II

DEVELOPMENTAL AND ADULT VARIATION IN NEURAL ORGANIZATION

The five chapters in Part II all focus on nervous system organization. This emphasis is important because, traditionally, comparative research tends to focus on similarities rather than differences (i.e., on conservation rather than variation). However, after the conserved features are known, the research focus can shift to the nonconserved features, the variable elements. In grappling with this variation, researchers often look for constraints and scaling principles (Striedter, 2005), and they seek to explain the variation in mechanistic terms.

In Chapter 4, Erin Jarvis and colleagues review the segmental variation in arthropod appendages (mainly mouthparts and limbs) and its control by *hox* genes. They note that *hox* genes also control segmental variation in the motor neurons that control the various appendages. This observation is important because it suggests that variation in *hox* gene expression patterns can coordinate evolutionary changes in appendage morphology with evolutionary changes in motor neurons, thus ensuring functionality. Pursuit of this idea will extend evo-devo (evolutionary developmental) biology, which has thus far focused primarily on body plan evolution, into the realm of neuroscience, which is just beginning to experience an evo-devo boom (Striedter et al., 2011).

Continuing in Chapter 5 the neuro-evo-devo theme, Luke McGowan and colleagues present results from an experiment in which they used intraventricular FGF2 injections to delay neurogenesis in the optic tectum of chicks. This manipulation increases tectum size to the point where parts of the tectum form folds, an interesting finding because delays in

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neurogenesis have likely led to cortical folding in large-brained mammals. However, the FGF2 injections also disrupt the normally smooth pattern of tectal lamination, which is unlikely to be adaptive. Intriguingly, McGowan et al. suggest that the laminar disruptions are causally linked to ruptures in the overlying pia mater. Collectively, these findings imply that evolutionary increases in the size of brain regions must be coordinated with expansions of the associated pia mater, which may be difficult when neural expansion is caused by a delay in neurogenesis.

In Chapter 6, Leah Krubitzer and Adele Seelke focus on variability in cortical organization, both within species and across mammalian taxa. In addition to describing this variability, they analyze its phylogenetic pattern and underlying mechanisms. In particular, they suggest that the cerebral cortex is constrained to vary in specific ways rather than being freely variable. This finding would explain why many features of cortical organization are broadly conserved and why some variants evolved repeatedly and independently in diverse lineages. What sorts of mechanisms generate this variation and its constraints? As Krubitzer and Seelke review, both intrinsic genetic and extrinsic activity-dependent mechanisms are at play. Furthermore, variation in one part of the nervous system can induce changes in distant, functionally related brain regions. For example, removal of the eyes during early development causes a dramatic reduction and functional respecification of the primary visual cortex. A similar cascade effect has been observed in blind mole rats. Thus, experimental manipulations of brain development can mimic at least some aspects of natural variation.

Jon Kaas continues in Chapter 7 the discussion of mammalian cortical variation, but his chapter is focused more explicitly on neocortical modules, which include cortical areas, patches, bands, stripes and interstripes, blobs and interblobs, and columns and minicolumns. Within each module, adjacent neurons tend to be activated by similar stimuli at similar locations or, for movement-related neurons, to control similar behaviors. Between modules, activity patterns change abruptly. These findings suggest that cortical modules are generated by Hebbian plasticity, which strengthens connections between neurons that fire simultaneously or nearly simultaneously. Although this form of plasticity is most often invoked as a mechanism for generating topographic maps within the brain, it can also explain the formation of abrupt boundaries, because such boundaries can maximize the overall probability that adjacent neurons fire concordantly. As Kaas suggests, the mechanisms for topographic map and module formation seem to exist throughout mammalian neocortex but also in some other brain regions, such as the frog's optic tectum.

In Chapter 8, Suzana Herculano-Houzel steps back from the organizational details of mammalian brains and focuses instead on the number of

neurons and nonneurons (primarily glia) found in the major brain regions of various mammals. Using the isotropic fractionator method, which involves homogenizing brain regions and counting stained cell nuclei in samples from the resulting homogenate, she discovered that neuron numbers scale differently (against brain region mass) in primates and rodents. This finding may explain why primates tend to be more intelligent than other mammals, even when brain mass is held constant: as brain size increases, primates have more neurons per gram of brain tissue than other mammals. Accordingly, Herculano-Houzel argues that absolute neuron number is a better predictor of "intelligence" than absolute brain size. She also points out that human brains contain almost exactly the number of neurons that one would predict, given the primate scaling rules. This conclusion would have pleased T. H. Huxley, if not Darwin himself. Moving beyond these findings, Herculano-Houzel proposes interesting ideas on the evolution of brain energy costs and their relationship to feeding behavior.

4

Evolving Specialization of the Arthropod Nervous System

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AND NIPAM H. PATEL*[†‡]

The diverse array of body plans possessed by arthropods is created by generating variations upon a design of repeated segments formed during development, using a relatively small "toolbox" of conserved patterning genes. These attributes make the arthropod body plan a valuable model for elucidating how changes in development create diversity of form. As increasingly specialized segments and appendages evolved in arthropods, the nervous systems of these animals also evolved to control the function of these structures. Although there is a remarkable degree of conservation in neural development both between individual segments in any given species and between the nervous systems of different arthropod groups, the differences that do exist are informative for inferring general principles about the holistic evolution of body plans. This review describes developmental processes controlling neural segmentation and regionalization, highlighting segmentation mechanisms that create both ectodermal and neural segments, as well as recent studies of the role of Hox genes in generating regional specification within the central nervous system. We argue that this system generates a modular design that allows the nervous system to evolve in concert with the body segments and their associated appendages. This information will be useful in future studies of macroevolutionary changes in arthropod body plans, especially in

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understanding how these transformations can be made in a way that retains the function of appendages during evolutionary transitions in morphology.

The phylum Arthropoda derives its name from the Greek words for "joint" and "foot" (or "leg"), and the remarkable functional diversity of these arthropod appendages has contributed to the notable evolutionary success of this animal group. The basic arthropod body plan consists of serially repeated body segments, with a pair of appendages on most of these segments. Individual segments (or groups of adjacent segments), along with their associated appendages, are often specialized for particular functions (Brusca and Brusca, 2003). These patterns of specialization vary enormously between arthropod species, and this flexible, modular body plan accounts for the superb mobility and specialized feeding modalities that have enabled arthropods to fill a wide variety of terrestrial and aquatic ecological niches. In turn, the great adaptability of arthropod body morphology may be a result of a highly coordinated patterning mechanism that uses a common regulatory network to align regional identity for the ectoderm, mesoderm, and nervous system along the body axis.

Genetic and molecular studies in the model arthropod, *Drosophila melanogaster*, have provided us with a detailed understanding of the mechanisms that subdivide the embryo into segments and provide regional identity to these units. The sequential action of maternal gradients and zygotic gap, pair-rule, and segment polarity genes sequentially subdivides the embryos into smaller and smaller units, ultimately organizing the pattern of segmentation. A portion of this segmentation network also regulates the expression of homeotic (Hox) genes, which provide regional identity to the developing segments to make segments distinct from one another (Fig. 4.1). Altering the expression patterns of these Hox genes leads to the transformation of one or more segments toward the identity of adjacent segments. Subsequent studies revealed a remarkable level of evolutionary conservation of these Hox gene transcription factors, and it appears that Hox genes play a well-conserved role in patterning regional identity along the antero-posterior axis in all bilaterian animals.

Whereas Hox genes have provided developmental biologists with an outstanding example of a deeply conserved mechanism of pattern formation, changes in these genes have also been implicated in the evolutionary process that has led to the diversification of body plans both between and within animal phyla. For example, comparisons of Hox gene expression and function within the various groups of arthropods led to a number of hypotheses regarding the possible role of these genes in the evolution of the arthropod body plan [reviewed in Hughes and Kaufman (2002)].
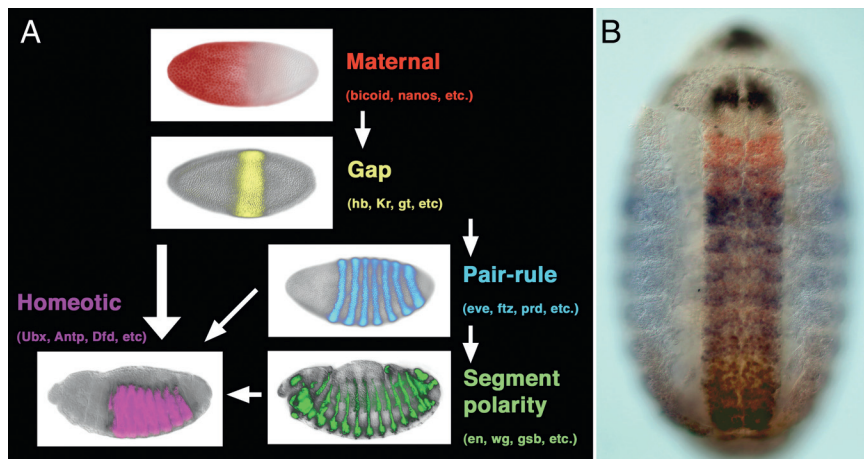
FIGURE 4.1 Early embryo patterning along the antero-posterior axis in *Drosophila*. (*A*) Hierarchy of maternal gradients and zygotic gap, pair-rule, and segment polarity genes establishes the repetition of segments, whereas the homeotic genes regionalize the body plan, making segments differ from one another. (*B*) Protein expression pattern produced by four (of the eight) Hox genes at midembryogenesis. Scr is in black, Antp in red, Ubx in blue, and Abd-B in brown. More intensely stained area in the middle is the central nervous system. Anterior is to the left in *A* and up in *B*. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

Indeed, work on Hox genes played a key role in the renaissance of evolutionary developmental biology ("evo-devo") during the past 30 years.

One example of the potential role of Hox genes in morphological evolution comes from work on *Ultrabithorax* (*Ubx*) in crustaceans. In this case, changes in the expression pattern of *Ubx* are associated with the evolution of a specific type of appendage, known as a maxilliped, which is a jaw-like feeding appendage that is part of the anterior thorax. Depending on the species, crustaceans possess anywhere from zero to three pairs of maxillipeds, and, as illustrated in Fig. 4.2, the point of transition from maxilliped-bearing segments to more posterior thoracic-type segments (usually used for locomotion) is correlated with the boundary of Ubx expression (Averof and Patel, 1997). This relationship between *Ubx* and the evolution of body patterning was moved beyond correlation with functional studies in the amphipod crustacean, *Parhyale hawaiensis*. A combination of misexpression and knockdown experiments revealed that the number of maxillipeds could be increased or reduced by knocking down or misexpressing *Ubx*, respectively, in *Parhyale* (Liubicich et al., 2009; Pavlopoulos et al., 2009).
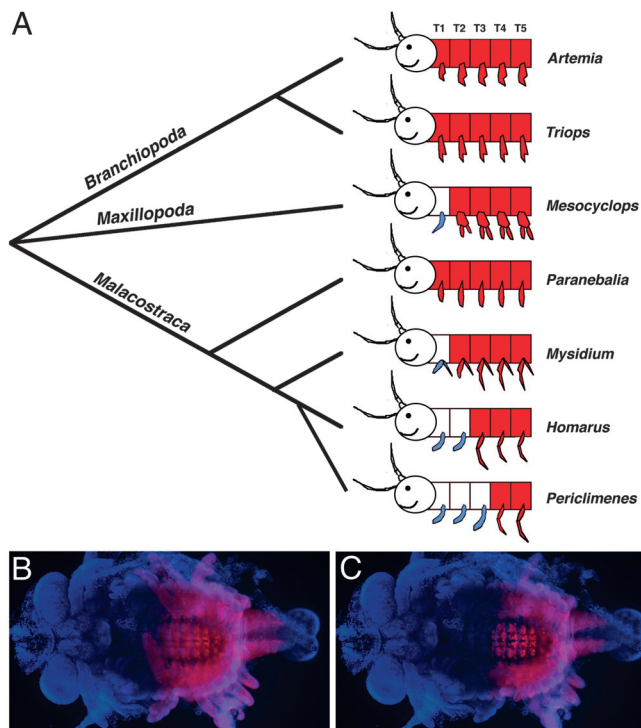
FIGURE 4.2 Correspondence between Ubx expression and the transition from feeding to locomotory appendages along the antero-posterior axis during crustacean evolution. (*A*) Ubx expression is shown in red, maxillipeds are shown in blue, and anterior is to the left. The anterior boundary of Ubx expression in various crustacean species corresponds to the transition point from feeding to locomotory appendages. The head appendages of Mn, MxI, and MxII are not shown, but would also be classified as feeding (jaw) appendages [adapted from Averof and Patel (1997)]. (*B* and *C*) Ubx protein expression (in red) in a marble crayfish embryo focused to highlight expression in the appendages (*B*) and the nervous system (*C*). In this species, there are maxillipeds in T1-T3, and Ubx expression begins at T4 in both the ectoderm and the nervous system. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

The change in the number of maxillipeds was not due to a change in the total number of appendages, but rather due to homeotic transformations altering the relative ratio of different appendage types, resembling the general pattern of differences seen between existing crustacean species.

Whereas these experiments result in the striking transformation of appendage morphology that mimics evolutionary transitions, reservations about the relevance of such Hox-mediated transformation to the natural process of evolution still remain. Such radical morphological transformations in a single step are probably unlikely to be adaptive, but it is reasonable to consider that gradual morphological changes would simply require incremental changes in the patterns and levels of Hox gene expression. Indeed, some crustaceans, such as mysids, possess appendages of intermediate morphology (between a standard maxilliped and a swimming leg) that are associated with intermediate levels and mosaic patterns of *Ubx* expression (Averof and Patel, 1997). Thus, it is likely that microevolutionary changes in Hox gene regulation could occur over time to lead to macroevolutionary changes in morphology.

A more important consideration is that even gradual transformation during evolution must occur in such a way that the appendage and associated segment remain functional and useful to the organism at each point in the transition. For this to happen, more than just the external morphology of the appendage needs to be altered. Coordinated changes must also be made in the musculature and nervous system associated with the transforming appendage. It is reasonable to assume that the segment must evolve as a whole, with coordination between the ectoderm, mesoderm, and nervous system. We suggest that the Hox gene system functions in arthropods in a manner that facilitates such a coordinated transformation. Our purpose here is to review the manner in which the nervous system is patterned in arthropods, highlighting first that the same system used for ectodermal segmentation, particularly at the level of segment polarity genes, contributes to generating the segmental organization of the nervous system, and second, that Hox genes play a major role in the regionalization of the nervous system just as they do for the ectoderm. Most of the data come from studies in *Drosophila*, but comparative studies have helped to define properties that are generally conserved in neural patterning across the phylum. In conclusion, we argue that the manner in which Hox genes function in the nervous system provides a mechanism to coordinate the different parts of the segment during evolutionary transitions.

NEUROGENESIS IN ARTHROPODS

In arthropods, neurogenesis takes place within a broad ventral domain called the ventral neuroectoderm (VNE), which is competent to form both ectoderm and neural precursor cells. In the VNE of insects, groups of four to eight cells within each hemisegment are recruited into a proneural fate by the achaete-scute complex, and stochastic interactions mediated by Delta-Notch signaling specify one of these cells to become a neural

stem cell, called a neuroblast (NB), and the remaining cells become epidermal (Goodman and Doe, 1993; Campos-Ortega, 1995). The specified NB then delaminates from the surrounding epithelium (Fig. 4.3*A*) and undergoes several rounds of asymmetric division perpendicular to the epithelium, thereby generating a column of cells called ganglion mother cells. Each ganglion mother cell divides once, symmetrically, to produce either two postmitotic neurons or two postmitotic glial cells (Doe and Goodman, 1985; Campos-Ortega, 1995). The lineage resulting from each NB is invariant.

Neuroblast formation and proliferation to form neurons and glia in malacostracan crustaceans are similar to those in insects, with some notable exceptions. Crustacean NBs remain within the VNE and do not delaminate from the epithelium (Fig. 4.3*B*) and NB specification appears to involve an invariant lineage pattern (Scholtz and Dohle, 1996), as opposed to the inductive system seen in insects.

Neurogenesis in both myriapods and chelicerates is fundamentally different from that seen in insects and crustaceans. Rather than specifying a single stem cell that buds off multiple neurons and glia, an entire cluster of cells is recruited into a neural fate (Fig. 4.3*C*) (Stollewerk et al., 2001; Dove and Stollewerk, 2003; Kadner and Stollewerk, 2004). Each cell within this cluster invaginates from the VNE, forming a conspicuous layer of cells beneath the presumptive ectoderm. In centipedes and spiders, each cluster consists of 5–9 cells (Stollewerk et al., 2001; Kadner and Stollewerk, 2004). In the millipede, clusters of up to 11 cells are observed (Dove and Stollewerk, 2003), which, because of the greater number of cells, are arranged in a grapelike as opposed to planar configuration and are less apically constricted than in spiders and chelicerates. In myriapods, each cell within the invaginated cluster divides equally, resulting in a column of cells within the embryo. However, spider clusters appear to proliferate preferentially within the apical presumptive ectoderm layer, before invagination (Weller and Tautz, 2003). Cell lineage tracing experiments have yet to be performed in chelicerates and myriapods to determine the relationships between neurons and glia within each cluster.

Despite these differences in the manner in which neural precursor cells form, across all arthropods each hemisegment generates ~30 NBs (insects and crustaceans) or clusters of precursors (in the case of chelicerates and myriapods) arranged in a stereotyped configuration of seven rows, with a characteristic number of NBs per row (Fig. 4.3*D*) (Doe and Goodman, 1985; Bossing et al., 1996; Stollewerk et al., 2001; Dove and Stollewerk, 2003; Kadner and Stollewerk, 2004). This configuration is serially repeated between segments and, at least for insects and crustaceans, is important for inferring NB homology between segments of the same animal and between segments of different animals (Boyan and Ball, 1993).
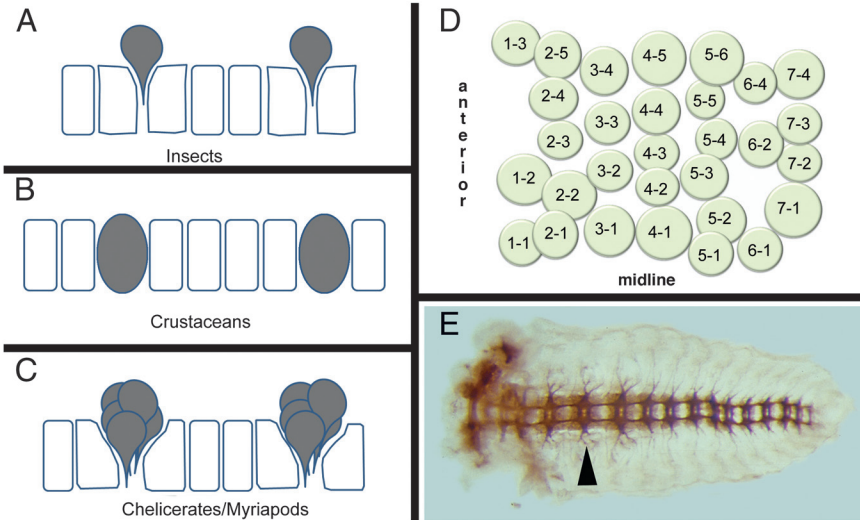
FIGURE 4.3 Arthropod neurogenesis. (*A-C*) Process of neuroblast formation in insects (*A*) and crustaceans (*B*) and precursor clusters in myriapods and chelicerates (*C*). Individual neuroblasts delaminate inward in insects, whereas they remain in the epithelia in crustaceans. In myriapods and chelicerates, instead of neuroblasts, clusters of cells move in to form neurons, although their arrangement is reminiscent of the neuroblast pattern seen in insects and crustaceans [adapted from Stollewerk and Chipman (2006)]. (*D*) Map of neuroblasts in a *Drosophila* hemisegment. The 30 neuroblasts are arranged in seven rows along the antero-posterior axis. (*E*) Nervous system (axon staining) of a grasshopper embryo showing the segmental arrangement of neural ganglia that is coincident with the segmental arrangement of the body ectoderm. Arrowhead points to the ganglion in the T2 segment.

SPECIFICATION OF NEUROBLAST IDENTITY CREATES SEGMENTAL NEUROMERES

In *Drosophila*, the NB array described above is arranged in a segmentally repeated pattern from the outset because of the action of the segmentation network that patterns all ectodermal derivatives. Indeed, much of the specification of the individual NBs occurs before, or just after, their delamination from the ectodermal layer. Detailed studies of the function of segment polarity genes reveal that this level of the segmentation hierarchy acts to pattern the NBs in a manner similar to its role in patterning the overlying ectoderm, although in a few cases it is possible to separate the function of segment polarity genes for patterning the neuroblasts vs. the ectoderm (Chu-LaGraff and Doe, 1993; Duman-Scheel et al., 1997).

Once specified, individual neuroblasts generate specific lineages of identified motor neurons, interneurons, and glial cells. Lineage specification involves the sequential expression of genes such as *hunchback*, *Kruppel*, *castor*, and *PDM* in ganglion mother cells [reviewed in Pearson and Doe (2004)] and cell–cell interactions between ganglion mother cell progeny. This process results in a specific and highly reproducible arrangement of ~600 neurons and glial cells within each segment of the nervous system. Many of these neurons are uniquely identifiable on the basis of morphological criteria such as cell body position and patterns of axonal projection, as well as on the basis of molecular criteria such as patterns of transcription factor and neurotransmitter expression (and some sets of glia are also uniquely identifiable on the basis of cell body position and trancription factor expression). By the midway point of *Drosophila* embryogenesis, the segmental organization of both the ectoderm (with associated appendage primordia) and the underlying central nervous system is clearly visible, and the same is true during the development of all arthropods. In most arthropods, the neural segments (neuromeres) condense into structures known as ganglia (Fig. 4.3*E*), and these ganglia remain located within their respective body segments (*Drosophila* is a notable exception in which the ganglia fuse and move anteriorly, but remain appropriately connected by nerves to their segments of origin).

NEUROMERES SHOW DISTINCT SEGMENT-SPECIFIC PROPERTIES UNDER HOX GENE CONTROL

Just as with the ectoderm, the individual neural segments are not equivalent. Whereas serially homologous neuroblasts of the thorax and abdomen generally produce the same progeny in each segment, there are at least seven lineages that show differences between segments, and it is the expression of Hox genes within the nervous system that controls these regional differences (Prokop et al., 1998; Technau et al., 2006; Rogulja-Ortmann et al., 2008; Kannan et al., 2010). During neurogenesis, Hox genes control NB lineage character by specifying cell number (by regulating both proliferation and apoptosis), cell type (specifying different types of neurons), and neural wiring (regulating axonogenesis). These differences ultimately give rise to segment-specific neural networks. Below, we focus on three individual NB lineages (Fig. 4.2) to demonstrate how Hox genes control NB fate at various stages throughout neurogenesis.

NB 1-1

Each neuroblast 1-1 (neuroblast occupying the first column and the first row) in the thoracic segments generates 8–14 cells, but NB 1-1s in the

abdominal segments generate only 5–6 cells. In addition, all thoracic NB 1-1 progeny are neurons, whereas the abdominal NB 1-1 produces both neurons and glia (Udolph et al., 1993; Bossing et al., 1996). This thoracic vs. abdominal neuromere fate difference for NB 1-1 is specified before NB delamination by *Ultrabithorax* (*Ubx*) and *abdominal A* (*abdA*); and these Hox genes are sufficient to induce an abdominal NB 1-1 fate when mis-expressed in the thorax (Prokop and Technau, 1994).

NB 6-4

In the embryonic thorax, the NB 6-4 lineage generates neurons and glial cells, whereas in the abdomen, the NB 6-4 lineage produces only glial cells (Schmidt et al., 1997). In the thoracic lineage, the absence of *abd-A* and *Abd-B* allows CycE to be expressed before the first division of the NB. CycE localizes to one daughter cell via asymmetric division of the neuroblast, which marks it for a neural fate; the absence of CycE in the other daughter cell promotes a glial fate. In the abdomen, *abd-A* and *Abd-B* directly repress CycE, and the NB divides symmetrically to produce only glial cells (Kannan et al., 2010).

NB 7-3

In embryonic segments of the labium and T3 to A8, the NB 7-3–generated motor neuron GW undergoes apoptosis, whereas in T1 and T2, GW is preserved (Rogulja-Ortmann et al., 2007). The segments in which GW survives correspond to the expression domain of *Antp*. Rogulja-Ortmann et al. (2008) demonstrate that an early antagonistic interaction between *Antp* and *Ubx* regulates the survival of GW during late embryogenesis. *Antp* is required for the survival of GW, whereas *Ubx* promotes apoptosis in this cell. In T3, where both *Antp* and *Ubx* are expressed, *Ubx* is strongly upregulated in late embryogenesis and counteracts the survival signal of *Antp*, resulting in GW apoptosis. The GW motor neuron of the labial segment never receives the *Antp* survival signal and thus undergoes apoptosis. The *Ubx*-directed apoptosis of GW is likely mediated by the proapoptotic gene *reaper*.

Toward the end of embryogenesis, neuroblast division ceases, and the majority of the NBs in abdominal segments undergo apoptosis, whereas in the thorax, very few NBs apoptose (Peterson et al., 2002; Rogulja-Ortmann et al., 2007). During the larval stage, neurogenesis begins once again as the quiescent neuroblasts begin dividing again (and are now known as postembryonic neuroblasts) (Prokop and Technau, 1991). The number of postembryonic neuroblasts (pNB) in each hemisegment varies along the anteroposterior axis, with ~23 pNBs in the thorax and only 3 pNBs

in the central abdomen (Bello et al., 2003). These region-specific differences between homologous pNBs reflect the greater sensory and motor complexity of the adult thorax relative to the abdomen, and again these differences are due to the activity of Hox genes. For example, the three abdominal pNBs transiently express *abd-A* during proliferation, which limits the number of cells they produce (Bello et al., 2003).

REGIONALIZED DIVERSITY OF MOTOR CIRCUITS

An important function of the nervous system is to control locomotion, which is achieved through a complex network of sensory neurons, interneurons, and motor neurons. The evolution of arthropods from a wormlike body plan to one with multijointed appendages implies the evolution of a more sophisticated nervous system with segment-specific innervation of individual muscles within the proximodistal axis of the appendages. To organize a series of muscle activations and coordinated movement, each motor neuron must develop a unique identity, extend axons to corresponding muscle targets, and grow proper dendritic trees that connect to sensory and interneurons. Regionalized locomotion is therefore supported by specialized functional networks that emerge during development. Here we discuss studies that show that the regulation by Hox genes of segment-specific neuronal patterning leads to specialized motor control.

Morphological diversity among segmental units of the nervous system is critical for proper axonal targeting and the formation of functional neuromuscular networks. This regionalized diversity is achieved, in part, by the selective cell death and survival of progenitor cells (as described above) and differentiated motor neurons. The regulation of apoptosis has become increasingly refined throughout evolution, and the key roles Hox genes play in the selective death and survival of neurons support their utility in the evolution of neuronal diversification along the anteroposterior axis (Miguel-Aliaga and Thor, 2004).

The antagonistic effects of *Ubx* and *Antp* regulate the survival of two differentiated motor neurons, GW and MNa, in late stages of *Drosophila* neurogenesis. *Antp* prevents cell death by blocking *reaper-* and *grim*-mediated apoptosis, whereas *Ubx*, which is strongly upregulated in the CNS at a late point in development, activates *reaper*-dependent cell death and executes apoptosis by counteracting the function of *Antp* (Rogulja-Ortmann et al., 2008). The segment-specific levels of *Ubx* and *Antp* may therefore enable the refinement of circuitry via the selective paring of motor neurons.

Hox genes may further specify neuronal morphology along the anteroposterior axis by influencing the selective removal of mature neurons.

Whereas developmental apoptosis typically occurs immediately after cell birth in *Drosophila* and other invertebrates, dMP2 and MP1 motor neurons undergo apoptosis only after axonal extension and the guidance of follower neurons has occurred. The MP1 pioneer neuron originates from the ventral midline after gastrulation and forms part of the CNS midline, whereas MP2 (progenitor of dMP2) originates from the ventral neuroectoderm and forms part of the lateral CNS. Postmitotic apoptosis of dMP2 and MP1 takes place only in anterior segments, and the selective survival in posterior segments A6–A8 is mediated by the differential expression of *Abd-B*, which cell autonomously represses the cell death activators *reaper* and *grim* (Miguel-Aliaga and Thor, 2004).

In the leg-bearing segments of *Drosophila*, motor neurons arise in segment-specific patterns during embryonic and postembryonic neurogenesis (Landgraf and Thor, 2006; Rogulja-Ortmann and Technau, 2008). In each of these segments, ~50 motor neurons arise from at least 11 independent lineages, but the majority of these motor neurons derive from only 2 lineages, referred to as Lin A and Lin B. Lin A motor neurons innervate the distal muscles, the femur and the tibia, whereas Lin B innervates the more proximal leg segments, coxa, trochanter, and femur. In addition to their critical role in motor neuron survival and specification during early development, Baek (2011) proposed that Hox genes, and the Hox cofactors *homothorax* (*hth*) and *extradentical* (*exd*), influence axon and dendritic targeting. *Pb*, *Antp*, *Ubx*, *hth*, and *exd* are differentially expressed during late larval and midpupal stages in adult leg *Drosophila* motor neurons within the CNS (see Fig. 4.4 for expression patterns of Hox genes within the larval and pupal CNS). When the expression of these Hox genes was eliminated, *Drosophila* leg motor neurons underwent apoptosis and axons showed arborization defects. Levels of Hox and Hox cofactor expression vary between individual Lin A motor neurons, and altering levels of *Antp* expression in Lin A cells results in axon targeting errors. By removing expression of the thoracic Hox genes (*Scr*, *Antp*, and *Ubx*) or *hth* function, the number of Lin A motor neurons in all three thoracic segments is reduced. For Lin B, *Antp* is also required for motor neuron survival, and *hth* is required for motor circuit development (Baek, 2011). In thinking about the manner in which Hox genes specialize regions of the *Drosophila* CNS, it is important to remember that *Drosophila* (as well as all other six-legged insects) appears to have evolved from an arthropod ancestor in which there were once legs on every segment; thus many aspects of motor neuron specialization in the *Drosophila* abdomen involve "sculpting" back from a thoracic-type pattern during development.

The influence of Hox in the specification of region-specific motor neurons is not limited to arthropods. In vertebrates, neurons are organized into distinct columns. Along the spinal cord, motor neurons acquire dis-
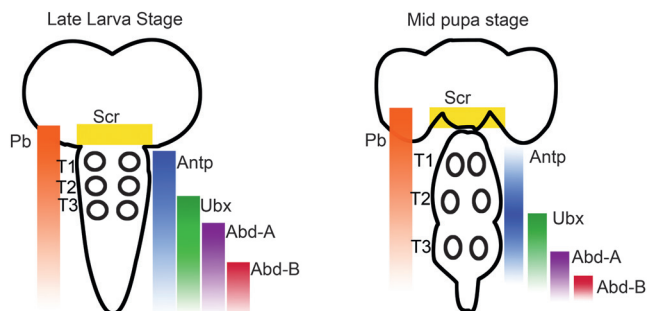
FIGURE 4.4 Summary of Hox gene expression patterns during the larval and pupal stages of *Drosophila.* The Hox genes *Antp, Ubx,* and *Pb* are expressed in the leg motor neuron containing thoracic segments (T1-T3; position of leg motor neurons indicated by circles). [Adapted from Baek (2011).]

tinct columnar identities relative to their position along the rostrocaudal (anteroposterior) axis, and each columnar subtype innervates distinct muscle targets [reviewed by Dasen et al. (2003)]. Interestingly, postmitotic motor neurons express Hox-c patterns relative to their rostrocaudal position (Liu JP et al., 2001), and these expression patterns appear to specify columnar fate. The misexpression of Hoxc6 (members of the *Antp* group of Hox genes) and Hoxc9 (members of the *Abd-B* group) elicits rostrocaudal shifts in thoracic- and limb-level identities, suggesting the role of Hox genes in the specification of motor neuron columnar subtypes (Dasen et al., 2003). Furthermore, the rostrocaudal positioning of the lateral motor column (LMC) by Hox6 initiates subsequent axon projections along the dorsoventral axis of a limb (Kania and Jessell, 2003), and the inactivation of *Hoxa10* and *Hoxd10* (members of the *Abd-B* group) causes defects in hind limb innervation (Wahba et al., 2001).

EXTENSION FROM *DROSOPHILA* TO OTHER ARTHROPODS

As described previously, there are some notable differences between the process of neurogenesis in *Drosophila* and that in other arthropods, which have interesting implications for the notion that homologous structures need not share identical developmental pathways. Homology is an important concept in understanding evolution, and a deeper insight into morphogenesis from a developmental and molecular approach may serve to strengthen an abstract definition by referencing concrete operational mechanisms. In the case of insects and crustaceans, there are very clear homologies at the level of neuroblasts, differentiated neurons, and axo-
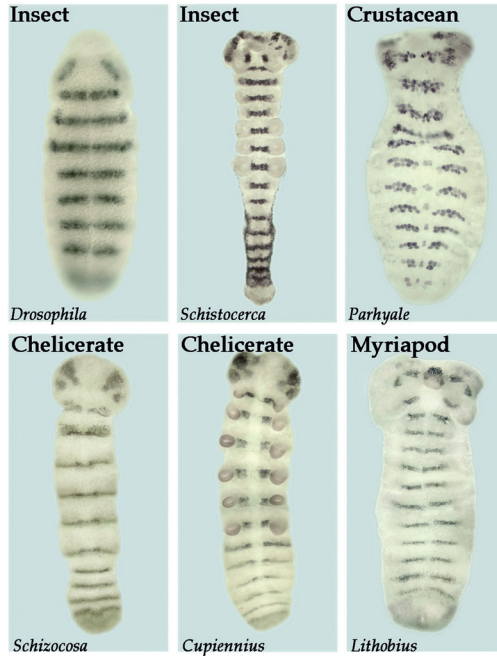
FIGURE 4.5 Similarity in segment polarity gene expression in the ectoderm and developing nervous system of various arthropods. The segment polarity genes function to maintain and refine segments within both the nervous system and the ectoderm of *Drosophila*. Shown here is the expression of the segment polarity gene *gooseberry* (*gsb*) in *Drosophila*. Similar patterns of striped expression of *gsb* homologs through both the ectoderm and the neurogenic region are seen in the grasshopper (*Schistocerca*), a crustacean (*Parhyale*), two species of spiders (*Schizocosa* and *Cupiennius*), and a centipede (*Lithobius*).

nal projections (Duman-Scheel and Patel, 1999). Further studies are still needed in myriapods and chelicerates to determine if one-to-one homologies can be extended to these arthropods. In either case, the segmental nature of the neuromeres is apparent for all arthropods. The early steps in the segmentation process vary significantly between arthropod groups, but there is significant conservation at the level of segment polarity gene expression. For example, the segment polarity gene *gsb* is expressed in the posterior portion of each ectodermal segment and in the underlying neuroblasts of rows 5 and 6. As shown in Fig. 4.5, the expression pattern of this gene is well conserved in insects, crustaceans, myriapods, and chelicerates. Thus, there are clear molecular similarities in the mechanisms that create the pattern of both body segments and neuromeres in all arthro-

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pods. The same also holds true for the genetic system that acts to make neuromeres and body segments different from one another. In this case, the conserved function of the Hox genes appears to control regionalization of both the external body segments (including appendages) and the nervous system in all arthropods studied so far.

SUMMARY

As we have described, the mechanisms of segmentation and body regionalization in arthropods function in a manner that allows developmental coordination between ectodermal structures, such as appendages, and the underlying nervous system. We suggest that subdivision of both the body into segments and the nervous system into neuromeres also provides evolutionary flexibility through modular design—any change in one will be mirrored by changes in the other. If segment number is varied by increasing the number of stripes of segment polarity gene expression, the number of neuromeres will also change so that there is still a one-to-one relationship between neural and ectodermal segments. Likewise, a homeotic shift that alters appendage morphology can simultaneously result in a shift in the pattern of neural regionalization. The next step will be to test these ideas in the context of arthropod evolution. Are homeotic-type shifts in appendage specialization during arthropod evolution accompanied by matching shifts in the nervous system that allow coordinated evolution of both appendage morphology and the neural mechanisms that control the locomotion of these appendages? For example, when a crustacean locomotory appendage is transformed to a feeding appendage, is the underlying neural pattern changed as well to ensure that the transformation is functional, not just morphological? Answering these questions will advance our understanding of how macroevolutionary changes in body plans might occur and ultimately help explain how complex nervous systems and behaviors evolve within animals.

ACKNOWLEDGMENTS

5

Expansion, Folding, and Abnormal Lamination of the Chick Optic Tectum After Intraventricular Injections of FGF2

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CHRISTINE J. CHARVET, AND GEORG F. STRIEDTER

Comparative research has shown that evolutionary increases in brain region volumes often involve delays in neurogenesis. However, little is known about the influence of such changes on subsequent development. To get at this question, we injected FGF2—which delays cell cycle exit in mammalian neocortex—into the cerebral ventricles of chicks at embryonic day (ED) 4. This manipulation alters the development of the optic tectum dramatically. By ED7, the tectum of FGF2-treated birds is abnormally thin and has a reduced postmitotic layer, consistent with a delay in neurogenesis. FGF2 treatment also increases tectal volume and ventricular surface area, disturbs tectal lamination, and creates small discontinuities in the pia mater overlying the tectum. On ED12, the tectum is still larger in FGF2-treated embryos than in controls. However, lateral portions of the FGF2-treated tectum now exhibit volcano-like laminar disturbances that coincide with holes in the pia, and the caudo-medial tectum exhibits prominent folds. To explain these observations, we propose that the tangential expansion of the ventricular surface in FGF2-treated tecta outpaces the expansion of the pial surface, creating abnormal mechanical stresses. Two alternative means of alleviating these stresses are tectal foliation and the formation of pial holes. The latter probably alter signaling gradients required for normal cell migration

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and may generate abnormal patterns of cerebrospinal fluid flow; both abnormalities would generate disturbances in tectal lamination. Overall, our findings suggest that evolutionary expansion of sheet-like, laminated brain regions requires a concomitant expansion of the pia mater.

Evolutionary increases in brain region volumes are common (Striedter, 2005). For example, the neocortex is disproportionately enlarged in primates relative to other mammals, and the telencephalon is disproportionately enlarged in parrots and songbirds relative to other birds (Stephan et al., 1981; Boire and Baron, 1994; Iwaniuk and Hurd, 2005; Striedter, 2005). Recent work in evolutionary developmental neurobiology has shown that these evolutionary increases in brain region volumes are often caused by delays in cell cycle exit of neuronal precursors (Finlay et al., 2001; Charvet et al., 2011). Among birds, for example, parrots and songbirds exhibit delayed telencephalic neurogenesis relative to chicken-like birds (Charvet and Striedter, 2008, 2009; Striedter and Charvet, 2008). Among mammals, cell cycle exit in the neocortex is similarly delayed in primates, which have a disproportionately enlarged neocortex (Clancy et al., 2000, 2001, 2007; Finlay et al., 2001).

Unfortunately, the downstream effects of delayed cell cycle exit on subsequent developmental processes and adult morphology remain poorly understood. One way to fill this gap in our knowledge is to experimentally recreate the key species differences in the laboratory by means of carefully selected developmental manipulations. A good example of this phenocopy approach was the creation of transgenic mice with a constitutively active form of β-catenin that prolongs proliferation, increases neocortical volume, and generates cortical folds (Chenn and Walsh, 2002). In another example, it has been shown that intraventricular injections of FGF2 in rats delay neocortical cell cycle exit, leading to dramatic increases in neocortex volume and neuron number (Vaccarino et al., 1999). Based in part on these experiments, it is becoming increasingly common to explain human cortical evolution and expansion in terms of delayed and prolonged precursor proliferation (Rakic, 1995a; Kriegstein et al., 2006).

The present study began as an attempt to phenocopy natural variation in telencephalon size among birds. Specifically, we reasoned that FGF2 injections into ventricles of embryonic chicks should, by analogy to the work in mammals (Vaccarino et al., 1999), increase telencephalon volume, effectively creating chickens with a telencephalon as large as that of parrots and songbirds (Striedter and Charvet, 2008; Charvet and Striedter, 2009). However, our FGF2 injections did not significantly alter telencephalon development. Instead, they increased the size of the optic tectum, disrupted tectal lamination, and created tectal gyri and sulci.

It is tempting to dismiss these induced alterations as mere pathologies of no evolutionary significance. However, developmental "monsters" have long been used by evolutionary developmental biologists to identify the kind of variation and generative principles with which natural selection must work (Alberch, 1989). Most of this work has used naturally occurring teratologies, but carefully selected developmental manipulations can likewise be useful. Conrad Waddington, for example, argued that experimental perturbations of development can be used to infer the shape of the epigenetic landscapes that constrain developmental and evolutionary variation (Waddington, 1957; Striedter, 1998), even if the results are deleterious.

In line with Waddington's approach, here we describe several changes in tectal development that are induced by FGF2 and provide a model to explain them. Our analysis suggests that experimental expansion of the optic tectum is disruptive mainly because it is not accompanied by an equivalent expansion of the pia mater. This mismatch causes tectal foliation and tears holes in pia, which then disrupt laminar development. The presumably maladaptive nature of these alterations probably explains why naturally occurring species differences in optic tectum size are based, as far as we know, on changes in brain patterning, rather than neurogenesis timing (McGowan et al., 2011). Our findings also suggest that the evolutionary expansion of mammalian neocortex must have required a concordant expansion of the neocortical pia.

RESULTS

We first report on changes in tectal volume, ventricular surface area, and proliferative-zone fraction at embryonic day (ED) 7, 3 days after the FGF2 injections (on ED4). We then describe how these alterations manifest on ED12. Finally, we describe qualitative changes in tectal shape and cytoarchitecture that result from FGF2 injection.

FGF2 Expands the Tectal Progenitor Pool

On ED7, FGF2-treated embryos exhibit an expanded tectum compared with controls (Fig. 5.1). Because of substantial variability in absolute volumes at this age, we express tectum volume relative to the rest of the brain (minus telencephalon and medulla), as estimated stereologically (*Materials and Methods*). By using these methods, we observed a 32% increase in normalized tectum volume [$t(11) = 3.9$; $P < 0.01$; $n = 13$] for the FGF2-treated chicks relative to controls. In contrast, telencephalon volume does not differ significantly between FGF2-treated and control embryos, relative to diencephalon-tegmentum volume [$t(11) = 1.3$; $P = 0.23$; $n = 13$]. FGF2
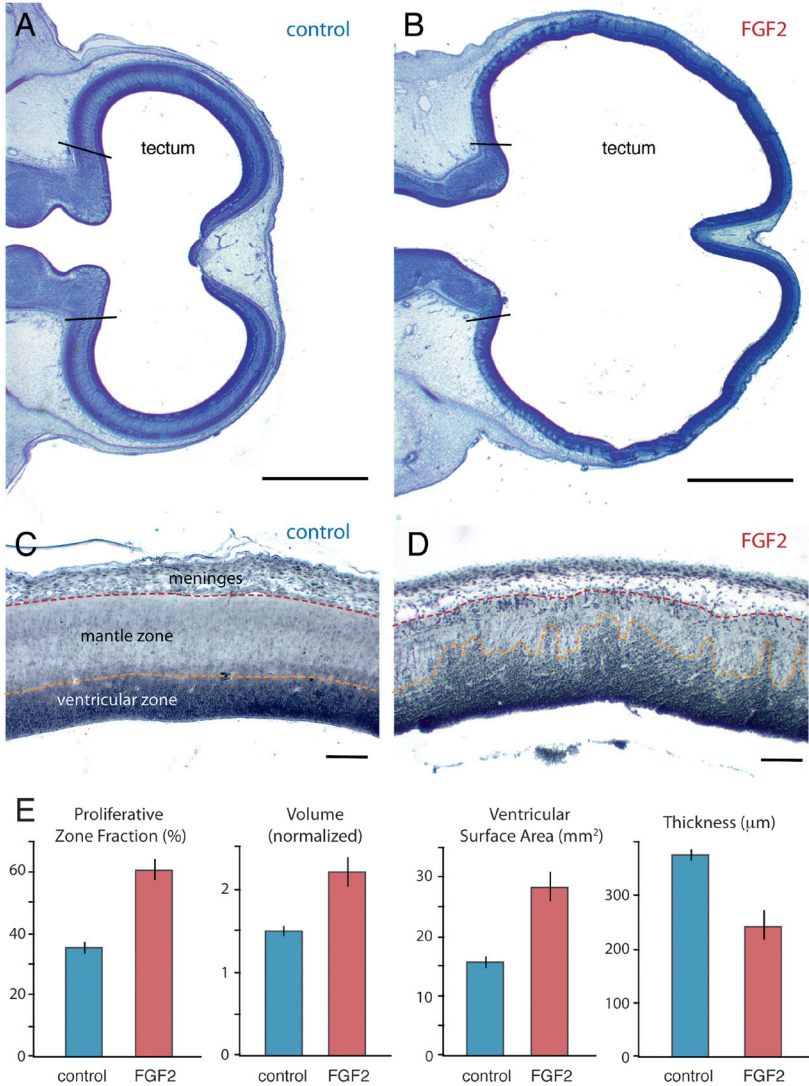
FIGURE 5.1 On ED7, the optic tectum is expanded in FGF2-treated embryos relative to controls, as illustrated here with Giemsa-stained horizontal sections (*A* and *B*; anterior is to the left). Staining with antibodies against PCNA (*C* and *D*) reveals that FGF2 treatment increases the proportion of proliferating cells in the tectum (i.e., PZF; *E*). Normalized tectum volume and tectal ventricular surface area are also larger in FGF2-treated birds than in controls, but tectal radial thickness is reduced. SE bars are shown. (Scale bars: *A* and *B*, 1 mm; *C* and *D*, 100 μm.) [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

injections also expand the tectum's ventricular surface area by 79% [$t(11)$ = 4.9; $P < 0.01$; $n = 13$] and reduce tectal thickness by 35% [$t(11) = -4.5$; $P < 0.01$; $n = 13$].

Postproliferative cells in the developing brain's mantle zone are less densely packed than their proliferating precursors in the ventricular zone (Fig. 5.1*C*). Therefore, the FGF2-induced tectal thinning is consistent with FGF2 delaying tectal cell cycle exit. To test this hypothesis, we computed the fraction of all tectal cells that is proliferative, rather than postproliferative. We have previously used this proliferative zone fraction (PZF) measure to demonstrate species differences in neurogenesis timing (Striedter and Charvet, 2008; Charvet and Striedter, 2009). Here we extend the approach by staining ED7 brains with proliferating cell nuclear antigen (PCNA), a relatively specific marker for proliferating cells (Valero et al., 2005) (Fig. 5.1*C* and *D*). As very few PCNA-negative cells were observed within the PCNA-positive zone (and vice versa), we calculated the tectum's PZF as the volume of the PCNA-positive zone divided by total tectum volume. Our analysis revealed that the tectum's PZF is 67% larger in FGF2-treated embryos than in controls [$t(9) = 7.2$; $P < 0.01$; $n = 11$; Fig. 5.1*E*).

FGF2-Induced Alterations Persist to ED12

On ED12, FGF2-treated embryos still exhibit abnormally large and thin optic tecta (Fig. 5.2). At this age, absolute tectum volume is 40% larger in FGF2-treated chicks than in controls [$t(14) = 2.4$; $P < 0.05$; $n = 16$]. Tectum volume relative to rest-of-brain volume (minus telencephalon) is increased by 57% [$t(14) = 5.9$; $P < 0.01$; $n = 16$], and tectum volume relative to the entire brain is boosted by 33% [$t(14) = 6.6$; $P < 0.01$; $n = 16$]. Again, telencephalon volume is not significantly different between FGF-treated embryos and controls, regardless of how this volume is measured (absolute, $P = 0.94$, $n = 16$; normalized, $P = 0.15$, $n = 16$; volume fraction, $P = 0.53$, $n = 16$). The tectum's ventricular surface area is increased by 181% in FGF2-treated chicks [$t(14) = 5.3$; $P < 0.01$; $n = 16$], but tectal thickness is reduced by 60% [$t(14) = -8.61$; $P < 0.01$; $n = 16$].

Qualitative Changes in Lamination, Folding, and Pial Integrity

The tectum of FGF2-treated embryos exhibits not only quantitative changes in volume and thickness, but also altered morphology. As mentioned earlier, FGF2-treated embryos on ED7 have an abnormally thin mantle zone in the optic tectum. This effect is most extreme in the lateral tectum, where the tectal surface approaches the developing skull. In this region, the ventricular zone of FGF2-treated embryos is not smooth, as in control embryos, but contains irregular radial protrusions that resemble
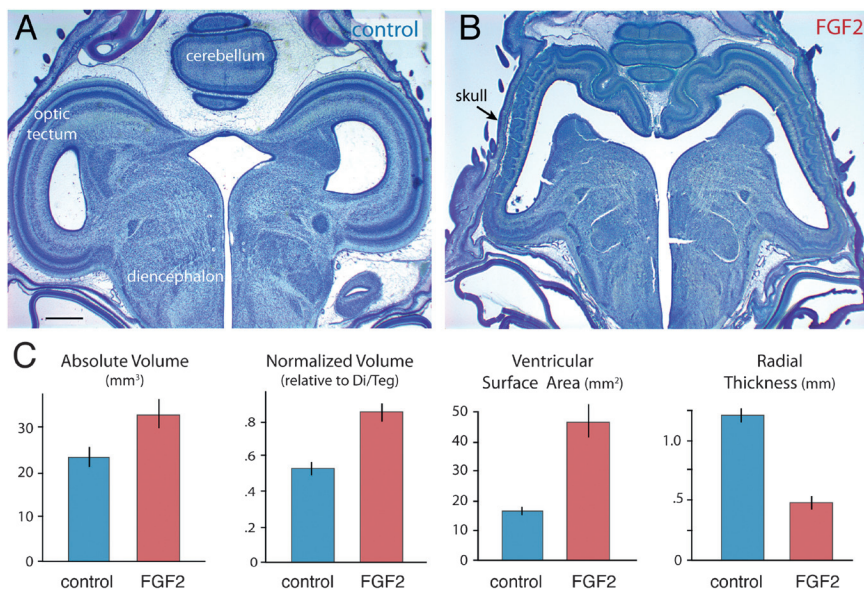
FIGURE 5.2 FGF2-treated embryos on ED12 have a significantly enlarged and abnormal tectum (*A* and *B*). Absolute and normalized tectum volumes, as well as the tectum's ventricular surface, are increased significantly in FGF2-treated chickens relative to controls (*C*). Tectal thickness of FGF2-treated chickens is reduced relative to controls. SE bars are shown. (Scale bar: 1 mm.) [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

mountains (Fig. 5.3*B*). These mountainous protrusions consist mainly of dividing precursor cells of the VZ (Fig. 5.1*C*). However, double labeling with anti-PCNA and bisbenzimide, a fluorescent counterstain, reveals that the tops of the cellular mountains in the FGF2-treated tecta consist primarily of postproliferative cells (Fig. 5.3*C* and *D*).

Nissl staining further revealed that the pia mater in the mountainous tectal region of FGF2-treated embryos is abnormally thin. To examine this more closely, we used an antibody against laminin, an ECM protein secreted by pial cells (Halfter et al., 2002; Siegenthaler et al., 2009). Control embryos on ED7 show a continuous layer of laminin at the tectum's outer surface (Fig. 5.3*E*). This layer of laminin is disorganized in the mountainous regions of the FGF2-treated tecta and exhibits numerous discontinuities (Fig. 5.3*F*).
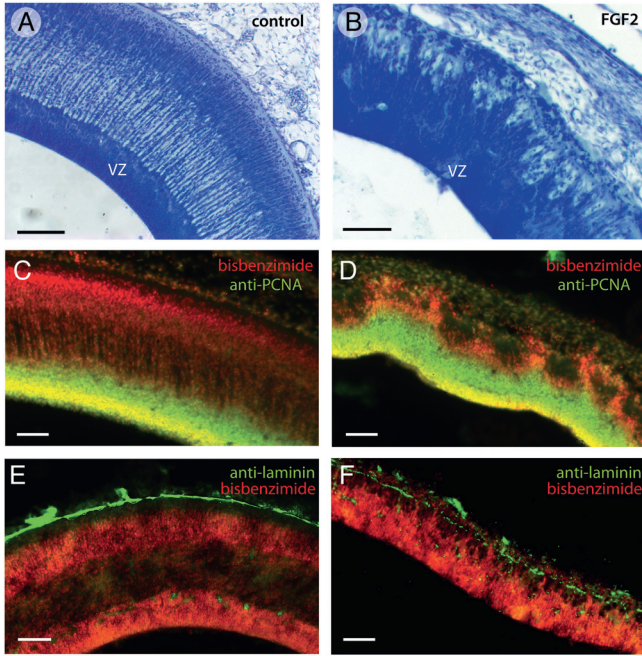
FIGURE 5.3 In lateral portions of FGF2-treated tecta on ED7, the superficial surface of the ventricular zone (VZ) is not smooth, as it is in control embryos (*A*), but irregular or "mountainous" (*B*). Double-staining with anti-PCNA to label proliferating cells and bisbenzimide to label all cell nuclei reveals that the tops of the mountains in the FGF2-treated tecta contain mainly postproliferative cells (*C* and *D*). Staining with antibodies against laminin, which delineates the pia mater, reveals that FGF2 treatment disrupts the pia overlying the mountainous tectal regions (*E* and *F*). (Scale bars: 100 μm.) [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

FGF2-induced abnormalities in tectal lamination are even more pronounced by ED12 (Fig. 5.4). At this age, the tectum of controls contains numerous laminae that are clearly delineated, smooth, and of constant thickness (Fig. 5.4*A*). Corresponding laminae can be identified in FGF2-treated animals, but some of the layers are thinned, especially in the lateral tectum. Furthermore, in these lateral tectal regions, layers vi and viii exhibit protrusions where neurons appear to have migrated too far in the radial dimension (Fig. 5.4*B*). The center of each such protrusion
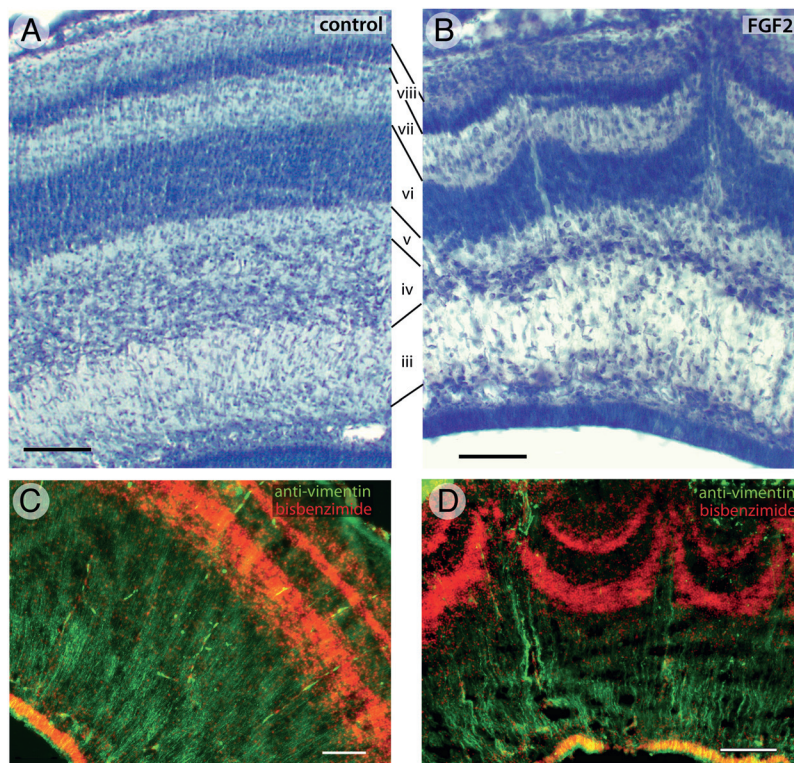
FIGURE 5.4 On ED12, the tectum of control embryos contains numerous laminae (*A*). The same laminae, numbered according to the nomenclature of LaVail and Cowan (1971a), are present in FGF2-treated animals, but some of them are thinner than normal (*B*). In addition, the lateral tectum of FGF2-treated embryos exhibits radial protrusions that resemble volcanoes (*B*). Running up through the center of each volcano are cell-sparse zones containing numerous vimentin-positive radial glia fibers (*D*). The radial glia are more homogenously distributed in control embryos (*C*). (Scale bars: 100 μm.) [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

contains a radially oriented cell-sparse zone. Analysis of serial transverse and sagittal sections reveals these protrusions to be elongate at their base but relatively short and punctate at their tips (McGowan et al., 2012, Fig. S1). Therefore, we refer to these protrusions as "volcanoes." Staining with antibodies against vimentin, an intermediate filament found mainly in radial glial cells, shows that the central channel of each volcano contains numerous radial glia processes (Fig. 5.4*D*). In control birds, these radial processes are more homogeneously distributed (Fig. 5.4*C*).
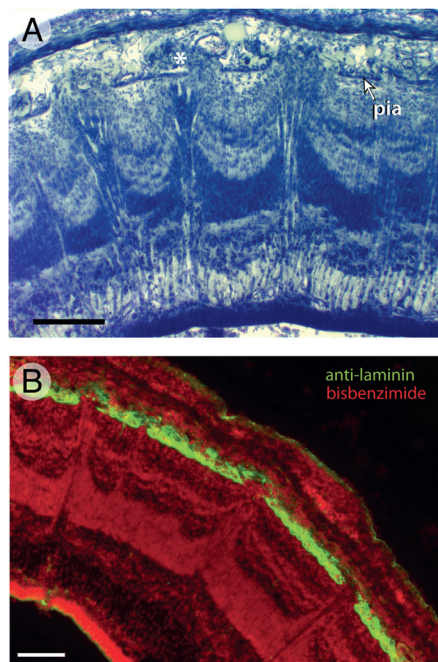
FIGURE 5.5 Individual volcanoes in FGF2-treated tecta are aligned with holes in the pia mater on ED10. This is evident in Giemsa-stained sections (*A*), but even more obvious in sections stained with antibodies against laminin (*B*). The image in *A* also depicts several cell clusters in the space between the pia and the overlying dura mater (asterisk). These ectopias typically extend through the pial holes from the top of individual volcanoes. (Scale bars: 100 μm.) [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

The volcano-like laminar disturbances at ED12 tend to be located in the lateral and dorsal tectum, where the tectal surface approaches the developing skull (McGowan et al., 2012, Movie S1). This location corresponds, at least roughly, to the position of mountainous laminar disturbances observed on ED7 (McGowan et al., 2012, Movie S2). In addition, the peaks of individual volcanoes at ED12 tend to be in register with discontinuities in, or regional thinning of, the overlying pia mater. This alignment between individual volcanoes and holes in the pia is even more obvious at ED10 (Fig. 5.5), suggesting that pial holes are partially repaired between ED10 and ED12. Ectopic cells are often found in the subdural space above each pial hole, especially on ED10 (Fig. 5.5*A*).
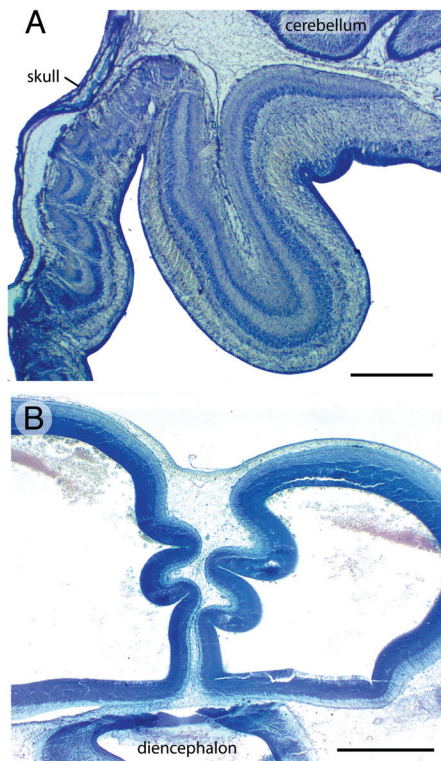
FIGURE 5.6 Folding of the caudomedial tectum in FGF2-treated embryos is seen most often at ED12 (*A*) but also in a few cases on ED7 (*B*). (Scale bars: 1 mm.) [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

Caudal to the volcanoes, the tectum of FGF2-treated embryos frequently exhibits folds resembling cortical gyri and sulci. The extent of folding is variable across embryos, but the folds are usually located in the caudomedial tectum, where the tectal surface lies far from the skull and, by ED12, close to the cerebellum [Fig. 5.6*A* and McGowan et al. (2012, Movie S1)]. Folding is more often seen at ED12 than at ED7, but a few FGF2-treated embryos exhibit prominent tectal folds even at ED7 [Fig. 5.6*B* and McGowan et al. (2012, Movie S2)]. In the folded parts of the tectum, the laminae are consistently smooth and devoid of volcanoes.

DISCUSSION

FGF2 is a secreted growth factor that in vitro prolongs proliferation and delays differentiation for various neuronal and glial precursors (Deloulme et al., 1991; Vescovi et al., 1993; Ray and Gage, 1994; Bouvier and Mytilineou, 1995). It also delays neurogenesis in vivo. Specifically, intraventricular injections of FGF2 in embryonic rats and mice increase neocortical neuron numbers (Ohmiya et al., 2001; Chenn and Walsh, 2002), as one would expect if FGF2 prolongs precursor proliferation. Several studies suggest that proliferation rate and neuronal migration are relatively unaffected by FGF2 manipulations in vivo (Raballo et al., 2000; Ohmiya et al., 2001; Chenn and Walsh, 2002; Kang et al., 2009), although in vitro studies have reported more complex effects (Martín et al., 2006).

Given the mammalian data, it is surprising that our injections of FGF2 into chicken embryos altered tectal, rather than telencephalic, development. The most likely explanation for this species difference is that some of the receptors binding FGF2 are only weakly expressed in the chick telencephalon at the age when we inject exogenous FGF2 (Walshe and Mason, 2000; Nishita et al., 2011). We also note that the rodent studies focused exclusively on neocortical development, leaving some uncertainty about whether FGF2 affects mammalian midbrain development.

Our injections of FGF2 on ED4 appear to delay tectal neurogenesis. The principal evidence for this conclusion is that the tectum's PZF, as determined from the PCNA-stained sections, is significantly higher in FGF2-treated embryos than in controls. Neuronal birth-dating studies to confirm the delay in neurogenesis are in progress. FGF2 treatment also affects neuronal migration in the lateral tectum, where the mountains and volcanoes are observed, but these effects are likely to be downstream consequences of the delay in tectal neurogenesis. Our principal evidence in favor of this hypothesis is that FGF2 injections on ED5 do not induce the migratory abnormalities seen after injections on ED4. However, at this point, we cannot exclude the possibility that FGF2 also affects other developmental parameters, such as cell cycle rate or developmental cell death.

An intriguing aspect of our findings is that FGF2 induces large folds in the caudomedial tectum, but laminar disruptions without folding in the lateral tectum. This differential effect is unlikely to be caused by a difference in FGF2 levels, as the embryonic tectum produces very little endogenous FGF2 (Martín et al., 2006) and the injected FGF2 appears to diffuse homogeneously through the cerebral ventricles. However, the differential FGF2 effect could be related to spatial differences in FGF2 receptor distribution (Walshe and Mason, 2000; Nishita et al., 2011) or to the normal rostroventral to caudodorsomedial gradient of neurogenesis observed within the avian tectum (LaVail and Cowan, 1971b). Alternatively, the

effects of FGF2 on tectal morphology may depend on interactions between the developing tectal surface and overlying nonneural tissues. The latter hypothesis is supported mainly by the observation that mountains and volcanoes are consistently observed in close apposition to the developing skull, whereas the macroscopic folds develop where the skull lies far from the tectum. Taking this observation into account, as well as the finding that FGF2 injections disrupt pial morphology in lateral tectum, we propose the following model to explain most of the observed FGF2 effects (Fig. 5.7).

Young embryonic brains consist mostly of radial glia progenitors that surround the ventricle and extend radial processes toward the pia mater (Kriegstein and Alvarez-Buylla, 2009). As these radial glia divide, the brain tissue expands tangentially (Rakic, 1995a; Kriegstein et al., 2006; Kriegstein and Alvarez-Buylla, 2009). At some point, radial glia begin to leave the cell cycle and become young neurons, which migrate away from the ventricular surface along the radial processes. The neurons are thought to stop their migration when they encounter a molecular signal, such as reelin or retinoic acid, secreted from specialized cells near the pial surface (Halfter et al., 2002; Siegenthaler et al., 2009) (Fig. 5.7*A*).

To explain our observations, we propose that intraventricular FGF2 injections cause the ventricular surface of the developing tectum to expand tangentially more quickly than the pial surface with its attendant laminin-positive basement membrane. Because the radial glia processes are attached to the pial surface (Halfter et al., 2002; Siegenthaler et al., 2009; Georges-Labouesse et al., 1998; Radakovits et al., 2009), the differential tangential expansion creates laterally directed tension between the ventricular and pial surfaces. One way to relieve this tension is to let the tectum buckle into the ventricle, creating macroscopic folds (Fig. 5.7*B*). Alternatively, the differential expansion of the pial and ventricular surfaces can be accommodated by stretching and thinning the pia, which can cause it to become perforated in some locations. We suggest that this second solution is adopted in the lateral tectum of FGF2-treated embryos, perhaps because adhesive interactions between the tectal surface and the overlying skull prevent the tectal infolding.

The formation of mountains and volcanoes in the lateral tectum is likely linked to the pial holes (or small tears), with which they are spatially aligned. One possibility is that the holes in the pia mater lead to gaps in signaling gradients that originate directly from pial cells or from cells associated with them (e.g., Cajal–Retzius cells). If the signal instructs young neurons when to stop their migration, neurons born beneath the gaps would migrate abnormally far, thereby forming the observed volcanoes as well as ectopias in the subdural space (Fig. 5.7*C*). In addition, or alternatively, cerebrospinal fluid may flow through the gaps in the pia and carry young neurons with it through bulk flow. This second hypothesis implies
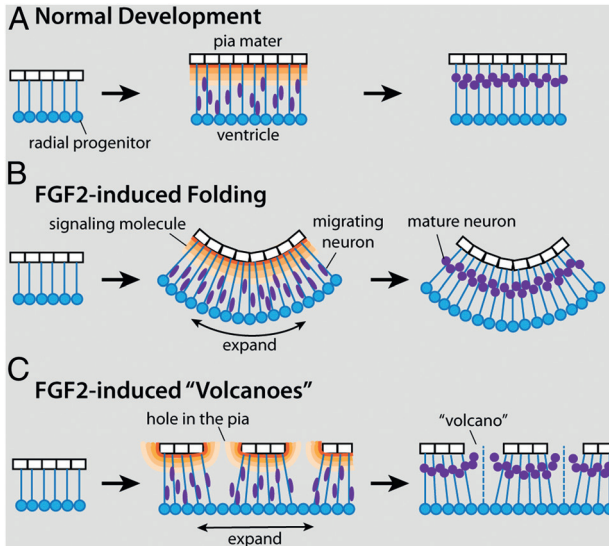
FIGURE 5.7 A working model to explain the two major effects of FGF2 injections on tectal development. (*A*) During normal development both radial glia progenitors (blue) and pia mater cells (white) proliferate, causing coordinate tangential expansion of the tectum's pial and ventricular surfaces. After exiting the cell cycle, young neurons (purple) migrate away from the ventricular surface until they encounter a molecular signal (orange) secreted by specialized cells near the pial surface. By delaying neurogenesis, exogenous FGF2 causes the ventricular surface of the developing tectum to expand tangentially more quickly than the pial surface can expand (*B* and *C*). One way to relieve the tension caused by this differential tangential expansion is to fold the tectum inward, into the ventricle (*B*). Alternatively, the pia can stretch and, in some places, break (*C*). The resultant holes in the pia create gaps in the signaling gradient, which in turn leads to aberrant migration and the formation of cellular volcanoes. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

that intraventricular pressure is relatively high and that the pia mater, rather than the ventricular surface (i.e., the ependymal layer), provides the major resistance to cerebrospinal fluid efflux. Evidence thus far exists only for the former assumption (Desmond et al., 2005). The vimentin-positive radial glia fibers running up the center of individual volcanoes (Fig. 5.4*D*) most likely represent glial processes that grew toward the pial surface after

the holes had formed, sometimes extending beyond the pial surface into the meningeal space (Halfter et al., 2002).

Our model ties together the two major types of changes seen in FGF2-treated tecta, namely tectal folding and volcano-like disturbances in tectal lamination. However, our model does not explain why the tectum remains abnormally thin on ED12, when one might expect tectal differentiation and migration to be largely complete (Crossland et al., 1975), even if tectal neurogenesis is briefly delayed. Perhaps exogenous FGF2 delays neuronal differentiation and migration more than we expect. To test this hypothesis, one would have to examine tectal thickness in older FGF2-treated embryos. Alternatively, FGF2-treated tecta may exhibit increased rates of developmental cell death. Finally, it is possible that tectal cell density is higher in the FGF2-treated embryos than in controls. These hypotheses have not yet been tested.

Another open question is whether evolution ever increased tectum size by delaying tectal neurogenesis. To our knowledge, there have been no published reports of foliated tecta in nature. Furthermore, we have previously shown that chicken-like birds expanded their midbrain tectum, relative to other birds, by shifting an early gene expression boundary, rather than by selectively delaying tectal neurogenesis (McGowan et al., 2011). This does not, of course, prove that evolution never increased tectum size by delaying neurogenesis. However, our present findings suggest that the avian tectum is vulnerable to morphological disruptions if tectal neurogenesis is delayed dramatically. To create viable increases in tectum size by delaying neurogenesis, those delays would have to be small or coupled with increased pial proliferation.

Finally, our study sheds light on the evolution of neocortical folding in mammals. The foliation in our FGF2-treated tecta resembles that observed in the neocortex of mice modified to exhibit increased neocortical progenitor proliferation (Chenn and Walsh, 2002; Kingsbury et al., 2003). However, in all these cases, the pial and ventricular surfaces are equally folded. In contrast, in naturally occurring cortical gyri the ventricular surface is much smoother (and smaller) than the pial surface (Welker, 1990; Kriegstein et al., 2006). Why does evolution prefer the latter mode of cortical foliation? One possible explanation is that involution of the ventricular surface would make it more difficult for axons to cross from one side of a gyrus to the other (Van Essen, 1997). It would also obstruct the path of long-range axons that normally pass down the center of individual cortical gyri (Prothero and Sundsten, 1984). Alternatively, the downside of the natural mode of cortical foliation is that it requires an enormous expansion of the pial surface because, without it, gyral growth would likely rupture the pia and disrupt lamination. Thus, the present study highlights that cortical foliation can be accomplished by various developmental means and that pial development is a critical variable.

MATERIALS AND METHODS

Fertile chicken eggs (*Gallus gallus domesticus*) were obtained from a commercial supplier and incubated in a rotating egg incubator (PROFI-I; Lyon Technologies) at 38° and 50% to 60% humidity. On ED4, 0.5 to 1 μL of human recombinant bFGF (100 ng/μL, dissolved in 0.1 M PBS solution and dyed with methylene blue; R&D Systems) was injected into the lateral or tectal ventricles. The injected FGF2 rapidly diffused throughout the ventricles, regardless of injection site. Control chicks were injected with 0.5 to 1 μL of dyed 0.1 M PBS solution. After injection, the eggs were resealed and transferred to the incubator until ED7, ED10, or ED12. The embryos were then immersion-fixed overnight in methacarn (by volume 60% methanol, 30% chloroform, 10% glacial acetic acid), dehydrated, embedded in paraffin, and sectioned at 18 μm. Approximately 40 to 70 evenly spaced sections from each brain were mounted onto Superfrost Plus slides (Fisher Scientific).

For morphometric measurements, sections were stained with Giemsa stain (Sigma-Aldrich) and coverslipped. Brain regions were delineated and volumes estimated using the Cavalieri method, as described previously (Striedter and Charvet, 2008). As defined here, the telencephalon includes the evaginated hemispheres and midline telencephalic structures; the tectum corresponds to what others have called the optic or dorsal tectum (Delgado et al., 2005). Telencephalon and tectum volumes for the ED12 embryos were normalized by comparing them to the rest of the brain, including diencephalon, pretectum, tegmentum, torus semicircularis, and hindbrain (but excluding tectum or telencephalon, respectively). The hindbrain was excluded from the normalization factor for the ED7 embryos, because it had not been sectioned completely in all the embryos. Ventricular surface area was estimated by summing ventricular surface lengths from a series of regularly spaced sections and multiplying the sum by the section spacing. The tectum's radial thickness was quantified by dividing tectum volume by the tectum's ventricular surface area.

To examine whether FGF2 injections delay tectal neurogenesis, we measured the proliferative and postproliferative zones in FGF2-treated and control chickens at ED7. As development proceeds, cells exit the proliferative ventricular zone and form a postproliferative mantle zone. As the mantle zone expands, the ventricular zone wanes. Therefore, a region's PZF is a good measure of how far neurogenesis has progressed; the higher the PZF, the more neurogenesis has been delayed (Striedter and Charvet, 2008). To estimate the tectum's PZF, we stained sections with antibodies against PCNA. Measurements were made on four equally spaced sections through each tectum and then averaged. Mounted sections were incubated with anti-PCNA (clone PC10; mouse; 1:500; Zymed), followed by a secondary antibody (anti-mouse IgG; 1:200; Vector Labs). They were then

processed with Vectastain ABC standard kits and Vector SG (Vector Labs). Additional sections were processed with Alexa Fluor secondary antibodies (Invitrogen) at a dilution of 1:300 and counterstained with bisbenzimide (Sigma-Aldrich). All statistical tests were performed in the program JMP (version 9; SAS).

For a more detailed analysis of the FGF2-induced morphological alterations, selected sections were stained with antibodies against laminin (clone 3H11; mouse; 1:20; Developmental Studies Hybridoma Bank) or vimentin (clone H5; mouse; 1:20; Developmental Studies Hybridoma Bank). For the anti-vimentin staining, brains were fixed for 2 h in 4% PFA, cryoprotected overnight in 30% sucrose, mounted in optimal cutting temperature compound (Tissue-Tek), and sectioned horizontally at 20 µm by using a Leica CM1850 cryostat. Following incubation with the primary antibodies, sections were incubated in Alexa Fluor secondary antibodies (Invitrogen) at a dilution of 1:300 and counterstained with bisbenzimide. To illustrate the locations of the folds and volcanoes (Movies S1 and S2), 3D animations were constructed from tracings of serial sections by using Neurolucida software (MBF Bioscience).

ACKNOWLEDGMENTS

6

Cortical Evolution in Mammals: The Bane and Beauty of Phenotypic Variability

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Evolution by natural selection, the unifying theory of all biological sciences, provides a basis for understanding how phenotypic variability is generated at all levels of organization from genes to behavior. However, it is important to distinguish what is the target of selection vs. what is transmitted across generations. Physical traits, behaviors, and the extended phenotype are all selected features of an individual, but genes that covary with different aspects of the targets of selection are inherited. Here we review the variability in cortical organization, morphology, and behavior that have been observed across species and describe similar types of variability within species. We examine sources of variability and the constraints that limit the types of changes that evolution has and can produce. Finally, we underscore the importance of how genes and genetic regulatory networks are deployed and interact within an individual, and their relationship to external, physical forces within the environment that shape the ultimate phenotype.

Evolution is the change in heritable, phenotypic characteristics within a population that occurs over successive generations. The notion that biological life evolves and that animal forms descend from ancient predecessors has been considered for centuries and, in fact, predates

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Aristotle (Aristotle et al., 2008). However, Charles Darwin was the first to articulate a scientific argument based on extensive observations for a theory of evolution through natural selection. Darwin's theory contains three basic tenets: individuals within a group are variable, variations are heritable, and not all individuals survive (Darwin, 1859). Survival is based on selective advantages that particular phenotypic characteristics or behaviors confer to some individuals within a given environmental context. Although in Darwin's time our understanding of the brain was in its infancy and Mendel's Laws of Inheritance were little appreciated, Darwin's assertions regarding evolution through natural selection of adaptive traits, were, and still are, compelling.

Recently our understanding of the mechanisms underlying evolution has become more sophisticated, and we appreciate that slight variations in gene sequence can be correlated with alterations of traits and behaviors within and across species. However, an important but often overlooked distinction is the difference between the targets of selection (i.e., phenotypic variations) vs. what natural selection passes on to the next generation (i.e., genes). Although genes are the heritable part of the equation and have a causal, although not always direct, link with some characteristic of the phenotype, genes are not the targets of selection. Genes are indirectly selected for because they covary with the targets of selection, and if the target of selection is adaptive, then genes or portions of the genome replicate and produce a long line of descendants. The direct target of selection is multilayered but can be thought to center around the individual and the unique phenotypic characteristics and behaviors that it displays. These characteristics include external morphology such as color, size, jaw configuration, digit length, and bone density, to name a few. This physical variability in the phenotype is also accompanied by variability in behavior, such as utilization of individual specialized body parts, as well as more complex whole-animal behavior such as intraspecies communication. Based on the assumption that the gene's success is due not only to the individual's success but to its effects on the world, Dawkins (1978) proposed the idea of an "extended phenotype," wherein a gene can find its expression in the body of the next generation or in a created environment that perpetuates its success. For example, bowers built by bowerbirds are variable and have variable success in attracting mates. Inasmuch as the structure of the bower is linked to the phenotypic expression of some behavior that has causal links to one or several genes, the bower is part of an extended phenotype of the bowerbird. Thus, phenotypic expression can occur outside of the individual's body and include inanimate objects used for niche construction and can even include the social niche constructed by differential behaviors of individuals within a population. Because the measure of evolutionary success is reproduction, it follows that the tar-

gets of selection must also include covert features of the phenotype that keep the individual alive long enough to reproduce, such as differential resistance to infection or adeptness at reading social cues.

Although our focus is how brains are altered through the course of evolution, brains, like genes, are not the direct targets of selection. Genes are the heritable components that covary with aspects of brain morphology, connectivity, and function, and in this context, provide a scaffold for brain organization. The brain in turn generates behavior. Ultimately, it is the behavior of a phenotypically unique individual along with its extended phenotype that are the direct targets of selection. Thus, although genes (not individuals) replicate themselves through generations, their link to selection is indirect and convoluted. Of course, an important question is how genes and aspects of brain organization covary with each other and with the targets of selection. Associated questions include these: How variable are features of brain organization? How variable is gene expression and gene deployment during development within a population? In addition, what factors contribute to this multilayered variability of the organism?

We address these questions from a comparative perspective. First we examine aspects of the cortical phenotype that are ubiquitous across species because of inheritance from a common ancestor (homology). We then describe how these characteristics vary across species. We contend that the ways in which homologous features vary provide an important insight into the subtler variations that might be present in individuals within a population. Finally we discuss the external and internal mechanisms that give rise to cross-species and within-species variation and the constraints these forces exert on evolution.

PHENOTYPIC SIMILARITY AND VARIABILITY ACROSS SPECIES

There is a general plan of neocortical organization that has been observed in all mammals investigated. This includes a constellation of cortical fields involved in sensory processing, such as primary visual (V1), somatosensory (S1), and auditory (A1) areas (Fig. 6.1) (Krubitzer, 2009). These homologous fields share similar patterns of connectivity from both the thalamus and other cortical fields, a common architectonic appearance, and neurons within these fields have similar properties (Krubitzer, 2007). These observed similarities allow us to infer the cortical organization of the common ancestor of all mammals (Fig. 6.1) and underscore the constraints imposed on the evolving nervous system. For example, the visual system in blind mole rats is used only for circadian functions, and not for visual discrimination. Yet, V1 is still present, as are geniculocortical connections (Cooper et al., 1993; Nemec et al., 2008). However, V1 is
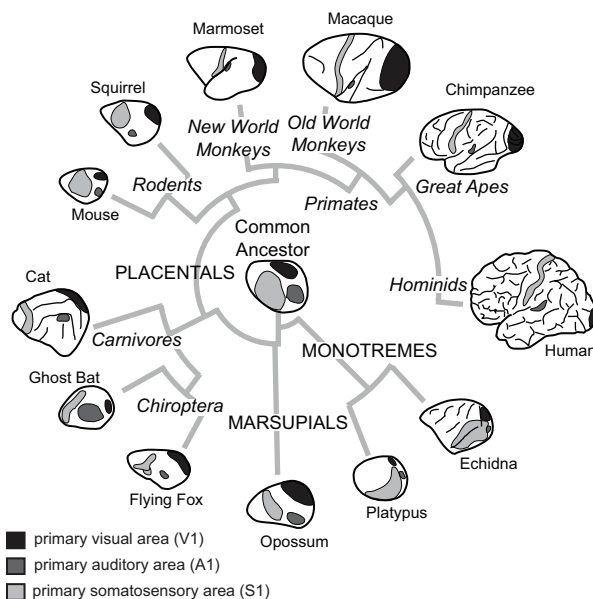
FIGURE 6.1 Cladogram of phylogenetic relationships for the major subclasses of mammals and some of the orders within each subclass. All species examined have a constellation of cortical fields that includes primary somatosensory, visual, and auditory areas (see grayscale codes). However, the relative size and location of this homologous network has been altered in different species.

greatly reduced in size, neurons in V1 respond to auditory stimulation, and subcortical connections of auditory pathways have been rerouted to the lateral geniculate (Heil et al., 1991; Doron and Wollberg, 1994; Bronchti et al., 2002). Comparative studies also allow us to appreciate deviations from this organization that have occurred over evolution.

Surprisingly, the systems-level alterations to the mammalian neocortex are limited (Fig. 6.2). One among these is a change in sensory domain allocation. This specialization begins in the periphery with a relative increase in the innervation of a sensory effector organ, followed by an increase in the size of subcortical structures that receive inputs from this effector organ, an increase in the amount of thalamic territory to which these structures project, and ultimately an expansion in the amount of neocortex devoted to processing inputs from a particular sensory system (Deschênes et al., 1998; Catania, 2011; Catania et al., 2011). Cortical fields within a sensory domain can also vary, both in their overall size and in the size of the representation (or cortical magnification) of specialized

Modifications to the Neocortex
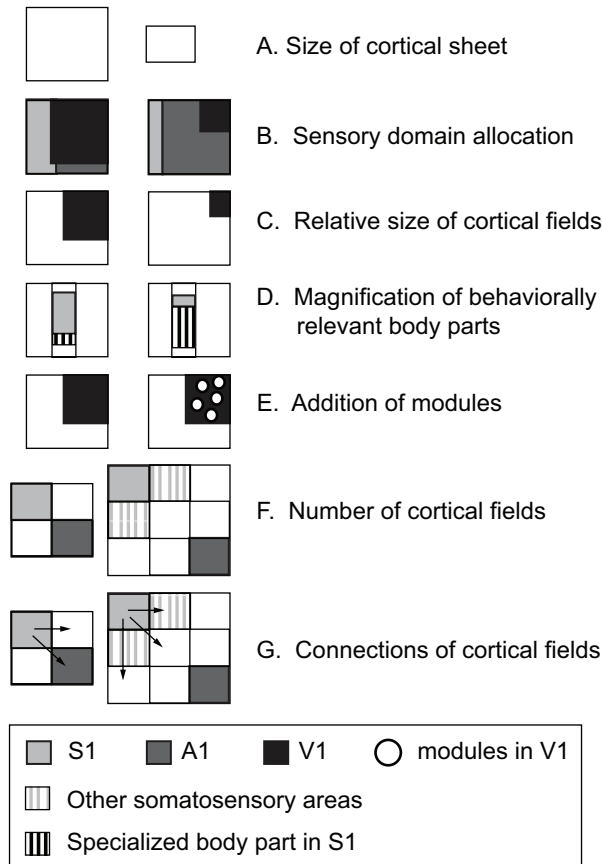
FIGURE 6.2 Schematic of the types of cross-species, systems-level modifications that have been observed in the neocortex. The outline of the boxes indicates the entire cortical sheet (e.g., *A*) and smaller boxes within represent either cortical domains (*B*), cortical fields (*C, E, F,* and *G*), or representations within cortical fields (*D*). Circles in *E* represent modules within cortical fields. These same types of changes have been observed across individuals within a species, but they are often less dramatic.

morphological features, such as the nose of a star-nosed mole or the bill of a platypus (Fig. 6.3). Cortical fields can vary in connectivity with cortical and subcortical structures, and the number of cortical fields varies across species. The persistence of both a common plan of organization, even

Cortical Magnification

A. Duck-billed platypus
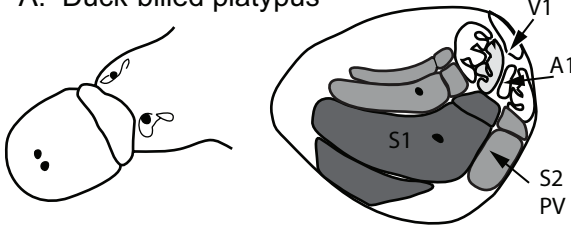
B. Star-nosed mole
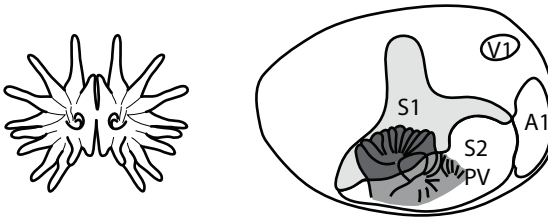
C. Raccoon

D. Rat
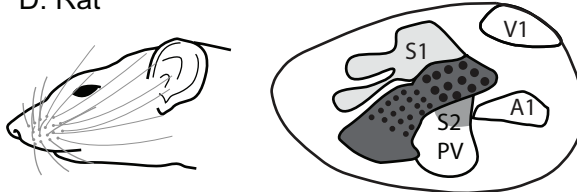
■ Specialized body part representation in S1

■ Specialized body part represenation in other areas

□ Other body part representations in S1

in the absence of use, and the limited ways in which this plan has been independently altered suggest that there are large constraints imposed on evolving nervous systems.

Species also vary in the peripheral morphology of homologous body parts and the use of these structures. A good example is the glabrous hand of humans, the pectoral fin of a dolphin, and the wing of a bat (Fig. 6.4). The hands of humans have undergone several important changes, including alterations in the size of the distal, middle, and proximal phalanges. The carpal and metacarpal joints, the articulation between the first and second carpals, and the metacarpophalangeal joints underwent significant change, as did the size and position of associated ligaments (Lewis, 1977). The distal digit tips also evolved a high concentration of tactile receptors with a high innervation density. These transformations allow for an expanded repertoire of grips, including a precision grip. Although these adaptations are proposed to have evolved for tool use (Marzke and Marzke, 2000), in modern humans the hand is also used for playing instruments and other nontool-related activities.

In dolphins the homolog of the primate hand is the pectoral fin. The fin has undergone several important morphological changes including a transition from bone to soft cartilaginous tissue, elongated digits with additional joints (hyperphalangy), atrophied triceps, immobilization of most of the joints, and lack of most connective tissue structures (Cooper et al., 2007). These alterations to the forelimb allow for different properties and functions associated with locomotion in water, such as increased lift, reduced drag, and the ability of execute turns and braking (Reidenberg, 2007). However, recent studies indicate that fins are also used in "flipper rubbing," which involves the physical contact between one dolphin's fin and another dolphin's body or fin and likely has important social functions (Dudzinski et al., 2009).

Finally, in bats, the wing is the homolog of the hand and fin. Digits 2–5 form the wing, and digit 1 is unattached from the rest of the wing and used for climbing. Although bats have little to no ability to grip or manipulate objects with this highly derived structure, wings are of course well

FIGURE 6.3 Examples of cortical magnification for the bill of the platypus (*A*), the nose tentacles of the star-nosed mole (*B*), the hand of the raccoon (*C*), and whiskers of the rat (*D*). Although the specialized effector is different in different species, the same principle of cortical magnification in somatosensory areas S1 and S2/PV apply.
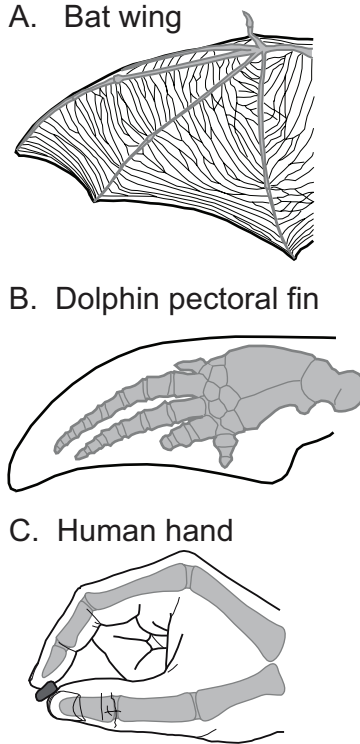
A. Bat wing

B. Dolphin pectoral fin

C. Human hand

FIGURE 6.4 The wing of a bat (*A*), pectoral fin of a dolphin (*B*), and hand of a human (*C*) are examples of homologous morphological structures that have undergone remarkable specialization in different lineages and serve different functions. Although they are used for very different purposes, they are organized around the same basic skeletal frame (gray).

adapted for self-propelled flight [see Zook (2007) for review]. Between the elongated digits, elastin-collagen bands or membranes have evolved. These are covered with small, specialized receptor assemblies, termed touch domes, which are exquisitely sensitive to very small changes in air pressure (Sterbing-D'Angelo et al., 2011). These structures are thought to be used for sensing wing membrane strain during sharp turns, monitoring boundary layer airflow, and locating, tracking, and assisting in the transfer of wing-captured prey to the mouth (Zook, 2007).

In species in which the neocortex has been explored and related to such extraordinary morphological specializations, corresponding alterations have been noted, including cortical magnification within sensory

areas [e.g., Nelson et al. (1980), Calford et al. (1985), Krubitzer et al. (2004)], and in some instances an extreme magnification in higher-order cortical areas, such as Area 5 in macaque monkeys (see Fig. 6.6*B*) (Seelke et al., 2011). Alterations in neural response properties [e.g., rapidly and slowly adapting direction selectivity (Sur et al., 1984; Ruiz et al., 1995; Sterbing-D'Angelo et al., 2011)], architectonic appearance [e.g., Qi and Kaas (2004)], and connectivity have also been observed. Thus, changes in aspects of cortical organization covary with alterations in peripheral morphology and the very unique behaviors associated with this morphology.

One can also compare body parts that are analogous, or have the same function. In human and nonhuman primates the hand is one of the main effector organs used to explore nearby objects or space. Other species use different effector organs for exploration, such as the platypus's bill, the rat's vibrissae, and the nose of the star-nosed mole. Although these structures may not be homologous they have a similar function, and in turn they share similar features of organization of the neocortex, which have emerged independently. In addition to cortical magnification of the main effector organ in different sensory areas (Fig. 6.3), similar but independently evolved patterns of connectivity have emerged between motor cortex and posterior parietal cortex, despite the differences in body parts used to explore the immediate environment.

Perhaps the most compelling example of this phenomenon is the independent evolution of an opposable thumb and precision grip in Old World monkeys and only one New World monkey, the cebus monkey. A repertoire of behaviors associated with this hand morphology includes complex manipulation of objects and tool use in the wild. In terms of neural organization, cebus monkeys have independently evolved a relatively larger cortical sheet, such that their encephalization (Gibson, 1986; Rilling and Insel, 1999) resembles that of distantly related Old World monkeys rather than their closely related sister groups, New World monkeys. In addition, they have independently evolved direct corticospinal projections to the ventral horn motor neurons that project to muscles of the digits (Bortoff and Strick, 1993) and have also independently evolved a cortical field, Area 2, associated with processing proprioceptive inputs (Padberg et al., 2007). This example illustrates two important points. First, hand morphology associated with specialized use covaries with cortical sheet size, cortical field addition, and corticospinal connections. Second, the independent evolution of these striking features of the morphological, behavioral, and cortical phenotype suggests that there are strong constraints on how complex brains and behaviors evolve.

The types of cross-species comparisons described above inform us about what types of phenotypic changes have occurred, how homologous aspects of brain organization vary across species, and clearly indicate that

evolution of brain, morphology, and behavior is constrained. However, they do not tell us how these phenotypic transitions occur and what factors contribute to or constrain phenotype diversity. Because cross-species variability had to begin as within-species variability, we can understand the process of speciation by looking at individual variability.

WITHIN-SPECIES VARIABILITY

Phenotypic variability within a population is the cornerstone of evolution by natural selection, yet most studies of neural organization and connectivity underscore the similarities across individuals within a group rather than their differences. As a result, there are few studies that directly examine and quantify naturally occurring differences in features of nervous system organization within a species. As noted in our introduction, we reasoned that the most likely place to observe measurable within-species differences is in the features of organization that demonstrate dramatic variability across species, like cortical field size and sensory domain allocation, and that are related to or covary with the targets of selection.

At a gross morphological level, animals with a large neocortex show variations in the size and configuration of sulcal patterns. Within-species variation is also observed in the size of cortical fields in rats (Riddle and Purves, 1995), opossums (Karlen and Krubitzer, 2006), squirrels (Campi and Krubitzer, 2010), and both nonhuman (Van Essen et al., 1986) and human primates (Dougherty et al., 2003). Intraspecies comparisons of the size of V1 in humans and nonhuman primates reveal a high degree of variability, ranging from 13% to 27% with respect to the entire visual cortex [see Karlen and Krubitzer (2007) for review]. In rats, Riddle and Purves (1995) observed that both the overall size of S1 and the proportion of cortex devoted to different body parts, such as the lip, barrel field, and forepaw, varied significantly across animals and even across hemispheres in the same rat. Our laboratory directly examined intraspecies variability in the primary sensory areas of opossums (*Monodelphis domestica*) and measured and compared their sizes across hemispheres for each animal and across individuals within a species. We found that the size of primary cortical areas was similar across hemispheres but varied considerably across individuals (Karlen and Krubitzer, 2006). Based on recent comparative studies in rodents, we propose this variability was mediated by environmental influences. Specifically, wild-caught *Rattus norvegicus* had a large V1 and a greater amount of variability in cortical field size than their laboratory counterparts (Campi and Krubitzer, 2010). Although these studies did not demonstrate large variability in overall cortical sheet size, the amount of cortex that was allocated to individual cortical fields was variable.

Within-species variability has also been observed in the internal organization of both sensory and motor maps. For example, Albus and Beckman (1980) observed notable differences in the visuotopic organization of V2 and V3 in cats. Variability in somatotopic organization has been reported for the hand representation in primates (Merzenich et al., 1987). In addition, although not always directly measured or the focus of a study, examination of somatotopic maps generated from functional mapping studies indicates that the representation of different portions of the body in adjacent somatosensory areas, such as 3a, 1, and 2, is variable across individuals within a primate species [e.g., Krubitzer et al. (2004) and Padberg et al. (2005)]. The differences in the somatotopic organization of these sensory areas are clearly present but not extreme. However, the within-species variability in topographic organization of higher-order areas, such as posterior parietal Area 5, is remarkable (Fig. 6.5*B*) [e.g., Seelke et al. (2011) and Padberg et al. (2005)]. Finally, when similar microstimulation parameters are used across animals, the functional organization of primary motor cortex (M1) is highly variable within many species, including mice (Tennant et al., 2011) (Fig. 6.5*A*), rats (Neafsey et al., 1986), squirrels (Cooke et al., 2011), and owl monkeys (Gould et al., 1986).

Individual differences have also been observed in smaller units of organization within a cortical field, termed modules. For example, in rats the succinic dehydroxinase-rich barrels and barrel-like structures that represent different body parts vary in size between individuals (Riddle and Purves, 1995). In owl monkeys and squirrel monkeys, myelin-rich isomorphs associated with the oral structures and digits vary in size (Fig. 6.5*D* and *E*) (Jain et al., 1998, 2001), as do the digit isomorphs for the digits in macaque monkeys, particularly D1 (Calford et al., 1985). Ocular dominance columns in V1 of squirrel monkeys can show extreme variability (Adams and Horton, 2003). In some monkeys they are discrete, stripe-like bands, in others they are smaller and less distinct, and in some monkeys they are nonexistent (Fig. 6.5*C*).

As noted in the previous section, homologous fields vary in their patterns of connectivity across phyla and even across species within an order such as rodents [see Krubitzer et al. (2011)]. Connectional studies of the neocortex in any mammal share two common features. First, if the sources of technical variability are minimized (e.g., placement of injection of anatomical tracer, age, rearing condition), the majority of connections for a given cortical field are similar across individuals. Second, the variability that does exist takes two forms: alterations in the density of common inputs and the presence of novel but sparse connections to some structures or areas in different individuals.

Recent studies also demonstrate that cellular composition varies within a population. For example, within the cortex of primates the total
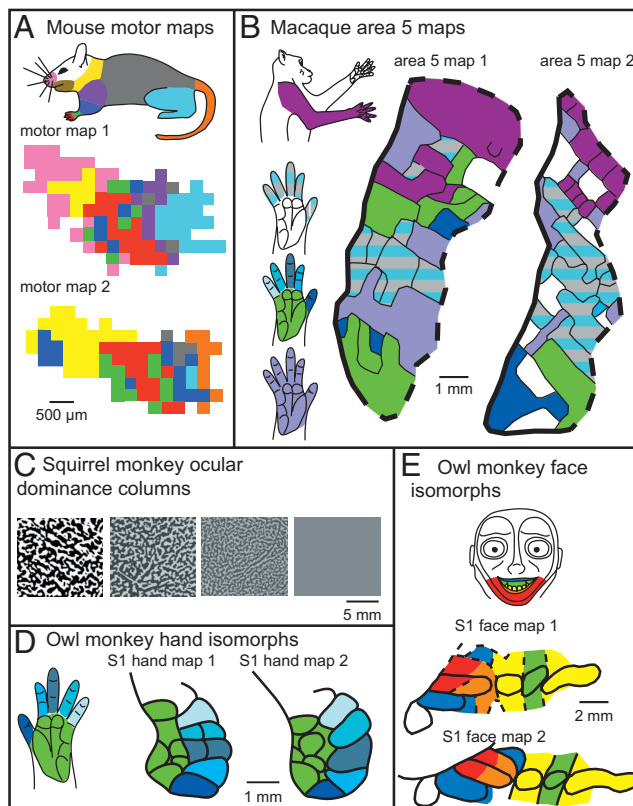
FIGURE 6.5 Examples of intraspecies variability for motor cortex in mice (*A*), Area 5 in macaque monkeys (*B*), ocular dominance columns in squirrel monkeys (*C*), S1 architectonic isomorphs in the owl monkey face representation (*D*), and hand representation (*E*). In mice, motor maps are grossly topographically organized but are locally fractured such that stimulation at adjacent sites did not necessarily cause movements of adjacent parts of the body. The example provided in *A* shows motor maps from two different individual mice. Each small square represents a microstimulation location that evoked a movement of a particular body part, color-coded according to the colored mouse body at top. In macaques (*B*), maps of posterior parietal Area 5 are highly variable and, like maps of motor cortex in *A*, they are fractured. Area 5 also demonstrates an extreme magnification of the forelimb since no other body parts are represented in this field. The portions of the hand and arm are color coded to represent the types of receptive fields found within maps in two individual macaque monkeys. In squirrel monkeys (*C*), ocular dominance columns as defined with cytochrome oxidase vary from highly distinct (left square) to nonexistent (far right square). Finally, the myeloarchitectonically

number of neurons varies between individuals by a factor of ~1.3 [calculated from Herculano-Houzel et al. (2007)]. In another study, wild-caught rats (*Rattus norvegicus*) were found to have a larger percentage of neurons and a greater density of neurons in V1 compared with laboratory rats of the same species (Campi et al., 2011).

Some of the within-species variations in cortical organization described above are undoubtedly linked with behavior, although the relationship is often nonlinear and indirect. However, examination of certain aspects of organization, such as the size and cellular composition of the primary visual area, are correlated with diel patterns and lifestyle of an animal. These, in turn, are linked to alterations in the visual system, such as the emergence of two-cone color vision and a highly laminated lateral geniculate nucleus in the highly visual, diurnal squirrel [see Campi and Krubitzer (2010) for review]. These alterations, which cross multiple levels of organization, provide some insight into the relationship between the brain and behavior. Although these brain–behavior relationships are interesting, there have been few studies of within-species variation that examined how sensory-mediated behavior covaries with some measurable aspect of the cortical phenotype. In contrast, studies of variability in behavior within a population abound.

Some of the best examples of behavioral/neural/genetic variation are in the field of behavioral neuroendocrinology. For example, numerous studies have demonstrated that GnRh (gonadotropin-releasing hormone) regulates reproduction through a cascade of intermediaries. This begins with regulation of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion by the anterior pituitary, which in turn stimulates sex steroid production and gametogenesis. These sexual steroids (estrogen and testosterone) then bind to receptors in the brain in regions that regulate sexual behaviors. Important for this review, the volume and pattern of GnRh secretion varies with external cues, such as photoperiod, food availability, stress, and conflict (Smale et al., 2005; Steinman et al., 2012), which in turn generates variable release of LH and FSH by the anterior pituitary and so on. Natural variation in genes that regulate this pathway

distinct isomorphic modules of the face (*D*) and hand (*E*) representations in S1 of owl monkeys vary in their specific size and shape between individual animals. Color codes of the hand and face correspond to their representations in cortical maps. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

has also been demonstrated in different individuals within populations of deer mice and white-footed mice (Heideman, 2004; Smale et al., 2005). Thus, variability in the brain and behavior can be generated through both external and internal cues.

Thus far, we have discussed features of the cortex such as cortical field size, connectivity, and cellular composition that vary between and within species and are correlated with, and likely covary with, the targets of selection (i.e., behavior). Given that genes or portions of the genome are linked to these neural phenotypic characteristics, which in turn are linked to behavior, it is not surprising that features such as the location, amount, and time of expression of the same gene or gene network are variable across individuals within a population.

Recent studies demonstrate that this variability is due to differential activation of genetic regulatory networks (Macneil and Walhout, 2011). These networks are composed of transcription factors and genes (nodes) as well as regulatory interactions (edges). The level of differential gene expression can be robust (persistent under perturbation) or stochastic (nondeterministic and flexible) and in turn generate phenotypic characteristics that differ in the extent to which they are variable within a population. Stochasticity of gene expression often results in more variable phenotypic characteristics of the individual, whereas robustness of a gene regulatory network often, but not always, results in less variability of a phenotypic characteristic. Not surprisingly, fundamental biological functions, such as the cell cycle, cell growth, and transcription, are generally governed by robust regulatory networks, suggesting that high variability for these key functions is nonadaptive. It seems likely that the basic, ubiquitous mammalian constellation of cortical fields with its homologous patterns of connections is regulated by robust networks, because these fields persist even in the absence of use. Other aspects of organization that are highly variable within and across species are likely stochastically regulated. In fact it has been suggested that there may be "core" gene regulatory networks that are conserved between species and that differential alterations in the nodes or the edges contribute to species-specific differences (Macneil and Walhout, 2011).

WHAT FACTORS CONTRIBUTE TO PHENOTYPIC VARIABILITY?

There are two important factors that contribute to phenotypic variability: genes and external signals, the latter consisting of the distribution of physical stimuli in a particular environmental context. Genes both intrinsic and extrinsic to the neocortex play an important role in shaping different features of cortical organization. Equally important are the patterns of sensory stimuli that the developing organism is exposed to, and

by extension, the patterned activity within and across major effectors such as the retina, skin, and cochlea.

Transcription factors such as Emx2, Pax6, and COUP-TFI regulate patterns of cell adhesion molecules [e.g., cadherins; see O'Leary and Sahara (2008) for review] and are graded in their expression across the developing cortical sheet (Fig. 6.6). Numerous studies have shown that transcription factors and their downstream target genes covary with aspects of cortical organization, such as cortical field size, location, and connectivity [see O'Leary and Sahara (2008) for review], and deletion or overexpression of these factors results in changes in gene expression, contractions and expansions in the sizes of cortical fields, and altered patterns of connectivity from the dorsal thalamus (Bishop et al., 2002) (Fig. 6.6). As we discussed previously, such genetic changes only indirectly affect behavior, the actual target of selection. The relationship between alterations in transcription factors and changes in the direct targets of selection is complex but has been demonstrated to some degree in the mouse. For example, overexpression of Emx2 increases the size of V1 but decreases the size of
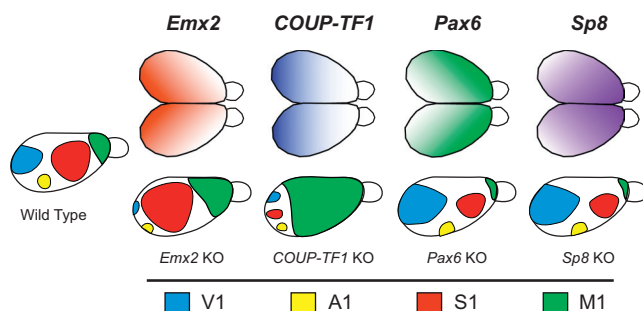
FIGURE 6.6 Graded patterns of expression of transcription factors (*Upper*) involved in aspects of arealization such as location and size of cortical fields. Overexpression (not shown) and knockout (KO; *Lower*) of these transcription factors generates radically different sizes and positions of cortical fields compared to wild-type mice (*Left*). Cortical fields are color-coded (see key at bottom). Deletions of *Emx2* result in a compression of caudal fields and an expansion of rostral fields, as do deletions of *COUP-TFI*. However, with the latter manipulation, motor cortex appears to be greatly expanded. These studies demonstrate how changes in gene expression may produce dramatic alterations to the cortical phenotype. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

somatosensory and motor areas (Hamasaki et al., 2004; Leingärtner et al., 2007). When these mice were tested on sensorimotor tasks that assessed hindlimb and forelimb coordination, they performed significantly worse than wild-type mice. This study establishes a clear link between genes, cortical field size, and behavior and demonstrates how alterations in patterns of expression of transcription factors and their downstream targets can generate relatively large degrees of phenotypic variability in the cortex, which in turn generates variability in the target of selection.

Genes extrinsic to the neocortex can also affect cortical organization. For example, homeobox genes from the *Hox* family are highly conserved across animals and are involved in forelimb development (Tallafuss and Bally-Cuif, 2002; Hirth and Reichert, 2007). Comparative studies between mice and bats indicate that expression of these genes is altered during development (Chen et al., 2005) and thought to be involved in transforming the forelimb into a wing (Cretekos et al., 2001; Sears et al., 2006). This process is multilayered. *Hoxd13* expression is posteriorly shifted in the developing forelimb at later developmental stages in bats compared with mice, which reduces some wing skeletal elements (Chen et al., 2005). Although bone morphogenic proteins (BMPs) trigger apoptosis of interdigit membranes in mouse fore- and hindlimbs and the bat hindlimb, in the bat forelimb BMPs are inhibited by *Gremlin* so that interdigit membranes are maintained (Weatherbee et al., 2006). This reduction in BMPs is accompanied by an increase in *Fgf8* in the apical ectodermal ridge and is responsible for the extended proximal to distal growth of the limb in the bat (Cretekos et al., 2007). BMP2 triggers proliferation and differentiation of chondroctyes, which increases digit length in bats (Sears et al., 2006). Thus, the amount, timing, and position of expression of genes during early forelimb development can induce dramatic alterations in the structure of the forelimb. As noted earlier, these alterations in forelimb morphology and the use of the forelimb covary with the size and internal organization of the cortical field. Compared with mice, bats have a larger forelimb representation within S1, and the topographic features of the wing representation within S1 relate uniquely to its altered position while the bat is at rest (Calford et al., 1985; Cretekos et al., 2007).

Although phenotypic diversity in cortical organization is generated by modifying these intrinsic and extrinsic genetic contingencies, these same contingencies also serve to constrain alterations to the phenotype. The complex relationship between morphogens, the transcription factors they regulate, and in turn the target genes that they regulate, has been well described by O'Leary and Sahara (2008). Most of these relationships are contingencies in which the actions of one node in a genetic regulatory network alter the trajectory of another node, which can potentially alter genetic regulatory networks associated with a completely different feature

of organization. Such integration limits the magnitude of viable changes that can be made via genetic mechanisms. Although small alterations at early stages of these contingencies (e.g., morphogen or transcriptional factor gradients) can have a large impact on the resultant cortical organization (e.g., change in cortical field size), alterations early in this cascade are also more likely to result in a nonviable phenotype. This is supported by the presence of certain cortical fields in some animals despite the lack of apparent functional use (Bronchti et al., 2002), the limited ways in which the cortical phenotype has changed, and the convergent evolution of similar features of organization despite very distant phylogenetic relationships. While we have given many examples of phenotypic diversity in the present review, we could provide an equally compelling argument that this diversity is fairly restricted if one considers all of the possible ways in which information could be processed and behavior generated.

Extrinsic factors also generate phenotypic variability within the cortex. For example, the activity from different sensory effectors during development, and throughout life, affects brain organization. Experiments from our laboratory in short-tailed opossums (*Monodelphis domestica*) in which both eyes were removed before cortical and subcortical connections were formed demonstrate that all of what would be visual cortex contained neurons that were responsive to somatosensory and/or auditory stimulation. Thus, sensory domain allocation was dramatically altered (Kahn and Krubitzer, 2002). In addition, architectonically defined V1 was significantly smaller, whereas S1 was significantly larger than in normal animals, and "V1" received altered projections from cortical and subcortical somatosensory and auditory structures (Karlen et al., 2006). Similar results have been observed in anophthalmic mice (Chabot et al., 2008) and blind mole rats (Cooper et al., 1993). In mutant mice in which the cochlea is dysfunctional but the eighth nerve is still present, all of cortex that would normally process auditory inputs contains neurons that respond to visual and somatosensory stimulation, and the size of A1 is significantly reduced, whereas the size of V1 is significantly increased (Hunt et al., 2005). Finally, as noted above, alterations in cortical field size and neuronal density are observed in the same species of rat reared in radically different environments (wild-caught vs. laboratory). Thus, loss of sensory receptor arrays, loss of sensory-driven activity, or reduced patterns of activity can alter cortical domain allocation, cortical field size, connectivity, and neuronal density.

Other studies specifically manipulate the sensory environment in which the animal is reared and examine the effects on neocortical areas. For example, when ferrets are exposed to early training on a single axis of visual motion, neurons in V1 become preferentially responsive to movement along that axis (Li et al., 2006). In rats, early and prolonged exposure
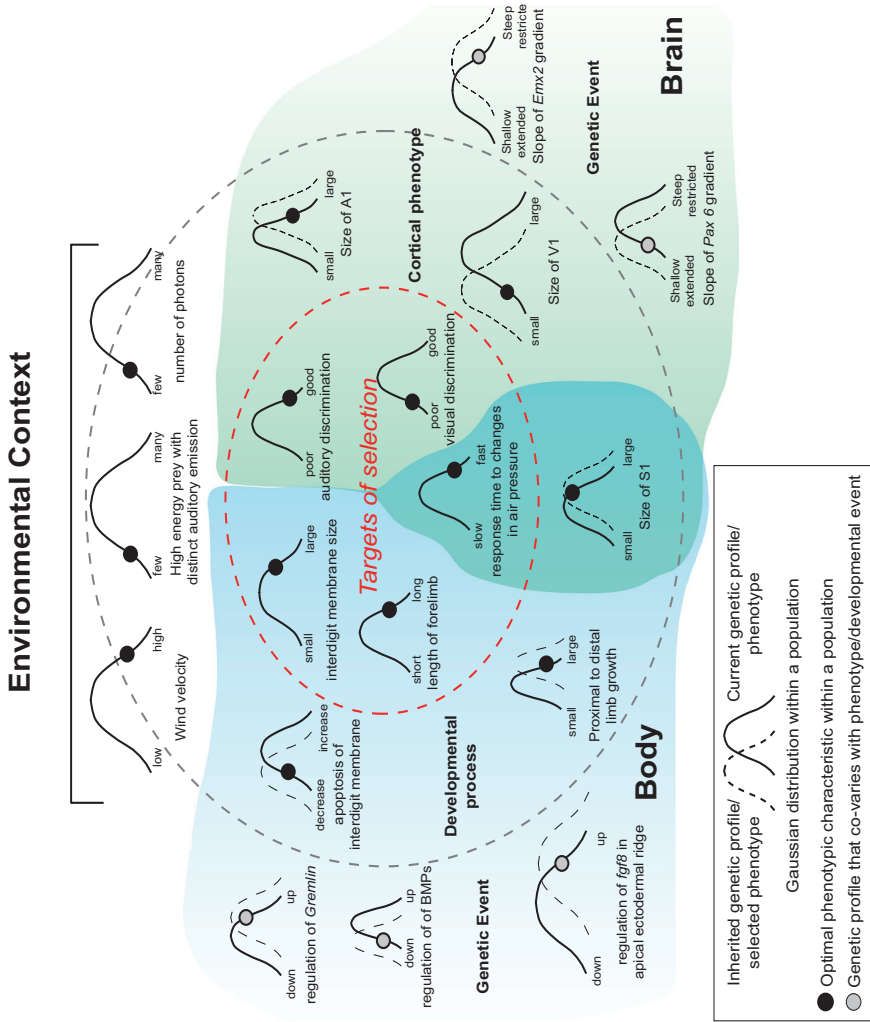
FIGURE 6.7 Schematic illustrating how genes, developmental processes, cortical phenotypes, and the targets of selection covary. The Gaussian curves represent the range of naturally occurring variability in a specific characteristic, with narrower curves representing robust characteristics and wider curves representing stochastic characteristics. The black and gray circles represent the location of the optimal characteristic along the current distribution (solid curve). Selection pressures will eventually push the population to a new distribution, centered around the optimal characteristic (dashed curve). In this example our species is an echolocating bat and our environmental context (*Top*) has low light, high wind velocity, and a small number of high-value prey items with restricted auditory emissions. Some of the targets of selection (Gaussian curves inside the innermost, dashed oval) would be characteristics of the forelimb that allow for flight such as the interdigit membranes with touch domes and the elongated portions of the forelimb. Additionally, behaviors such as fast response time and good auditory discrimination would be selected for. Visual discrimination ability would have a neutral effect in this context. Cortical phenotypic characteristics (located between the dark gray and the innermost dashed lines) that underlie auditory and tactile discriminatory ability would include an increase in the size of S1 and A1, as well as an increase in the wing representation within S1. This latter cortical phenotype is related to the morphological and use-dependent changes to the limb. Underlying developmental processes associated with wing formation include a decrease in apoptosis in the interdigit membrane and the growth of the limb. At the far perimeter (*Far Left* and *Far Right*) of this illustration are the genetic events that covary with aspects of the body and brain phenotypes. For the brain this could include the gradient of transcription factors and changes in the boundaries of their downstream target genes (not shown). For the body this includes changes in the regulation of morphogens (e.g., downregulation of BMPs) and growth factors (upregulation of *fgf8*). The light gray shading on the left corresponds to factors associated with the forelimb morphology, and the light gray shading on the right corresponds to factors associated with brain organization. These are not mutually exclusive but interact to some extent (overlapped shading). This illustration is a simplified version of the multiple layers of events that contribute to a phenotypic characteristic that is the target of selection. However, it demonstrates how covariation between the targets of selection, phenotypic organization, and genetic events could lead to inheritance of genes that generate a population of future individuals with a unique combination of phenotypic characteristics.

to a particular auditory tone results in increased cortical magnification for that frequency in A1 (Zhang et al., 2001). These changes in the internal organization of a sensory field and neuron response properties are similar to the types of differences observed across species and can be induced early in development by altering the sensory environment in which the animal develops.

Thus, a high degree of phenotypic variability can be induced without invoking genetic mechanisms that control brain development. The cortex has evolved to match the sensory environment in which it develops and produce highly adaptive behavior for that context. Although we have focused this review on how sensory systems and cortical areas are modified, if one considers both social and cultural influences on the brain as complex patterns of sensory stimuli that groups of brains generate, then the same rules of construction and modification apply. However, as with genes, the environmental factors that generate phenotypic variability also serve to constrain the types of changes that can be made to the brain. For example, although photons can be differentially distributed in an aquatic, cave, or terrestrial environment, they have the same intrinsic properties, are uniformly defined as a discrete quantum of electromagnetic energy, are always in motion, and in a vacuum travel at the speed of light. These immutable characteristics of a stimulus that the nervous system must detect, transduce, and ultimately translate, constrain the evolution and construction of the effector organ that initially captures some portion of the spectrum of this energy, and also impacts how higher-level structures transmit specific information about its presence, magnitude, and dispersal within an environment.

CONCLUSIONS

We have discussed phenotypic variability across and within species and conclude that the ways in which animals and brains change are limited and predictable. Further, we show that a specific characteristic, such as the size of a cortical field, can be generated by different genetic mechanisms and/or activity-dependent mechanisms. Thus, similar features of organization that have independently arisen in different lineages may not have similar underpinnings. Examination of variability at multiple levels of organization indicates that although genes are not directly related to a specific behavior, they covary with aspects of body and brain organization, which in turn covary with the targets of selection (Fig. 6.7). For example, the wing of a bat is constructed in development through complex interactions between genes and morphogens. Slight variations in the amount, location, and timing of these factors can generate phenotypic diversity within a population. The presence of the highly derived wing with its

array of specialized touch domes covaries with both the size of the fore-limb representation and neural response properties in S1. Together such morphological and cortical specializations are critical for detecting and processing inputs that provide motor cortex with information necessary to produce fine muscle control during self-propelled flight. It is the resulting morphology and behavior, the efficiency with which a bat navigates, captures, and consumes insects using a wing of a given size, shape, tensor properties, and receptor distribution, that are the targets of selection.

In addition there are genetic regulatory networks in the neocortex that are responsible for providing the scaffold of organization that includes a constellation of cortical fields and their connectional relationships that all mammals share. These networks can vary to produce phenotypic change in cortical field size, relative location, and connectivity within individuals in a population. This in turn generates changes in sensory-mediated behaviors, and as in the example above, it is behavior, not genes or features of cortical organization, that are the targets of selection (Fig. 6.7). Given this complex, multilayered relationship between genes, brains, bodies, the environment, and the targets of selection, the dialect of the current scientific culture, which proposes to study "the gene" for autism, language, memory, or any other class of complex behaviors, is inaccurate and certainly misleading.

Although variability is the cornerstone of evolution, it is difficult to find studies that specifically examine and quantify naturally occurring variability in any aspect of neural organization. As the title indicates, such variability is unwelcome in most studies. We strive to underscore common features or the sameness of our data and reduce the error bars on our histograms. For experimentation purposes, variability is in fact "the bane of our existence." However, this same variability provides a deep insight into how evolution proceeds and the complex, sometimes tortuous path of phenotypic change. Although the evolution of future forms is not completely known, we can predict the types of changes that will occur and know with certainty that at all levels of organization, there will be variability.

ACKNOWLEDGMENTS

7

Evolution of Columns, Modules, and Domains in the Neocortex of Primates

JON H. KAAS

The specialized regions of neocortex of mammals, called areas, have been divided into smaller functional units called minicolumns, columns, modules, and domains. Here we describe some of these functional subdivisions of areas in primates and suggest when they emerged in mammalian evolution. We distinguish several types of these smaller subdivisions. Minicolumns, vertical arrays of neurons that are more densely interconnected with each other than with laterally neighboring neurons, are present in all cortical areas. Classic columns are defined by a repeating pattern of two or more types of cortex distinguished by having different inputs and neurons with different response properties. Sensory stimuli that continuously vary along a stimulus dimension may activate groups of neurons that vary continuously in location, producing "columns" without specific boundaries. Other groups or columns of cortical neurons are separated by narrow septa of fibers that reflect discontinuities in the receptor sheet. Larger regions of posterior parietal cortex and frontal motor cortex are parts of networks devoted to producing different sequences of movements. We distinguish these larger functionally distinct regions as domains. Columns of several types have evolved independently a number of times. Some of the columns found in primates likely emerged with the first primates, whereas others likely were present in earlier ancestors. The sizes and shapes of columns seem to depend on the balance of neuron activation patterns and molecular signals during development.

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Neocortex is an important part of the brain that varies in size from a small cap on the rest of the forebrain (Kaas, 2007) to approximately 80% of the brain in humans (Azevedo et al., 2009). The varied functions of neocortex depend on the cortical areas, the so-called "organs of the brain" (Brodmann, 1909) that are specialized for processing different inputs and providing different outputs. Cortical areas can be hard to define and identify, and their exact number in any species is uncertain. However, it is clear that the number of cortical areas varies across extant taxa, from approximately 20–30 or so to perhaps more than 200 in humans (Kaas and Preuss, 2008). Because the first mammals had little neocortex and likely few cortical areas, interest in the evolution of neocortex across the great radiation of mammals has largely focused on the issue of modifying and adding cortical areas. Some of the cortical areas proposed for primates are shown in Fig. 7.1. However, areas are often composed of smaller subdivisions, the cortical columns or modules, and these subdivisions within areas modify and expand the functions of areas. Thus, an understanding of how different types of neocortex evolved depends not only on determining the numbers and types of cortical areas that exist but also on the modifications of the internal organization of areas that occur in the various lines of evolution, including modifications in columnar organization. Here we review the types of columnar subdivisions of cortical areas that have been proposed (Hendrickson, 1985; Purves et al., 1992; Mountcastle, 1997; da Costa and Martin, 2010) and then consider how and when such modules might have evolved. The phyletic distributions of the types of columns in extant mammals allow one to infer when such columns evolved (Hennig, 1966; Striedter, 2005). Primates, rodents, tree shrews, and lagomorphs are all placed within the superorder Euarchontoglires. Thus, we are especially interested in how types of columns are distributed within the primate radiation, but also whether they are present in the closest relatives of primates. Because the shapes of columns are not always columnar, they also are called modules.

MINICOLUMNS

One of the defining features of neocortex is that it consists of layers and various sublayers of neurons specialized for different steps in processing; neurons in radial (vertical) arrays across the layers are more densely interconnected than neurons along the layers (Casagrande and Kaas, 1994; Nieuwenhuys, 1994b; Kaas, 2010). As a result, neurons in narrow vertical arrays share many response properties, especially the location of the receptor fields of neurons on the sensory receptor surface. This arrangement has great functional importance, and it is likely responsible for the impressive flexibility and powers of neocortex. Developmentally, minicolumns reflect
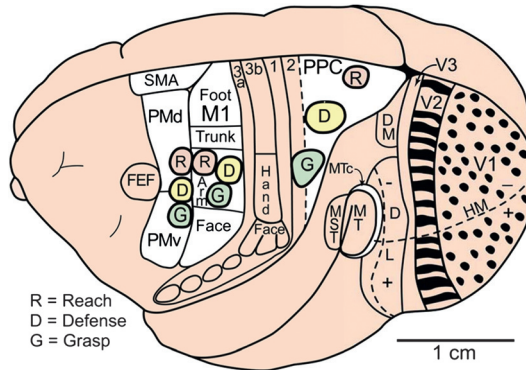
FIGURE 7.1 Some of the proposed cortical areas of primates shown on a dorsolateral view of the left cerebral hemisphere. Modular subdivisions of some of these areas are discussed in the text. Visual areas include the first, second, and third areas (V1, V2, V3), dorsomedial (DM or V3a) and dorsolateral visual areas (DL or V4), the middle temporal area (MT), the MT crescent (MTc), and the medial superior temporal (MST) area. The representation of the zero horizontal meridian (HM) divides the representation of the upper (+) and lower (−) visual hemifields. Motor areas include primary motor cortex (M1), ventral (PMv), and dorsal (PMd) premotor cortex, the supplementary motor area (SMA), and the frontal eye field (FEF). Somatosensory areas include the four areas of anterior parietal cortex (3a, 3b, 1, 2), with the region representing tactile inputs from the hand indicated in area 3b (S1). Modular subdivisions in V1 (dots) and V2 (bands) are shown in black (see text). Ovals mark the locations of proposed reach, defense, and grasp domains in motor and posterior parietal cortex (PPC). Based on Gharbawie et al. (2011a).

the radial migration of clones of excitatory neurons from progenitors in the ventricular and subventricular zones (Rakic, 1995b), as radially arranged sister neurons preferentially develop synapses with each other (Yu et al., 2009). These vertical arrays of interconnected neurons across the cortical layers have been called minicolumns (Mountcastle, 1957, 2003). Minicolumns are sometimes visible as vertical arrays of neurons separated somewhat by neuropil (Buxhoeveden and Casanova, 2002; DeFelipe et al., 2002). Minicolumns are thought to be 30–50 μm in diameter, although functional boundaries between them are not likely to be sharp owing to the spread of apical dendrites of pyramidal cells and the extents of axon arbors of cortical neurons and subcortical activating inputs. Because minicolumns are clearly visible in a number of cortical areas, and across mammalian species, including monotremes, they may have originated when the ancestors of all extant mammals with a cortex of six layers emerged.

Classic Columns

Mountcastle (1957) introduced the concept of cortical columns after reporting that recordings along microelectrode trajectories tangential to the surface of somatosensory cortex encountered short sequences of neurons that responded either to light touch on the skin (superficial skin receptors) or touch with pressure (deep receptors). This grouping of cortical neurons according to how they respond to sensory stimuli led to the concept of a patchwork of alternating columns of neurons that extend across all cortical layers, with each type of column activated by a different somatosensory input. The subsequent evidence for such alternating patches of neurons activated by either deep or superficial receptors of the skin and deeper tissue has been limited, and they do not seem to exist in area 3b (S1 proper) of somatosensory cortex of monkeys. Instead, there is evidence for a modular arrangement of groups of neurons in layer 4 that responds to activation by inputs relayed from either slowly adapting or rapidly adapting cutaneous receptors of the skin (Sur et al., 1981, 1984). There is also evidence for at least a partial segregation of territories activated by slowly adapting and rapidly adapting receptors in area 1 of somatosensory cortex of monkeys (Friedman et al., 2004). However, given these limited observations, we can say little about the phyletic distribution of slowly adapting and rapidly adapting cortical columns, or their evolution, even in primates.

More can be said about the blob and interblob surround organization of primary visual cortex (V1) in primates (Fig. 7.2). All primates seem to have a pattern of cytochrome oxidase (CO)-rich blobs (reflecting high metabolic activity) within interblob surrounds of lower CO levels (Horton, 1984; Horton and Hedley-Whyte, 1984; Preuss and Kaas, 1996). Neurons in the blobs respond to color, are less selective for stimulus orientation, and have higher firing rates than neurons between the blobs (Livingstone and Hubel, 1984; Hendrickson, 1985; Felleman, 2008; Lu and Roe, 2008; Economides et al., 2011). However, blobs and interblob regions are found not only in primates with trichromatic or dichromatic color systems but also in nocturnal primates with only one functional type of cone in the retina (Wikler and Rakic, 1990). The blobs and interblobs are also distinguished by different patterns of inputs from the visual thalamus, intrinsic connections, and connections with other visual areas (Livingstone and Hubel, 1984; Casagrande and Kaas, 1994). In macaque monkeys, most of these connections are well developed in newborns (Barone et al., 1996; Baldwin et al., 2012). The segregation of groups of neurons by differences in response characteristics that are mediated by differences in activating inputs fits the classic definition of cortical columns, although the blobs and interblobs do not occupy equal territories, and the interblob territory is continuous. The blob and surround pattern evolved in the immediate
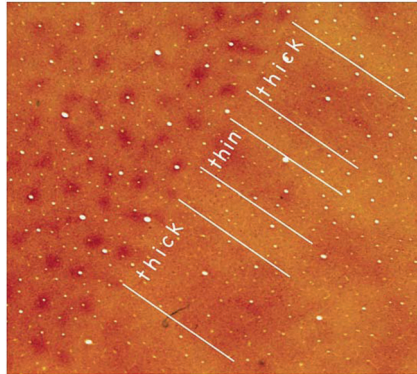
FIGURE 7.2 Anatomically defined columns in visual cortex of primates. Sections of primary visual cortex (V1) and the adjoining second visual area (V2) of a macaque monkey have been cut parallel to the brain surface and processed for CO, a marker of neurons with high metabolic requirements. The brain sections provide a "surface view" of parts of V1 and V2. In V1, there is a pattern of CO-rich "blobs" (also called "puffs" or "patches") surrounded by cortex that expresses less CO, the interblob territory. In V2 an alternating pattern of CO-dark bands, separated by CO-light bands, cross the width of V2. The CO-dark bands are of two types, thick and thin. Thus, there are three types of bank-like structures in V2 that can be anatomically distinguished. Because the CO blobs and interblobs, as well as the CO-dense thick, thin, and interbands have neurons that differ in response properties, they can be considered classic columns. A pattern of CO-dense and CO-light bands is also present in the third cortical visual area, V3, along the outer border (on the right) of V2. Compare with Fig. 7.1.

ancestors of primates, or in archaic primates, given that none of the close relatives of primates, tree shrews, rodents, and lagomorphs have blobs in V1.

Classic columns are also found in the second visual area, V2, of most primates, where V2 is characterized by a repeating series of CO-dense thick stripes and CO-dense thin stripes separated by CO-pale interstripes. These band-like stripes cross the narrow width of V2, and they seem to exist in all anthropoid primates (Kaas, 2003). The three types of stripes differ in anatomical connections and have neurons with different response properties. The stripes and differences in connections are apparent in newborn macaques (Barone et al., 1996; Baldwin et al., 2012). Although the CO-dense stripes are not consistently distinguishable as thick or thin, they can be identified by functional differences, with neurons in the thick stripes sensitive to binocular disparities and stimulus orientation, the neurons in the thin stripes sensitive to luminance and color, and neurons

in pale stripes sensitive to stimulus orientation (Hubel and Livingstone, 1987; Livingstone and Hubel, 1988; Lu and Roe, 2007; Felleman, 2008; Kaskan et al., 2009). The thick stripes project to visual area MT, whereas the other bands project to DL (V4). In prosimian primates, CO stripes in V2 are only weakly apparent, and such stripes are not present in V2 of tree shrews and rodents (Kaas, 2003). Thus, aspects of the stripe pattern may have evolved in early primates, whereas such stripes became fully developed as anthropoid primates emerged.

Although the V1 blob and interblob regions, as well as the V2 stripes, do not look like cylindrical pillars, they otherwise conform to the expectations of classic cortical columns. Other such classic columns undoubtedly exist (Mountcastle, 1997), but they largely remain to be explored. One such example is in the MT crescent, MTc, a visual area that forms a belt around the middle temporal visual area, MT (Fig. 7.1). This poorly understood visual area is composed of a series of CO-dense puffs in a single row, like beads on a string in a belt of CO-pale tissue (Kaas and Morel, 1993). The significance of these puffs and surrounds in MTc, which have different connections with other visual areas, remains to be determined.

Unbounded Columns That Represent Sectors of a Continuous Stimulus Dimension

Several cortical areas have repeating representations of stimulus orientations for different portions of the visual field (Hubel and Wiesel, 1963). Most notably, primary visual cortex of primates, carnivores, and tree shrews have repeating "pinwheel" patterns of cortex, in which stimulus orientation is systematically represented from vertical to horizontal lines and edges and back again (Bonhoeffer and Grinvald, 1991; Fitzpatrick, 1996; Kaschube et al., 2010). Groups of neurons most sensitive to one stimulus orientation or another can be selectively activated, the activity pattern optically imaged, and regions of cortex sensitive to different orientations color coded to produce colorful illustrations of arrays of orientation "columns." These "columns" differ from classic columns in that they have no borders because the orientations of stimuli change continuously without disruption. Thus, the illustrated "borders" between orientation columns are arbitrary. In addition, all "orientation columns" are selective for the same stimulus features, and thus these columns are not of the classic type, which are segregated by different classes of activating inputs. However, each entire array of orientation-selective neurons, the pinwheel for a given location in the visual field, can be considered as a larger domain or hypercolumn (Hubel and Wiesel, 1972, 1977). Orientation hypercolumns are widespread in visual cortex of primates: they also have been identified in V2 stripes, V3, V4 (DL), and MT (Kaskan et

al., 2009, 2010; Tanigawa et al., 2010). Neurons in orientation-selective hypercolumns may be divided for each orientation column into halves, preferring one or the other direction of motion perpendicular to the preferred orientation (Kaskan et al., 2010). The grouping of neurons by their preferences for stimulus orientation seems to be a trait that emerged first in V1 in the common ancestors of tree shrews and primates, because tree shrews also have orientation hypercolumns. However, the more distant relatives, rodents and rabbits, have orientation-selective neurons in visual cortex but not orientation-selective columns (Kaschube et al., 2010). Carnivores have independently evolved orientation hypercolumns in V1. Possibly, the presence of orientation hypercolumns in V1 is a prerequisite for the evolution of such hypercolumns in other visual areas, as found in primates. Orientation hypercolumns have not been reported for areas of extrastriate cortex of tree shrews. Thus, the extrastriate hypercolumns for stimulus orientation may have emerged with the first primates.

Other proposed modules of V2 in primates include subregions of thin stripes selective for different hues (Xiao et al., 2003; Roe, 2004). These hue-selective subregions are not classic columns because they are not separated by columns that are most sensitive to another stimulus feature, and they have arbitrary boundaries.

There is only limited evidence for the existence of classic columns in auditory cortex. All mammals seem to have primary cortical auditory areas that represent the receptors of the cochlea in a linear manner so that neurons are arranged in one dimension across a cortical area from being most sensitive to low-frequency sounds on one end, to high-frequency sounds on the other (Kaas, 2011). Thus, there are no modular divisions based on sound frequency, although isofrequency bands with arbitrary borders have been described. However, bands of primary auditory cortex where neurons that are excited from both ears (EE bands) alternate with bands of cortex with neurons that are excited by the contralateral ear and inhibited by the ipsilateral ear (EI bands) have been reported for cats (Merzenich and Kaas, 1980). The EE and EI bands extend across the isofrequency contours. Because EE and EI bands have neurons of differing functional properties, they qualify as classic columns (although shaped like bands). Such bands have not been identified in auditory cortex of primates.

Modules Representing Separated Parts of Sensory Surfaces

Another type of module, one that also would not qualify as a classic column, concerns separations of groups of neurons in somatotopic maps of the body surface, or retinotopic maps of the two eyes, in areas of cortex. The best-known example is the rows and columns of "barrels" in primary

somatosensory cortex of rats and mice, where a barrel-like structure represents each of the large sensory whiskers on the side of the face (Woolsey et al., 1975). The digits and pads of the feet also relate to separated groups of neurons (Dawson and Killackey, 1987).

The many studies of the "barrel field" of mice and rats have revealed that differences in neural activity are important in the formation of barrels, such that the number of barrels varies with the number of facial whiskers. Molecular factors also alter the formation of barrels, as revealed in mutant mice (Erzurumlu and Kind, 2001). Such segregations of cortical neurons by body part are found in primary somatosensory cortex of many species, but are perhaps most apparent in the somatosensory cortex of the star-nosed mole, where the highly innervated tactile rays of the nose are each separately represented in three areas of somatosensory cortex (Catania and Kaas, 1996). In primary somatosensory cortex of New World and Old World monkeys (Jain et al., 1998; Qi and Kaas, 2004), and possibly other anthropoid primates, the representations of the digits are separated from each other by narrow cell-poor septa, with a more conspicuous septum separating the representation of digit 1 (thumb) from that of the face. Such separated representations of digits in area 3b of primates are variable and have not been described in prosimian primates. Septa that separate representations of digits are more apparent in macaque monkeys than in New World owl monkeys and squirrel monkeys.

It could be argued that the narrow septal regions that separate the cortical barrels, bands, and other modules related to body parts do have neurons that differ in connections, such as having corpus callosum connections, and thus there is an alteration of functional types of columns in the classical sense. However, the septa are cell-poor, narrow regions that are primarily there to reflect disruptions of the receptor sheet. Yet, these narrow septa may be opportunistically occupied by late-developing sources of input. Because the septa that form module borders reflect junctions in neuron activity patterns during sensory activation, these septa are most apparent early in sensory hierarchies where short response latencies to sensory stimuli are maintained.

The retina of each eye is a continuous sensory surface, except for the nerve head and a narrow septum corresponding to the nerve head, which disrupts layers of the lateral geniculate nucleus that receive projections from the contralateral eye (Kaas et al., 1973). In cortex, the ocular dominance "columns" in primary visual cortex of primates fall into the category of modules based on disruptions of the sensory surface, because the retina of the two eyes have independent activity patterns prenatally. Thus, the afferents from the hemiretina of each eye terminate in separate layers in the lateral geniculate nucleus of the visual thalamus, and then these layers project in retinotopically matched patterns to primary visual

cortex to either congruently overlap or to separate locally in variable patchy-to-banding patterns in layer IV while maintaining some level of retinotopy, depending on species (Florence and Kaas, 1992; Horton and Adams, 2005). Ocular dominance columns, first revealed in microelectrode recordings (Hubel and Wiesel, 1968), and axon termination patterns from layers of the lateral geniculate nucleus (Wiesel et al., 1974) can also be demonstrated by differences in activity levels after blocking activity from one eye (Horton, 1984; Takahata et al., 2009a). The segregation of eye-related afferents is very weak in some primates, such as nocturnal prosimian galagos and owl monkeys (Kaskan et al., 2007; Takahata et al., 2011), and highly variable patterns exist in New World monkeys, even across individuals within a species (Horton and Adams, 2005). Ocular dominance patterns may reflect a high degree of segregation of thalamic afferents in layer 4 of primary visual cortex, as in Old World monkeys (Fig. 7.3), apes, and humans, or reflect such a low level of separation that they are anatomically cryptic and only revealed by relative differences in
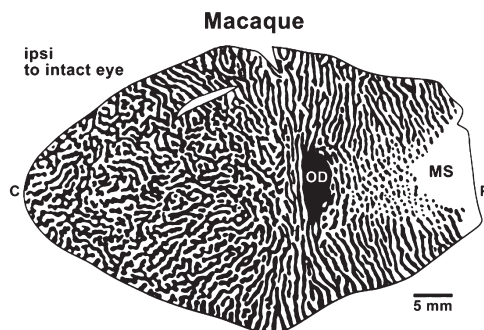
FIGURE 7.3 Ocular dominance columns (bands) in a flat surface view of primary visual cortex (V1) of an Old World macaque monkey as reflected by distribution of terminations of lateral geniculate axons related to each eye in cortical layer 4. Regions of black receive inputs from the ipsilateral eye, including the region of the optic disk of the retina that produces a gap in the projection of the hemiretina of the contralateral eye (OD in cortex). The monocular segment (MS) of V1 is activated by the monocular segment of the contralateral visual hemifield that is seen only by the contralateral eye. Foveal and central vision is represented to the left, and the extreme of peripheral vision is represented to the right. The ocular dominance bands break up into a dot-and-surround pattern in the part of V1 that represents peripheral vision as the inputs from the contralateral eye (white) become proportionately greater, and form the larger surrounds. Modified from Florence and Kaas (1992).

neural responses to each eye as revealed in optical imaging experiments (Kaskan et al., 2007) or the expression pattern of activity-dependent genes (Takahata et al., 2009b). Ocular dominance "columns" are absent in the closest relatives of primates, tree shrews, rodents, and rabbits, and thus are a feature of visual cortex that evolved in early primates but became more pronounced in Old World monkeys, apes, and humans. Obvious ocular dominance columns have evolved independently in carnivores (Anderson et al., 1988), and they likely exist in other taxa.

Domains: Larger Functional Divisions of Cortical Areas

Primary motor cortex and dorsal and ventral premotor areas are widely recognized as valid cortical areas, and each of these areas has a somatotopic representation of small movements of body parts that are revealed by brief trains of near-threshold pulses of electrical current. However, cortical motor areas representing major body parts, such as the forelimb, have a locally fractured somatotopy so that different movement zones, roughly the size of minicolumns, are mixed and repeated (Fig. 7.4). Thus, the forelimb region mixes zones for digit, wrist, elbow, and shoulder movements in a puzzling arrangement (Gould, 1986; Donoghue et al., 1992; Qi et al., 2000) that is unlike that of primary sensory representations, which closely reflect the organization of the sensory sheet. However, the somatosensory representation of tactile projections to the cerebellar cortex forms a fractured representation of the body surface (Shambes et al., 1978), much like the representations in motor cortex. The explanation for these adjoining patches of cerebellar cortex devoted to various nonadjacent body parts was that neurons in groups of such patches could interact to form "action-involved structures" for directing movement patterns.

It has long been known that longer trains of electrical pulses at higher current levels evoke more complex movement sequences from motor cortex than do short trains at threshold levels (Leyton and Sherrington, 1917). More recently, Graziano et al. (2002) have used longer (0.5 s) trains of electrical pulses to define different regions or domains (Fig. 7.1) in motor cortex where different ethologically relevant movement can be evoked (climbing, reaching, grasping, defense of the head, hand-to-mouth). Matching movement domains have been identified in posterior parietal cortex (Cooke et al., 2003; Stepniewska et al., 2005; Gharbawie et al., 2011a,b). In primary motor cortex, several different domains for functionally distinct movement patterns are found in separate parts of the forelimb representation, perhaps offering some explanation for the mosaic of minicolumns for different but related small movements and muscle twitches that are revealed by short trains of pulses at threshold levels of stimulating current (Fig. 7.4). Thus, circuits within a domain may evoke
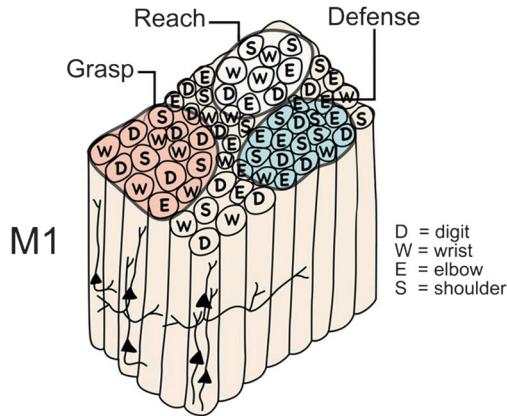
FIGURE 7.4 Proposed functional organization of the hand–forearm segment of primary motor cortex (M1) in monkeys and other primates. Although M1 has an overall somatotopy, the local somatotopy is fractured to form a mosaic of radial rows of neurons that evoke small, specific movements when electrically stimulated with brief trains of electrical pulses at threshold levels of current. Thus, neuron arrays or minicolumns for digit movement may adjoin those for wrist, elbow, or shoulder movements. Subsets of these minicolumns seem to be grouped to function in the production of more complex, ethologically relevant movement sequences, such as grasping, reaching, or defending the head against a blow. We refer to these larger divisions of motor, premotor, and posterior parietal cortex (Fig. 7.1) as domains (Gharbawie et al., 2011a).

sequences of movements involving the different body parts represented within the domain.

Functionally matched domains for at least some of the complex movement patterns of primary motor cortex also exist in premotor cortex and in posterior parietal cortex. The domains in posterior parietal cortex may be parts of larger cortical areas. The domains in frontal and posterior parietal cortex have similar spatial arrangements in prosimian galagos, two species of New World monkeys, and Old World macaque monkeys, and there is indirect evidence for them in humans (Kaas et al., 2011). Thus, they likely exist in all primates. Such domains for complex movements may also exist in motor cortex of the relatives of primates, tree shrews and rodents, where M1 also has a fractured somatotopy (Remple et al., 2007; Cooke et al., 2011). However, posterior parietal cortex is no more than a narrow strip of cortex in tree shrews and rodents and is unlikely to contain a series of primate-like domains.

Other areas of cortex may also have larger functionally distinct regions within cortical areas. For example, some of the face-selective and object-

selective regions of temporal cortex in macaque monkeys and humans resemble domains (Tsao et al., 2003, 2008a; Pinsk et al., 2005; Rajimehr et al., 2009). Likewise, the large visual area termed V4 or DL has been divided into large regions or domains of neurons that are either color selective or orientation selective (Tanigawa et al., 2010), although these large regions might also be considered separate cortical areas (Cusick and Kaas, 1988; Stepniewska and Kaas, 1996).

How Do Columns and Modules Emerge in Development?

A number of factors likely contribute to the functional organization of cortex, but at the modular level, activity-dependent selection of coactive afferents together with cellular signals that are position dependent probably are two of the most important variables (Erzurumlu and Kind, 2001; Sur and Leamey, 2001; Kaas and Catania, 2002). There is considerable evidence to support this conclusion, but some of the most impressive evidence comes from studies that created three-eyed frogs (Constantine-Paton and Law, 1978; Katz and Constantine-Paton, 1988). In frogs, each optic tectum normally receives inputs from only the contralateral eye, but when a third eye is added experimentally to one side of the head during embryonic development, both eyes on that side compete for territory in the same contralateral optic tectum. The projections from each of these eyes respond to molecular signals that tend to produce the same retinotopic pattern in the optic tectum, but local groups of tectal neurons favor inputs from one eye or the other. The result is that the afferents from the two eyes form alternating bands or stripes that resemble the ocular dominance bands in cats and anthropoid primates. The borders between these bands in the optic tectum and visual cortex correspond to locations where abrupt differences in activity patterns occur, and they do not develop or they degrade when activity is blocked (Cline et al., 1987). Obviously, the ability to form ocular dominance bands did not evolve via natural selection in the optic tectum of frogs for some future function. Instead, the developmental factors that produced these columns were present for other reasons that are not clear but apparently are widely important in nervous system development (Katz and Constantine-Paton, 1988). The capacity for module formation seems to be inherent in all cortical tissue, as well as in other tissue such as the optic tectum or superior colliculus, where inputs of different activation patterns compete for location with an overall global map. Thus, ocular dominance bands and other configurations, as well as orientation modules and other types of columns, including those based on discontinuities of the receptor sheet, have emerged independently in several lines of mammalian evolution. For some of these types of modules, asking what they do (Horton and Adams, 2005) may be the wrong

question. Instead, we might ask, what else is achieved in neural tissue by the mix of activity-dependent and position-dependent factors that select and group synaptic contacts when these factors coexist at particular developmental times? Purves et al. (1992) have suggested that some of the columns that have been described in cortex are "by-products" of synaptic development. If so, what is the product?

8

The Remarkable, Yet Not Extraordinary, Human Brain as a Scaled-Up Primate Brain and Its Associated Cost

SUZANA HERCULANO-HOUZEL

Neuroscientists have become used to a number of "facts" about the human brain: It has 100 billion neurons and 10- to 50-fold more glial cells; it is the largest-than-expected for its body among primates and mammals in general, and therefore the most cognitively able; it consumes an outstanding 20% of the total body energy budget despite representing only 2% of body mass because of an increased metabolic need of its neurons; and it is endowed with an overdeveloped cerebral cortex, the largest compared with brain size. These facts led to the widespread notion that the human brain is literally extraordinary: an outlier among mammalian brains, defying evolutionary rules that apply to other species, with a uniqueness seemingly necessary to justify the superior cognitive abilities of humans over mammals with even larger brains. These facts, with deep implications for neurophysiology and evolutionary biology, are not grounded on solid evidence or sound assumptions, however. Our recent development of a method that allows rapid and reliable quantification of the numbers of cells that compose the whole brain has provided a means to verify these facts. Here, I review this recent evidence and argue that, with 86 billion neurons and just as many nonneuronal cells, the human brain is a scaled-up primate brain in its cellular composition and

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metabolic cost, with a relatively enlarged cerebral cortex that does not have a relatively larger number of brain neurons yet is remarkable in its cognitive abilities and metabolism simply because of its extremely large number of neurons.

I f the basis for cognition lies in the brain, how can it be that the self-designated most cognitively able of animals—us, of course—is not the one endowed with the largest brain? The logic behind the paradox is simple: because brains are made of neurons, it seems reasonable to expect larger brains to be made of larger numbers of neurons; if neurons are the computational units of the brain, then larger brains, made of larger numbers of neurons, should have larger computational abilities than smaller brains. By this logic, humans should not rank even an honorable second in cognitive abilities among animals: at about 1.5 kg, the human brain is two- to threefold smaller than the elephant brain and four- to sixfold smaller than the brains of several cetaceans (Tower, 1954; Marino, 1998). Nevertheless, we are so convinced of our primacy that we carry it explicitly in the name given by Linnaeus to the mammalian order to which we belong—*Primata*, meaning "first rank," and we are seemingly the only animal species concerned with developing entire research programs to study itself.

Humans also do not rank first, or even close to first, in relative brain size (expressed as a percentage of body mass), in absolute size of the cerebral cortex, or in gyrification (Hofman, 1985). At best, we rank first in the relative size of the cerebral cortex expressed as a percentage of brain mass, but not by far. Although the human cerebral cortex is the largest among mammals in its relative size, at 75.5% (Rilling and Insel, 1999), 75.7% (Frahm et al., 1982), or even 84.0% (Hofman, 1988) of the entire brain mass or volume, other animals, primate and nonprimate, are not far behind: The cerebral cortex represents 73.0% of the entire brain mass in the chimpanzee (Stephan et al., 1981), 74.5% in the horse, and 73.4% in the short-finned whale (Hofman, 1985).

The incongruity between our extraordinary cognitive abilities and our not-that-extraordinary brain size has been the major driving factor behind the idea that the human brain is an outlier, an exception to the rules that have applied to the evolution of all other animals and brains. A largely accepted alternative explanation for our cognitive superiority over other mammals has been our extraordinary brain size compared with our body size, that is, our large encephalization quotient (Jerison, 1973). Compared with the trend for brain mass to increase together with body mass across mammalian species in a fashion that can be described mathematically by a power law (von Bonin, 1937), the human species appears

to be an outlier, with a brain that is about sevenfold larger than expected from its body mass compared with mammals as a whole (Jerison, 1977), or threefold larger than expected compared with other primates (Jerison, 1985), although how we came to be that way has not been well accounted for in the literature.

Why should a larger-than-expected brain bring about larger cognitive abilities? That notion is based on the idea that an "excess brain mass," relative to the brain mass necessary to operate the body, would endow the behavior of more encephalized animals with more complexity and flexibility (Jerison, 1985). The most encephalized species should also be the most cognitively able, and that species, finally, was our own.

However, the notion that higher encephalization correlates with improved cognitive abilities has recently been disputed in favor of absolute numbers of cortical neurons and connections (Roth and Dicke, 2005), or simply absolute brain size (Deaner et al., 2007). If encephalization were the main determinant of cognitive abilities, small-brained animals with very large encephalization quotients, such as capuchin monkeys, should be more cognitively able than large-brained but less encephalized animals, such as the gorilla (Marino, 1998). However, the former animals with a smaller brain are outranked by the latter in cognitive performance (Deaner et al., 2007).

It remains possible that the source of incongruence between our cognitive abilities and brain size is an unwarranted comparison of species across orders. Such comparisons are based on the notion, implicit in most comparative studies to date, that different brains are just scaled-up or scaled-down versions of a common basic plan, such that larger brains always have more neurons than smaller brains and two brains of a similar size always have comparable numbers of neurons. However, this notion is in disagreement with the observation that animals of similar brain size but belonging to different mammalian orders, such as the cow and the chimpanzee (both at about 400 g of brain mass), or the rhesus monkey and the capybara (at 70–80 g of brain mass), may have strikingly different cognitive abilities and behavioral repertoires. Thus, either the logic that larger brains always have more neurons is flawed or the number of neurons is not the most important determinant of cognitive abilities. The appealing alternative view that total connectivity, gauged from the total number of synapses in the brain, should be a direct determinant of brain processing capabilities runs into the same difficulty. Although this possibility remains to be examined systematically, the few pieces of evidence available in the literature suggest that synaptic density is constant across species (Cragg, 1967; Beaulieu and Colonnier, 1985; Schüz and Palm, 1989; Schüz and Demianenko, 1995). If that is indeed the case, the total numbers of brain synapses would be simply proportional to brain size and the differences

in cognitive abilities between brains of a similar size would, again, be left unaccounted for.

On the other hand, it is possible that the relationship between brain size and number of brain neurons is determined by rules that have varied in evolution, and visual examination of brain sizes in the mammalian radiation does suggest that large brains appeared several times independently in most of the mammalian orders (Fig. 8.1). In this scenario of independent evolution of large brains in different mammalian orders, not all mammalian brains are necessarily built as larger or smaller versions of the same plan, with proportionately larger or smaller numbers of neurons. This scenario leaves room for similarly sized brains across orders, such as the cow and the chimpanzee brains, to contain very different numbers of neurons, just as a very large cetacean brain might contain fewer neurons than a gorilla brain. In that case, size comparisons between the human brain and nonprimate brains, larger or smaller, might simply be inadequate and uninformative, and our view of the human brain as an outlier, an extraordinary oddity, may have been based on the mistaken assumption that all brains are made the same.

Here, I will explore the different relationships that apply across mammalian orders between brain structure size and numbers of neuronal cells (i.e., their order- and structure-specific neuronal scaling rules); the shared relationships across orders between brain structure mass and numbers of nonneuronal cells and nonneuronal cell density (i.e., their shared nonneuronal scaling rules); the concerted scaling across mammalian brains of numbers of neurons in the cerebral cortex and cerebellum, despite the increase in relative size of the former in larger brains; the constraints imposed by the primate neuronal scaling rules on cortical connectivity; the relationship between brain metabolism and number of neurons; and, finally, how humans compare with other mammals in these aspects, and what that recent evidence implies about human brain evolution.

NOT ALL BRAINS ARE MADE THE SAME: NEURONAL SCALING RULES

Testing the possibility that large brains have evolved as different functions of their numbers of neurons across mammalian orders became possible when we determined the numbers of cells that compose the brain of over 30 species belonging to three mammalian orders (Herculano-Houzel, 2011b). These studies were made possible by the development of the isotropic fractionator, an unbiased nonstereological method created in our laboratory that provides cell counts based on suspensions of free nuclei derived from tissue homogenates from whole brains divided into anatomically defined regions (Herculano-Houzel and Lent, 2005).
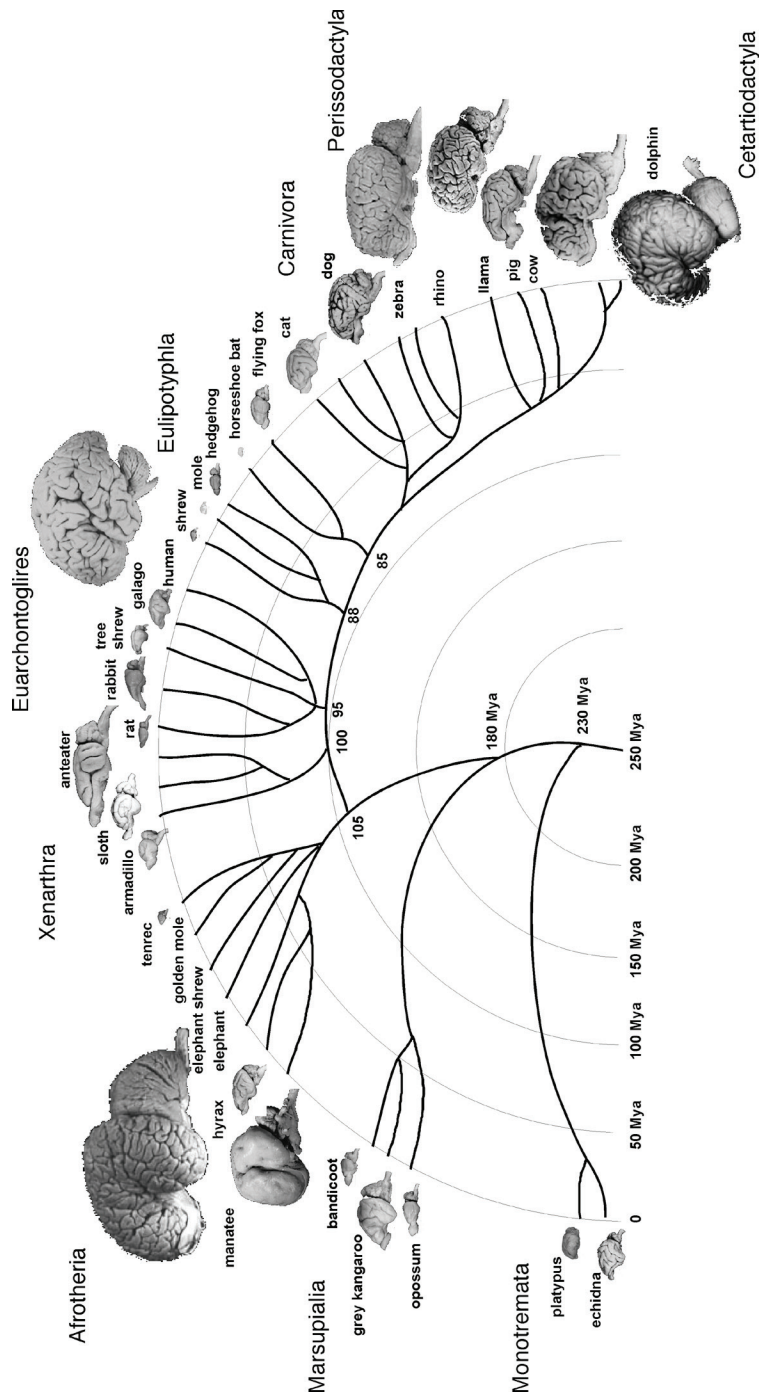
FIGURE 8.1 Large brains appear several times in the mammalian radiation. Example species are illustrated for each major mammalian group. Mammalian radiation based on Murphy et al. (2004) and Kaas (2007). Brain images from the Wisconsin and Michigan State Comparative Mammalian Brain Collections (www. Brainmuseum.org).

Applying the isotropic fractionator, we found that the proportionality between brain mass and number of brain neurons (i.e., the neuronal scaling rule for the brains of a group of animals) is different across brain structures and mammalian orders [reviewed in Herculano-Houzel (2011b)] (Fig. 8.2). In rodents, variations in brain size outpace variations in the number of brain neurons: rodent brains vary in mass as a power function of the number of brain neurons raised to a large exponent of 1.5 (Herculano-Houzel et al., 2006, 2011) (Fig. 8.2, *Upper Left*). In primates and insectivores, in contrast, brain size increases linearly as a function of its number of neurons, or as a power function with an exponent of ~1.0 (Herculano-Houzel et al., 2007; Azevedo et al., 2009; Sarko et al., 2009; Gabi et al., 2010) (Fig. 8.2, *Upper Left*). This means that a 10-fold increase in the number of neurons in a rodent brain results in a 35-fold larger brain, whereas in a primate or insectivore, the same increase results in a brain that is only 10- or 11-fold larger (Herculano-Houzel, 2009). Different neuronal scaling rules also apply separately to the cerebral cortex, cerebellum, and rest of the brain across mammalian orders (Figs. 8.2, *Upper* and 8.3A). This happens as the rate of variation in neuronal density with increasing structure size differs across brain structures and mammalian orders (Fig. 8.3B), indicating that average neuronal size varies rapidly with numbers of neurons in some and slowly or not at all in others (Herculano-Houzel, 2011b). For instance, the cerebral cortex grows across rodent species as a power function of its number of neurons with a large exponent of 1.7 (Herculano-Houzel et al., 2011), which means that a 10-fold increase in the number of cortical neurons in a rodent leads to a 50-fold increase in the size of the cerebral cortex. In insectivores, the exponent is 1.6, such that a 10-fold increase in the number of cortical neurons leads to a 40-fold larger cortex. In primates, in contrast, the cerebral cortex and cerebellum vary in size as almost linear functions of their numbers of neurons (Herculano-Houzel et al., 2007; Gabi et al., 2010), which means that a 10-fold increase in the number of neurons in a primate cerebral cortex or cerebellum leads to a practically similar 10-fold increase in structure size, a scaling mechanism that is much more economical than in rodents and allows for a much larger number of neurons to be concentrated in a primate brain than in a rodent brain of similar size (Fig. 8.3A).

SHARED SCALING RULES: NONNEURONAL CELLS

In contrast to the structure- and order-specific neuronal scaling rules, the numerical relationship between brain structure mass and the respective number of nonneuronal cells seems similar across all structures and species analyzed so far, spanning about 90 million years of evolution (Figs. 8.2, *Lower* and 8.3C): the larger a structure is, the more nonneuronal cells
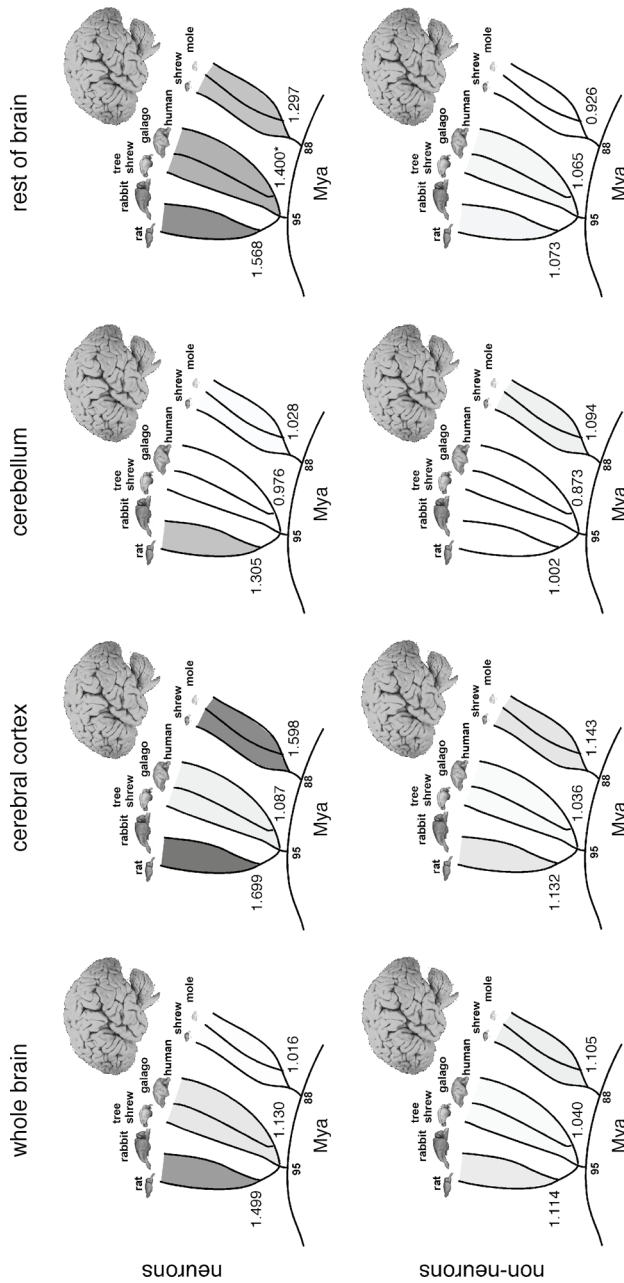
FIGURE 8.2 Comparison of allometric exponents for total brain mass, cerebral cortex mass, cerebellar mass, and rest of brain mass as a function of numbers of neurons (*Left*) or nonneuronal cells (*Right*). Exponents, given at the base of the radiation of each individual group (Glires, Primata/Scandentia, and Eulipotyphla), are illustrated by the intensity of the shading. Data from Herculano-Houzel et al. (2006, 2007, 2011), Azevedo et al. (2009), Sarko et al. (2009), and Gabi et al. (2010); exponents from Herculano-Houzel (2011b).
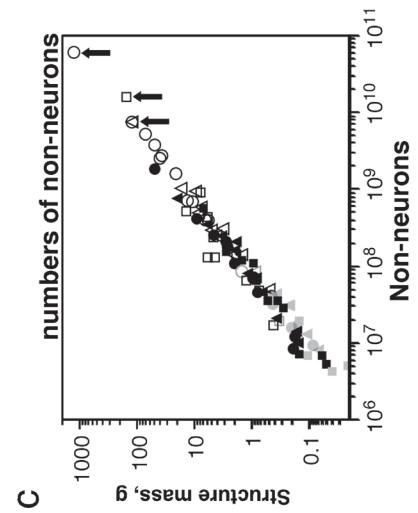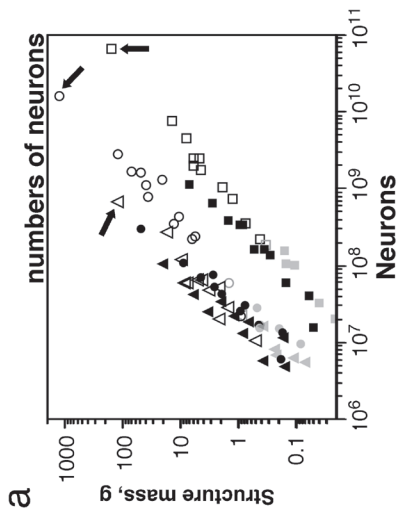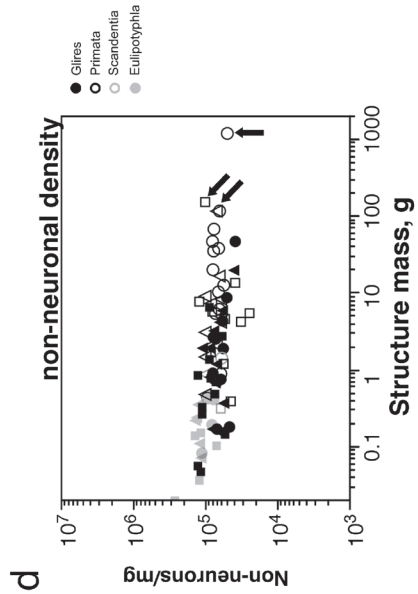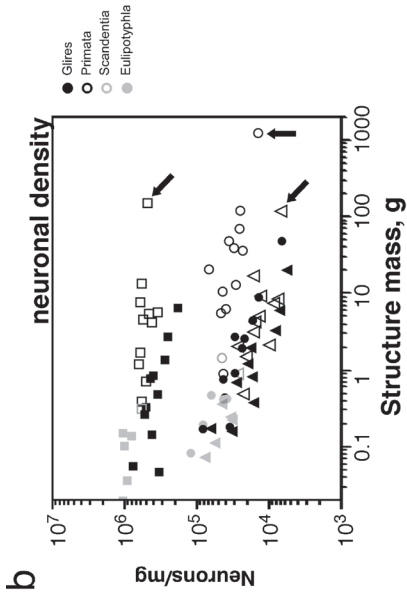
FIGURE 8.3 Shared nonneuronal scaling rules and structure- and order-specific neuronal scaling rules for mammalian brains. Each point represents the average values for one species (insectivores, filled gray symbols; rodents, filled black symbols; primates, open black symbols; scandentia, open gray symbols). Arrows, human datapoints. Circles, cerebral cortex; squares, cerebellum; triangles, rest of brain (excluding the olfactory bulb). (A) Clade- and structure-specific scaling of brain structure mass as a function of numbers of neurons. Allometric exponents: cerebral cortex, 1.699 (Glires), 1.598 (insectivores), 1.087 or linear (primates); cerebellum, 1.305 (Glires), 1.028 or linear (insectivores), 0.976 or linear (primates); rest of brain, 1.568 (Glires), 1.297 (insectivores), 1.198 (or 1.4 when corrected for phylogenetic relatedness in the dataset, primates). (B) Neuronal cell densities scale differently across structures and orders, but are always larger in primates than in Glires. Allometric exponents: cerebral cortex, −0.424 (Glires), −0.569 (insectivores), −0.168 (primates); cerebellum, −0.271 (Glires), not significant (insectivores and primates); rest of brain, −0.467 (Glires), not significant (insectivores), −0.220 (primates). (C) Mass of the cerebral cortex, cerebellum, and rest of brain varies as a similar function of their respective numbers of nonneuronal cells. Allometric exponents: cerebral cortex, 1.132 (Glires), 1.143 (insectivores), 1.036 (primates); cerebellum, 1.002 (Glires), 1.094 (insectivores), 0.873 (primates); rest of brain, 1.073 (Glires), 0.926 (insectivores), 1.065 (primates). (D) Average density of nonneuronal cells in each structure does not vary systematically with structure mass across species. Power functions are not plotted so as not to obscure the datapoints. Allometric exponents from Herculano-Houzel (2011b). Data from Herculano-Houzel et al. (2006, 2007, 2011), Azevedo et al. (2009), Sarko et al. (2009), and Gabi et al. (2010).

it has, in a nearly linear manner, such that nonneuronal cell density does not vary systematically with structure size (Fig. 8.3*D*). This implies that glial and endothelial cells have not been free to vary in size as mammalian brains evolve, a finding suggesting that the functions of these cells must be tightly regulated, allowing very little room for changes in evolution (Herculano-Houzel, 2011b).

SHARED SCALING RULES: CEREBRAL CORTEX AND CEREBELLUM

Larger brains possess larger cerebral cortices and cerebella but with a slightly faster increase in the size of the former compared with the latter, such that over five orders of magnitude, larger brains possess relatively larger cerebral cortices, whereas the relative size of the cerebellum fails to increase with brain size (Stephan et al., 1981). If the size of these structures were similar functions of their numbers of neurons, relatively larger cerebral cortices should hold increasingly larger percentages of brain neurons across species. Based on this implicit assumption, the discrepancy in the scaling of relative cerebral cortical and cerebellar size in larger brains has been used as an argument favoring the functional importance of relative neocortex expansion in brain function and evolution (Hofman, 1985; Clark et al., 2001; Jerison, 2007).

Strikingly, we found that the increase in relative size of the cerebral cortex in larger brains does not reflect a relatively larger number of cortical neurons compared with the whole brain, or with the cerebellum. Larger cortices do have larger numbers of neurons, of course (Fig. 8.3*A*); however, and in contrast to the increasing volumetric preponderance of the cerebral cortex in larger mammalian brains, numbers of neurons in the cerebral cortex increase coordinately and linearly with numbers of neurons in the cerebellum across mammalian species of different orders (Fig. 8.4*A*), regardless of how much the cerebral cortex comes to dominate brain size (Fig. 8.4*B*). This coordinated scaling happens with a relatively stable numerical preponderance of about four neurons in the cerebellum to every neuron in the cerebral cortex, even though these structures change in size following different cellular scaling rules across rodents, primates, and Eulipotyphla (Herculano-Houzel, 2010) (insectivores; Fig. 8.4*A*). This is illustrated by the finding that in most mammalian species examined so far, including humans, the cerebral cortex contains about 20–25% of all brain neurons, regardless of its relative size [which can reach 82% of the brain in humans (Herculano-Houzel, 2010)]. Thus, for a variation in brain size of five orders of magnitude, the ratio between numbers of cerebral cortical and cerebellar neurons varies relatively little and does not correlate with brain size. This is a strong argument against neocorticalization
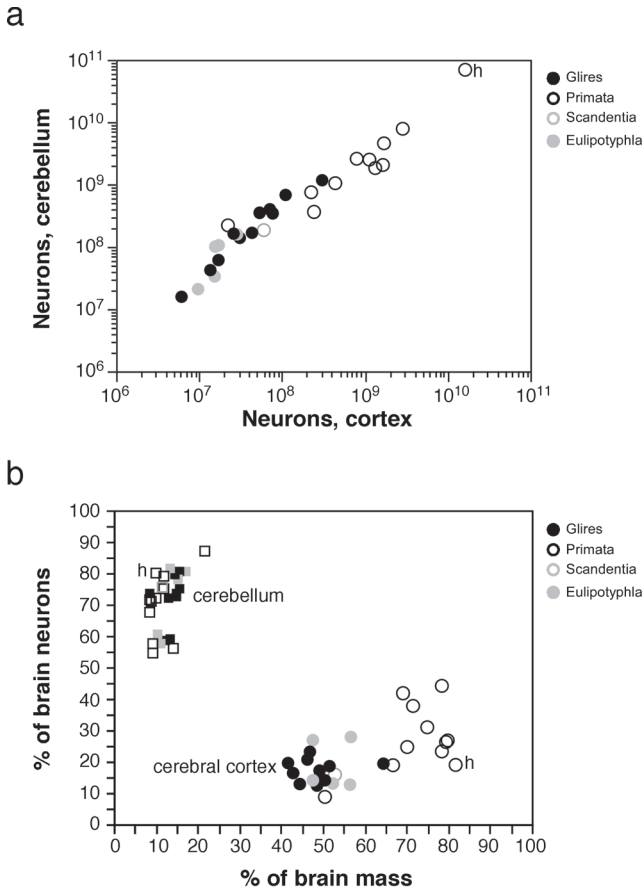
FIGURE 8.4 Coordinated scaling of the number of neurons in the cerebral cortex and cerebellum of mammals. (*A*) The number of neurons in the cerebellum covaries with the number of neurons in the cerebral cortex across all species in a way that can be described as a linear function of slope 4.2 ($p < 0.0001$, $r^2 = 0.995$). (*B*) Increased relative cortical mass does not reflect an increased relative number of brain neurons. Each point represents the average values for one species (insectivores, filled gray symbols; rodents, filled black symbols; primates, open black symbols; scandentia, open gray symbols). Circles, relative mass and relative number of brain neurons in the cerebral cortex; squares, relative values for cerebellum. All Spearman correlation *p*-values > 0.2. Data from Herculano-Houzel et al. (2006, 2007, 2011), Azevedo et al. (2009), Sarko et al. (2009), and Gabi et al. (2010); h, human datapoints.

(in what concerns numbers of neurons) and in favor of the coordinated increase in numbers of neurons across the cortex and cerebellum related to the behavioral and cognitive (not only sensorimotor) functions that corticocerebellar circuits mediate as brain size increased on multiple, independent occasions in evolution. The coordinated addition of neurons to cerebral cortex and cerebellum thus argues for coordinated corticocerebellar function and a joint evolution of the processing abilities of the two structures (Whiting and Barton, 2003; Ramnani et al., 2006; Balsters et al., 2010), a view also supported by the concerted increase in size of the prefrontal cerebral cortex, prefrontal inputs to the corticopontine system, and prefrontal-projecting cerebellar lobules in primates (Ramnani et al., 2006; Balsters et al., 2010). The issue then becomes accounting for how the cerebral cortex increases in size faster than the cerebellum as both gain neurons coordinately. As examined next, this differential scaling is probably related to how connectivity through the underlying white matter scales in the two structures, one of which carries massive long-range connections across cerebral cortical areas both within and across the hemispheres that are essential for the operation of associative networks (Wen and Chklovskii, 2005), whereas the other is mostly composed of centrifugal and centripetal connections, with associative connections mostly restricted to the gray matter of the cerebellum (Bush and Allman, 2003). As a result, the cerebral subcortical white matter gains volume faster than the cerebellar white matter in larger brains (Zhang and Sejnowski, 2000; Bush and Allman, 2003), because overall neuronal size (including dendrites and axonal arborizations) increases faster in the cerebral cortex than in the cerebellum, as both gain neurons coordinately.

CEREBRAL CORTEX EXPANSION, GYRIFICATION, AND CONNECTIVITY

Even if expanding without gaining relatively more of the total number of brain neurons, the mammalian cerebral cortex does vary in size over five orders of magnitude, albeit as different functions of its number of neurons across mammalian orders (Herculano-Houzel, 2011b). Cortical expansion is commonly envisioned as occurring laterally, through the increase of the number of progenitor cells in the subventricular zone and the consequent addition of radial columns containing a constant number of neurons across species (Rakic, 1988). A number of models of cortical expansion in evolution assume such a uniform distribution of neurons across species, based on the initial findings of Rockel et al. (1980) of a constant number of ~147,000 neurons beneath 1 mm^2 of cortical surface of various mammalian species. A second common assumption in evolutionary models of cortical expansion is that a constant fraction of cortical neurons sends axons into the white matter; that is, cortical connectivity

does not scale with brain size (Prothero, 1997; Zhang and Sejnowski, 2000; Wang et al., 2008), although some models predict a decrease in cortical connectivity through the white matter in larger cortices (Stevens, 1989; Ringo, 1991; Karbowski, 2001, 2003).

Contrary to the expectation of a uniform number of neurons beneath a given cortical surface across species (Rockel et al., 1980), cortical expansion in primates occurs with at least a threefold variation in these numbers across species (Herculano-Houzel et al., 2008). Moreover, cortical connectivity through the white matter (i.e., the fraction of gray matter neurons that sends or receives an axon through the white matter) indeed decreases as the cortex gains neurons (Herculano-Houzel et al., 2010). Larger primate cortices increase in size proportionally to the number, N, of neurons in the gray matter, of which a decreasing fraction (proportional to $N^{0.841}$) sends axons into the white matter. Given the average axonal length in the primate white matter to increase with $N^{0.242}$, and given our inference that the average axonal diameter does not change appreciably with N (Herculano-Houzel et al., 2010), we predict that the volume of the white matter should increase with $N^{1.114}$, which is close to the scaling exponent obtained experimentally (Herculano-Houzel et al., 2010). The expansion of both the gray and white matter of the brains of primates thus occurs with a decreasing connectivity fraction and a largely invariant average axonal diameter in the white matter, which might also explain the increasing gyrification of larger cortices through the increasing tension of axons coursing in the white matter [reviewed in Mota and Herculano-Houzel (2012)].

A decrease in long-range connectivity, favoring local connectivity, in larger primate brains is expected from the nearly linear increase in cortical size as the brain gains neurons, given that, all things being equal (including connectivity), cortical volume should increase with its number of neurons raised to the power of 4/3. A decrease in connectivity in larger primate brains is compatible with the view that the cerebral cortex displays among its neurons the connectivity properties of a small-world network, that is, a network in which distance between nodes (neurons) is small, with mostly local connectivity and only a relatively small number of long-range connections (Watts and Strogatz, 1998). Evidence that the cortex is connected and functions as a small-world network at the neuronal level has been found recently (Grinstein and Linsker, 2005; Bassett et al., 2006), even though the cerebral cortex may be densely connected at the level of functional areas (Markov et al., 2011). There is converging evidence that the cerebral cortex also scales as a small-world network at the neuronal level, growing through the addition of nodes that are densely interconnected locally (through horizontal connections in the gray matter) but only sparsely interconnected globally, through long fibers (in the white matter), which still guarantees fast global communication (Ringo, 1991; Changizi, 2001; Sporns and Kötter, 2004; Sporns and Zwi, 2004). A

decrease in neuronal connectivity is indeed an expected feature of growing small-world networks (Argollo de Menezes et al., 2000).

HUMAN BRAIN AS A SCALED-UP PRIMATE BRAIN

Despite common remarks in the literature that the human brain contains 100 billion neurons and 10- to 50-fold more glial cells [e.g., Helmuth (2001), Kandel et al. (2004), Nishiyama et al. (2005)], no references are given to support these statements; to the best of my knowledge, they are none other than ballpark estimates (Williams and Herrup, 1988). Comparing the human brain with other mammalian brains thus required first estimating the total numbers of neuronal and nonneuronal cells that compose these brains, which we did a few years ago (Azevedo et al., 2009). Remarkably, at an average of 86 billion neurons and 85 billion nonneuronal cells (Azevedo et al., 2009), the human brain has just as many neurons as would be expected of a generic primate brain of its size and the same overall 1:1 nonneuronal/neuronal ratio as other primates (Gabi et al., 2010). Broken down into the cerebral cortex, cerebellum, and rest of the brain, the neuronal scaling rules that apply to primate brains also apply to the human brain (Azevedo et al., 2009) (Fig. 8.3*A* and *C*, arrows). Neuronal densities in the cerebral cortex and cerebellum also fit the expected values in humans as in other primate species (Fig. 8.3*B*), and the ratio between nonneuronal and neuronal cells in the whole human brain of 1:1 (not 10:1, as commonly reported) is similar to that of other primates (Azevedo et al., 2009). The number of neurons in the gray matter alone of the human cerebral cortex, as well as the size of the subcortical white matter and the number of nonneuronal cells that it contains, also conforms to the rules that apply to other primates analyzed (Herculano-Houzel et al., 2010). Most importantly, even though the relative expansion of the human cortex is frequently equated with brain evolution, which would have reached its crowning achievement in us (Rakic, 2009), the human brain has the ratio of cerebellar to cerebral cortical neurons predicted from other mammals, primate and nonprimate alike (Fig. 8.4*A*). Therefore, the observed compliance of the human brain to the same neuronal scaling rules that apply to nonhuman primates [including great apes (Herculano-Houzel and Kaas, 2011)] makes the human brain simply a scaled-up primate brain: In what regards its number of neurons, our brain cannot be considered extraordinary in the sense of being an outlier.

HUMAN ADVANTAGE

Observing that the human brain is a scaled-up primate brain in its number of neuronal and nonneuronal cells is not to say that the human

brain is not at an advantage compared with other mammals. What needs to be considered is that the human cognitive advantage over other animals may reside simply in the total number of brain neurons (Herculano-Houzel, 2009, 2011a), and this may be the consequence of humans being primates and, among these, the species with the largest brain (Herculano-Houzel, 2012). Because of the different proportionality between brain size and number of brain neurons between primates and rodents, a primate brain contains more neurons than a similarly sized rodent brain (Herculano-Houzel, 2011b). For instance, the human brain has about sevenfold more neurons than the 12 billion neurons that a hypothetical rodent brain of 1.5 kg would be expected to have, according to the neuronal scaling rules that apply to rodent brains (Herculano-Houzel et al., 2006, 2011; Herculano-Houzel, 2009). Moreover, the primate advantage in numbers of brain neurons compared with a similarly sized rodent brain becomes increasingly larger with increasing brain size. Although direct measurements of numbers of neurons are not yet available for whole elephant and whale brains, one can speculate on how those numbers might differ depending on the particular neuronal scaling rules that apply. Hypothetically, if cetacean brains scaled similar to primate brains [which is unlikely, given their steep decrease in neuronal density with increasing brain size (Tower, 1954)], a whale brain of 3.65 kg would be predicted to have a whopping 212 billion neurons. In contrast, if cetacean brains scaled similar to rodent brains [which is a more likely scenario, given the very low neuronal densities in cetacean and elephant brains (Tower, 1954)], that same brain would only hold about 21 billion neurons, which is fewer than the 28 billion and 33 billion neurons that we have predicted for the chimpanzee and gorilla brains, respectively (Herculano-Houzel, 2009; Herculano-Houzel and Kaas, 2011).

Compared with other primates, the human brain is therefore not exceptional in its number of neurons, nor should it be considered an evolutionary outlier. If absolute brain size is the best predictor of cognitive abilities in a primate (Deaner et al., 2007), and absolute brain size is proportional to number of neurons across primates (Herculano-Houzel et al., 2007; Gabi et al., 2010), our superior cognitive abilities might be accounted for simply by the total number of neurons in our brain, which, based on the similar scaling of neuronal densities in rodents, elephants, and cetaceans, we predict to be the largest of any animal on Earth (Herculano-Houzel, 2009).

SCALING OF GLIA/NEURON RATIOS AND METABOLISM

Although neurons are generally considered the most important cell type for the generation of cognition, the role of glial cells in brain physiology is more and more recognized (Barres, 2008). One parameter tradition-

ally considered a functionally relevant indicator of the neuron/glia relationship is the ratio between numbers of glial and neuronal cells in brain tissue (the G/N ratio). The G/N ratio used to be considered to increase uniformly with brain size, which would be uniformly accompanied by larger neurons (Haug, 1987; Marino, 2006). Instead, as could be expected from the uniform nonneuronal scaling rules but structure- and order-specific neuronal scaling rules, we found that the nonneuronal/neuronal ratio (which serves as an approximation of the maximal G/N ratio) does not increase homogeneously with increasing brain size or increasing size of brain structures, as originally thought (Fig. 8.5*A*). However, the G/N ratio increases in a strikingly homogeneous manner with decreasing neuronal density across brain structures in all mammalian species examined so far, which indicates that the G/N ratio does indeed accompany average neuronal size [reviewed in Herculano-Houzel (2011b)] (Fig. 8.5*B*). The finding that glial cells are not nearly as numerous in the human brain as once believed is therefore highly significant: it shows that the human brain, like that of every other mammal observed so far, obeys the same uniform scaling relationship between the G/N ratio and neuronal density (Herculano-Houzel, 2012). Such a universal relationship between G/N ratios and neuronal size, conserved across brain structures and species over 90 million years of evolution, suggests that this ratio reflects a functionally fundamental and evolutionarily conserved aspect of brain morphology (Herculano-Houzel, 2011b).

The increased G/N ratio with increased neuronal size is traditionally believed to reflect an increased metabolic need of larger neurons (Attwell and Laughlin, 2001). Once numbers of neurons composing different rodent and primate brains were available, it became possible to estimate how the average metabolic cost per neuron scales with brain size and neuronal density. Contrary to expectations, dividing total glucose use per minute in the cerebral cortex or whole brain (Karbowski, 2007) by the number of brain neurons revealed a remarkably constant average glucose use per neuron across the mouse, rat, squirrel, monkey, baboon, and human, with no significant relationship to neuronal density and, therefore, to average neuronal size (Herculano-Houzel, 2011c). This is in contrast to the decreasing average metabolic cost of other cell types in mammalian bodies with increasing cell size (Porter and Brand, 1995a,b; West et al., 2002), with the single possible exception of muscle fibers (Hulbert and Else, 1989). The higher levels of expression of genes related to metabolism in human brains compared with chimpanzee and monkey brains (Cáceres et al., 2003; Uddin et al., 2004) might therefore be related not to an actual increase in metabolism per cell but to the maintenance of average neuronal metabolism in the face of decreasing metabolism in other cell types in the body.
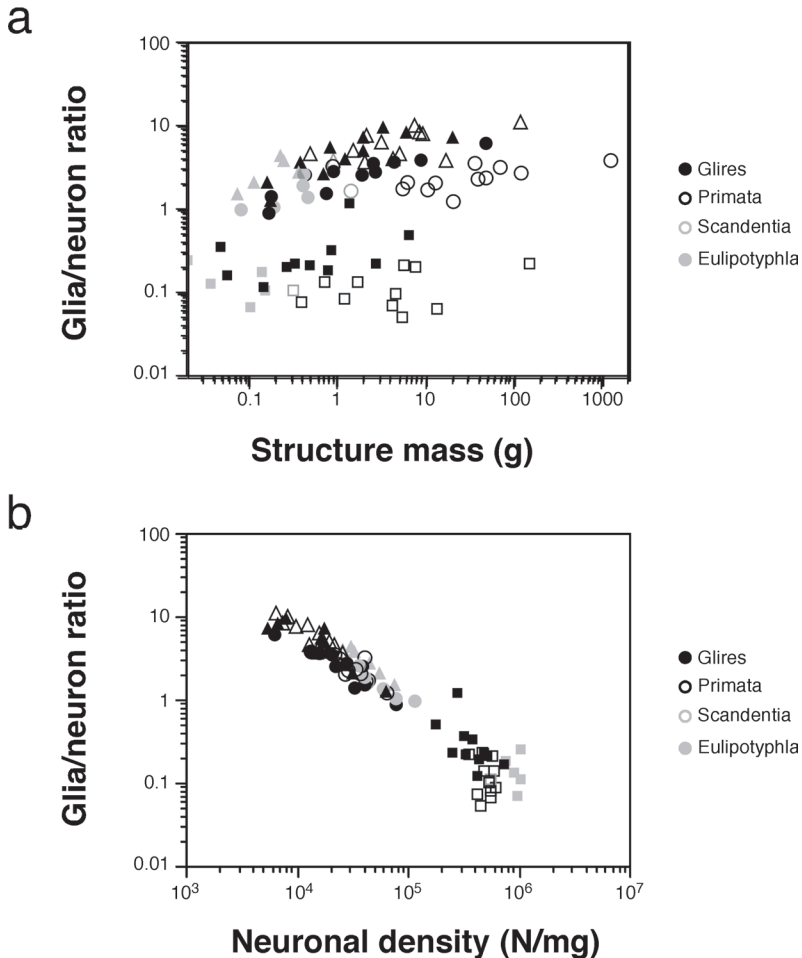
FIGURE 8.5 Glia/neuron ratio scales differently across structures and orders with structure mass, but scales homogeneously with neuronal density. Each point represents the average other cell/neuron ratio (which approximates the glia/neuron ratio) and structure mass (*A*) or neuronal density (*B*) in the cerebral cortex (circles), cerebellum (squares), or RoB (triangles) of a species (insectivores, filled gray symbols; rodents, filled black symbols; primates, open black symbols; scandentia, open gray symbols). Notice that in contrast to the scattered distribution across species and structures in (*A*), datapoints are aligned across species and structures in the bottom plot, suggesting that it is smaller neuronal densities (i.e., larger average neuronal cell size), not larger structure mass, that is accompanied by a larger glia/neuron ratio. Data from Herculano-Houzel et al. (2006, 2007, 2011), Azevedo et al. (2009), Sarko et al. (2009), and Gabi et al. (2010).

That the average energetic cost per neuron does not scale with average neuronal cell size has important physiological implications. First, considering the obligatory increased cost related to a larger surface area (Attwell and Laughlin, 2001), the evolution of neurons with a constant average energetic cost regardless of their total cell size implies that the relationship between larger neuronal size and a larger G/N ratio must not be related to increased metabolic needs, as usually assumed. Instead, we have proposed that this relationship ensues simply from the invasion during early development of a parenchyma composed mostly of neurons of varying sizes (in different brain structures and species) by glial cells of relatively constant size across structures and species (Herculano-Houzel, 2011c). Second, the constant average energetic cost per neuron across species implies that larger neurons must compensate for the obligatory increased metabolic cost related to repolarizing the increased surface area of the cell membrane. This compensation could be implemented by a decreased number of synapses and/or decreased rates of excitatory synaptic transmission (Karbowski, 2007). Synaptic homeostasis and elimination of excess synapses [e.g., during sleep (Gilestro et al., 2009)], the bases of synaptic plasticity, might thus be necessary consequences of a tradeoff imposed by the need to constrain neuronal energetic expenditure (Herculano-Houzel, 2011c).

Another consequence of a seemingly constant metabolic cost per neuron across species is that the total metabolic cost of rodent and primate brains, and of the human brain, is a simple, linear function of their total number of neurons (Herculano-Houzel, 2011c) (Fig. 8.6), regardless of average neuronal size, absolute brain size, or relative brain size compared with the body. At an average rate of 6 kcal/d per billion neurons (Herculano-Houzel, 2011c), the average human brain, with 86 billion neurons, costs about 516 kcal/d. That this represents an enormous 25% of the total body energetic cost is simply a result of the "economical" neuronal scaling rules that apply to primates in comparison to rodents, and probably to other mammals in general: For a similar brain size, more neurons will be found in a primate brain than in possibly any other mammalian brain (Herculano-Houzel, 2009, 2011a). It is intriguing to consider, therefore, that our remarkable cognitive abilities, at a remarkable relative energetic cost, might be mostly the result of a very large number of neurons put together in a not extraordinary fashion but, instead, according to the same evolutionary scaling rules that apply to other primates.

COST OF BEING HUMAN

Humans are not the largest living primates: Gorillas overlap with or exceed humans in body size, but their brains amount to about one-third
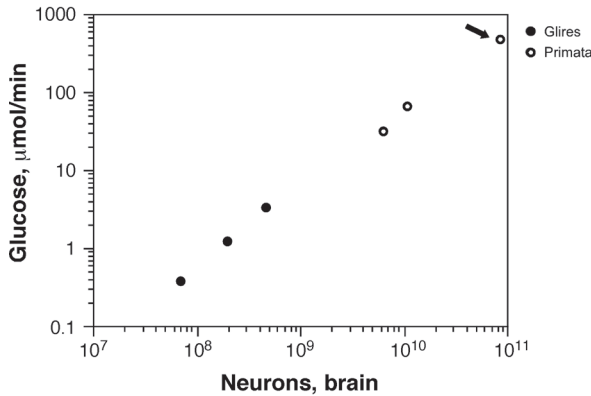
FIGURE 8.6 Total brain metabolism (measured as µmol of glucose consumed per minute) scales as a linear function of the total number of neurons in the brain across rodents and primates alike, including humans (arrow). The function plotted is a power function of exponent 0.988, not significantly different from 1.0. Data from Herculano-Houzel (2011c).

of the size of the human brain (Marino, 1998), making our comparatively larger brain size appear an oddity, given our body mass (Marino, 1998; Rilling, 2006; Gazzaniga, 2008). Why does the largest primate not also have the largest brain, if brain and body size are usually well correlated across species?

In the relationship between brain size, body size, and number of brain neurons, body mass is much freer to vary than the other two variables (Gabi et al., 2010). Across primates, the exponent that describes the brain–body scaling relationship is highly dependent on the species sampled, whereas the neuronal scaling rules that apply to primate brains are insensitive to the choice of species (Gabi et al., 2010). Moreover, body mass should not be considered as a variable determining, or contributing directly to, brain size (Herculano-Houzel, 2011a), even though it is often correlated with brain size, particularly given that body size evolution, such as body size divergence between chimpanzees and gorillas, can occur through changes in late growth that will be accompanied by little parallel change in brain size (Shea, 1983; Riska and Atchley, 1985).

The evolution of the hominin brain, and of the human brain in particular, may thus have involved two parallel but not necessarily related phenomena: an increase in brain size and number of neurons, obeying the same cellular scaling rules that apply to other primates, and a moderate increase in body size, compared with gorillas and orangutans, whose body size increased greatly compared with other primates that diverged

earlier from the common ancestor (Herculano-Houzel and Kaas, 2011). We and others (Shea, 1983; Riska and Atchley, 1985; Byrne, 1995; Deacon, 1997b; Herculano-Houzel and Kaas, 2011) have proposed that it might not be the case that humans have a brain that is too large for their body; rather, it might be that great apes evolved a body size (diverging from the brain–body relationship that applies to earlier diverging primates as well as to later diverging humans) that may not be directly related to their brain size, a trend in evolution that was not pursued in the *Homo* lineage.

There is, however, an additional possibility to be explored, and that is that great apes do not have larger brains to match their larger bodies because they cannot afford the metabolic cost of supporting the larger number of neurons. The great apes lineage appears to have favored marked increases in body size rather than brain size from the common ancestor with the *Homo* lineage, whereas the *Homo* lineage seems to have favored a large brain, with a large number of neurons, instead of a large body. The absence of animals in the fossil record with both a very large (human-like) brain and a very large (great ape–like) body is consistent with the possibility that it is not metabolically possible to have both.

Growing a large body comes at a cost. Although large animals require less energy per unit of body weight, they have considerably larger total metabolic requirements that, on average, scale with body mass raised to an exponent of ~3/4 (Kleiber, 1932; Schmidt-Nielsen, 1984; Martin, 1990; Bonner, 2006). Thus, large mammals need to eat more, and they cannot concentrate on rare, hard-to-find, or catch foods (Conroy, 1990). Adding neurons to the brain, however, also comes at a sizable cost, as reviewed above: 6 kcal/d per billion neurons (Herculano-Houzel, 2011c). In primates, whose brain mass scales linearly with its number of neurons, this implies that total brain metabolism scales linearly with brain volume or mass, that is, with an exponent of 1, which is much greater than the much cited 3/4 exponent of Kleiber (1932) that relates body metabolism to body mass. The discrepancy suggests that, per gram, the cost of primate brain tissue scales faster than the cost of nonneuronal bodily tissues, which calls for a modification of the "expensive tissue hypothesis" of brain evolution (Aiello and Wheeler, 1995), according to which brain size is a limiting factor. Given the steep, linear increase in brain metabolic cost with increasing numbers of neurons, we conclude that metabolic cost is a more limiting factor to brain expansion than previously suspected. In our view, it is not brain size but, instead, absolute number of neurons that imposes a metabolic constraint on brain scaling in evolution, because individuals with larger numbers of neurons must be able to sustain their proportionately larger metabolic requirements to keep their brain functional.

The larger the number of neurons, the higher is the total caloric cost of the brain, and therefore the more time required to be spent feeding

to support the brain alone, and feeding can be very time-consuming (Owen-Smith, 1988). Based on their brain mass [estimated from cranial capacity (De Sousa and Woods, 2007)], we predicted that total numbers of neurons in the brain increased from 27 billion to 35 billion neurons in *Australopithecus* and *Paranthropus* species to close to 50–60 billion neurons in *Homo* species from *Homo rudolfensis* to *Homo antecessor*, to 62 billion neurons in *Homo erectus*, and to 76–90 billion neurons in *Homo heidelbergensis* and *Homo neanderthalensis* (Herculano-Houzel and Kaas, 2011), which is within the range of variation found in modern *Homo sapiens* (Azevedo et al., 2009). It can thus be seen how any increase in total numbers of neurons in the evolution of hominins and great apes would have taxed survival in a limiting, if not prohibitive, way, given that it probably would have to occur in a context of already limiting feeding hours: The added 60 billion brain neurons from an orangutan-sized hominin ancestor to modern *Homo* require an additional 360 kcal/d, which is probably not readily available to great apes on their diet.

It has been proposed that the advent of the ability to control fire to cook foods, which increases enormously the energy yield of foods and the speed with which they are consumed (Carmody and Wrangham, 2009; Carmody et al., 2011), may have been a crucial step in allowing the near doubling of numbers of brain neurons that is estimated to have occurred between *H. erectus* and *H. sapiens* (Wrangham, 2009). The evolution of the human brain, with its high metabolic cost imposed by its large number of neurons, may thus only have been possible because of the use of fire to cook foods, enabling individuals to ingest in very little time the entire caloric requirement for the day, and thereby freeing time to use the added neurons to their competitive advantage.

CONCLUSION: REMARKABLE, YET NOT EXTRAORDINARY

Despite our ongoing efforts to understand biology under the light of evolution, we have often resorted to considering the human brain as an outlier to justify our cognitive abilities, as if evolution applied to all species except humans. Remarkably, all the characteristics that appeared to single out the human brain as extraordinary, a point off the curve, can now, in retrospect, be understood as stemming from comparisons against body size with the underlying assumptions that all brains are uniformly scaled-up or scaled-down versions of each other and that brain size (and, hence, number of neurons) is tightly coupled to body size. Our recently acquired quantitative data on the cellular composition of the human brain and its comparison to other brains, both primate and nonprimate, strongly indicate that we need to rethink the place that the human brain holds in nature and evolution, and to rewrite some basic concepts that

are taught in textbooks. The human brain has just the number of neurons and nonneuronal cells that would be expected for a primate brain of its size, with the same distribution of neurons between its cerebral cortex and cerebellum as in other species, despite the relative enlargement of the former; it costs as much energy as would be expected from its number of neurons; and it may have been a change from a raw diet to a cooked diet that afforded us its remarkable number of neurons, possibly responsible for its remarkable cognitive abilities.

Part III

FROM NEURAL CIRCUIT EVOLUTION TO ADAPTIVE BEHAVIOR

The five chapters of Part III aim to link evolutionary changes in neural circuits to the evolution of behavior. In Chapter 9, James Newcomb and colleagues describe the neural circuits underlying swimming behavior in various Nudipleura (sea slugs). As it turns out, some nudipleuran species have evolved the ability to swim by undulating their bodies either from side to side or dorsoventrally. Importantly, these different types of swimming evolved independently in several different lineages, allowing for interesting comparisons of their underlying circuitry. Specifically, Newcomb et al. report that nonhomologous swimming behaviors can be mediated by neural circuits that include homologous (as well as nonhomologous) neurons and that clearly homologous swimming in closely related species may involve nonhomologous neurons. These findings show that, even for homologous behaviors, it is difficult to predict how conserved the underlying circuits are. An important implication of this finding is that one cannot homologize behaviors merely on the basis of how similar their underlying circuits are. This conclusion extends a theme first mentioned by Northcutt (Chapter 3): homology at one level of biological organization need not imply homology at other hierarchical levels.

In Chapter 10, Andrew Bass and Boris Chagnaud review the literature showing that the premotor neurons controlling sound production tend to be derived from caudal rhombomere 8 in the hindbrain of many different vertebrates, including fishes and amphibians. Something about these neurons makes them especially well suited for complex, often rhythmic, pattern generation and for the coordination of diverse muscles, includ-

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ing the muscles related to breathing. Bass and Chagnaud further point out that in toadfishes the hindbrain vocal motor neurons lie adjacent to motor neurons innervating the pectoral fins. This finding suggests that the neural circuitry for sound production shares a long evolutionary (and developmental) history with the circuits controlling the pectoral fins and, in tetrapods, the forelimbs. This hypothesis may seem far-fetched at first; however, pectoral fins are used for sound production in a number of fishes, and forelimbs are clearly used for gestural communication in humans. If correct, the hypothesis implies a deep homology between behaviors that seem quite disparate but involve homologous neural circuits and, presumably, homologous developmental genes.

James Goodson and colleagues in Chapter 11 examine variation in neuropeptide expression across multiple brain regions involved in avian social behavior. More specifically, the paper focuses on differences in peptide expression among four emberizid songbird species, examining their correlation with seasonal changes in territoriality and/or flocking behavior. The analysis gets complicated, because variation in the degree of territoriality may be caused by reduced aggression or increased gregariousness (i.e., flocking), which likely involve different neural mechanisms. However, clever species selection allows the authors to identify one set of differences in neuropeptide expression that is most likely linked to differences in aggression and another set that correlates with differences in flocking behavior. As the authors admit, the conclusions are based on just a few species and, therefore, tentative. However, the study undeniably reveals an unexpectedly large degree of variation in peptide levels both across species and within species (i.e., seasonal variation). This variation is probably a driving force behind the variation in behavior, although it may also be a consequence. Experimental manipulations are needed to discriminate between these two hypotheses.

In Chapter 12, Lucia Jacobs develops ideas about the role of the hippocampus in navigation. She suggests that olfaction played a crucial early role in the evolution of spatial orientation, providing information about spatial gradients (in odor plumes) as well as local cue constellations (locale-specific odorant mixtures). The hippocampus became specialized to process and integrate these two kinds of information. Subsequently, these functions were extended to other sensory modalities. An interesting corollary of this hypothesis is that the size of the olfactory system should correlate more tightly with an organism's ability to navigate by olfactory cues than with its capacity for odor discrimination. The hypothesis might also explain why olfactory brain regions scale less tightly than other regions with overall brain size. Perhaps the evolutionary shift to multimodal navigation allowed the olfactory system to be reduced. Jacobs predicts that the olfactory system should be larger in species that must

predict when and where their food will be available than in species that feed opportunistically.

In Chapter 13, Kenneth Catania reports on two natural but highly unusual feeding behaviors. First, Catania reviews the incredibly rapid and efficient hunting behavior of star-nosed moles. Using optimal foraging theory, he shows that these small predators are specialized for rapidly finding and eating small aquatic invertebrates. Their star-shaped "nose" evolved to help them in this task, as did a series of related specializations in the brain, including an expanded somatosensory cortex. Catania then turns to an aquatic snake that has evolved a fascinating trick for catching fish. It uses a tiny muscular contraction of its body to trigger a nearby fish's escape response in such a way that the hapless fish tends to swim directly into the snake's wide-open fangs. Even more remarkable, the snakes can anticipate the trajectory of the escape response, intercepting a fish before it gets away. Because this predictive ability is found even in naive snakes that have never caught (or missed catching) a fish, it seems to be innate (i.e., unlearned). Why did the fish retain their stereotyped escape response, given that the snakes can exploit it? The answer may be that snakes are relatively rare, and the escape response serves the fish well when dealing with most other threats.

9

Homology and Homoplasy of Swimming Behaviors and Neural Circuits in the Nudipleura (Mollusca, Gastropoda, Opisthobranchia)

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How neural circuit evolution relates to behavioral evolution is not well understood. Here the relationship between neural circuits and behavior is explored with respect to the swimming behaviors of the Nudipleura (Mollusca, Gastropoda, Opithobranchia). Nudipleura is a diverse monophyletic clade of sea slugs among which only a small percentage of species can swim. Swimming falls into a limited number of categories, the most prevalent of which are rhythmic left–right body flexions (LR) and rhythmic dorsal–ventral body flexions (DV). The phylogenetic distribution of these behaviors suggests a high degree of homoplasy. The central pattern generator (CPG) underlying DV swimming has been well characterized in *Tritonia diomedea* and in *Pleurobranchaea californica*. The CPG for LR swimming has been elucidated in *Melibe leonina* and *Dendronotus iris*, which are more closely related. The CPGs for the categorically distinct DV and LR swimming behaviors consist of nonoverlapping sets of homologous identified neurons, whereas the categorically similar behaviors share some homologous identified neurons, although the exact composition of neurons and synapses in the neural circuits differ. The roles played by homologous identified neurons in categorically distinct behaviors differ. However, homologous identified neurons also play different roles even in the swim CPGs of the two LR swimming species.

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> Individual neurons can be multifunctional within a species. Some of those functions are shared across species, whereas others are not. The pattern of use and reuse of homologous neurons in various forms of swimming and other behaviors further demonstrates that the composition of neural circuits influences the evolution of behaviors.

Behavior and neural mechanisms can be considered to represent two different levels of biological organization (Lauder, 1986, 1994; Striedter and Northcutt, 1991; Rendall and Di Fiore, 2007). Nevertheless, the evolution of behavior and the evolution of neural circuits underlying behavior are intertwined. For example, it has been suggested that the properties of neural circuits affect the evolvability of behavior); the evolution of particular behaviors could be constrained or promoted by the organization of neural circuits (Airey et al., 2000; Bendesky and Bargmann, 2011; Carlson et al., 2011; Katz, 2011; Yamamoto and Vernier, 2011). Darwin and the early ethologists recognized that behaviors, like anatomical features, are heritable characters that are amenable to a phylogenetic approach (Darwin, 1876; Whitman, 1899; Heinroth, 1911; Lorenz, 1981). The use of behavioral traits to determine phylogenies has been validated several times (Wenzel, 1992; De Queiroz and Wimberger, 1993; Proctor, 1996; Stuart et al., 2002), and the historical debates about homology and homoplasy of behavior have been thoroughly reviewed (Lauder, 1986, 1994; Wenzel, 1992; Foster et al., 1996; Proctor, 1996; Rendall and Di Fiore, 2007). Examining the neural bases for independently evolved (i.e., homoplastic) behaviors within a clade could provide insight into fundamental aspects of neural circuit organization. However, it is difficult enough to determine the neural basis for behavior in one species. Doing this in several species with quantifiable behaviors is even more challenging.

Studies of the neural bases of swimming behaviors in the Nudipleura (Mollusca, Gastropoda, Opisthobranchia) offer such a possibility. These sea slugs exhibit well differentiated categories of swimming behaviors, and their nervous systems have large individually identifiable neurons, allowing the neural circuitry underlying the swimming behaviors to be determined with cellular precision.

Here we will summarize what is known about the phylogeny of Nudipleura, their swimming behaviors, and the neural circuits underlying swimming. We will also provide data comparing the roles of homologous neurons. We find that neural circuits underlying the behaviors of the same category are composed of overlapping sets of neurons even if they most likely evolved independently. In contrast, neural circuits underlying categorically distinct behaviors use nonoverlapping sets of neurons. Fur-

thermore, homologous neurons can have different functions in different behaviors and even in similar behaviors.

PHYLOGENY OF NUDIPLEURA

The Nudipleura form a monophyletic clade within Opisthobranchia (Gastropoda) that contains two sister clades: Pleurobranchomorpha and Nudibranchia (Waegele and Willan, 2000; Wollscheid-Lengeling et al., 2001; Göbbeler and Klussmann-Kolb, 2010) (Fig. 9.1). Molecular evidence suggests that the two sister groups separated approximately 125 Mya (Göbbeler and Klussmann-Kolb, 2010). Nudibranchia (or, informally, nudi-branchs), which are shell-less and have a slug-shaped appearance with "naked gills," were traditionally classified as their own order. The most recently agreed-upon taxonomic classification system for nudibranchs uses unranked clades instead of orders, suborders, and superfamilies (Bouchet and Rocroi, 2005). There are at least 2,000 to 3,000 identified nudibranch species (Behrens, 2005). Studies that used morphological and molecular data support the monophyly of Nudibranchia (Waegele and Willan, 2000; Wollscheid-Lengeling et al., 2001; Vonnemann et al., 2005; Dinapoli and Klussmann-Kolb, 2010; Göbbeler and Klussmann-Kolb, 2010; Pola and Gosliner, 2010).

Within Nudibranchia, there are two monophyletic clades (Waegele and Willan, 2000): Euctenidiacea (Anthobranchia) (Thollesson, 1999; Valdes, 2003) and Cladobranchia (Pola and Gosliner, 2010). Euctenidiacea includes Doridacea, which is larger than Cladobranchia, subdividing into 25 families (Thollesson, 1999). Within Cladobranchia, Bornellidae forms a sister group to the other subclades (Pola and Gosliner, 2010). Aeolidida is a monophyletic clade with Lomanotidae as a sister group (Pola and Gosliner, 2010). What was traditionally called Dendronotida forms a para-phyletic grouping. A recent study was unable to include the nudibranch *Melibe* in Cladobranchia because of a 12-bp deletion in its genome (Pola and Gosliner, 2010). However, its natural affinity with *Tethys* in terms of shared derived characteristics strongly suggests that it belongs in Clado-branchia, as we have indicated in Fig. 9.1. There are several additional unresolved relations in Nudibranchia, most notably in Dendronotida and Doridacea. Consideration of locomotor behavior and neural circuits may help resolve these relations.

CATEGORIES OF LOCOMOTOR BEHAVIOR

Crawling is the primary form of locomotion for all Nudipleura (Audesirk, 1978; Audesirk et al., 1979; Chase, 2002). The majority of spe-cies crawl via mucociliary locomotion; cilia on the bottom of the foot beat

Clade   Family   Genus

Bornellidae — *Bornella* - LR

Dironidae — *Dirona* - NS

Tritoniidae
- *Tritonia* - DV
- *Marionia* - DV
- *Tochuina* - NS

Scyllaeidae
- *Notobryon* - LR
- *Scyllaea* - LR

Dendronotidae — *Dendronotus* - LR

Tethydidae
- *Melibe* - LR
- *Tethys* - LR

Phylliroidae
- *Phylliroë* - LU
- *Cephalopyge* - LU

Lomanotidae — *Lomanotus* - LR

Flabellinidae
- *Cumanotus* - BS
- *Flabellina cynara* - BS
- *Flabellina iodinea* - LR
- *Flabellina telja* - LR
- *Flabellina trophina* - NS

Aeolidiidae — *Aeolidella* - BS

Glaucidae
- *Hermissenda* - LR
- *Pteraeolidia* - LR

Armina - NS

Dorididae — *Aphelodoris* - DV

Discodorididae
- *Diaulula* - NS
- *Discodoris* - DV
- *Sebadoris* - DV/DU

Chromodoridae
- *Archidoris* - NS
- *Hypselodoris* - NS
- *Cadlina* - NS

Goniodorididae — *Trapania* - LR

Hexabranchidae — *Hexabranchus* - DV/DU

Polyceridae
- *Plocamopherus* - LR
- *Tamja* - LR
- *Nembrotha* - LR
- *Triopha fulgarans* - LR
- *Triopha catalinae* - NS

Pleurobranchidae
- *Pleurobranchaea* - DV
- *Tomthompsonia* - ?
- *Burthella* - NS?
- *Pleurobranchus* - AU
- *Berthellina* - NS?
- *Euselenops* - F
- *Bathyberthella* - NS?

Clade labels: Nudipleura, Nudibranchia, Cladobranchia, Aeolidida, Dendronotida, Euctenidiacea, Doridacea, Doridoidea, Polyceroidea, Pleurobranchomorpha

and propel the animal over a surface of secreted mucus. The speed of crawling is affected by efferent serotonergic and peptidergic neurons that control the ciliary beat frequency (Audesirk, 1978; Audesirk et al., 1979; Willows et al., 1997). Some species also use muscular crawling, which relies on waves of contraction or extension and contraction of the foot. Crawling is a trait shared with most Opisthobranchia and is therefore plesiomorphic to the Nudipleura. Only three nudibranch species do not crawl because they are truly pelagic: *Phylliroë atlantica*, *Phylliroë bucephala*, and *Cephalopyge trematoides* (Lalli and Gilmer, 1989). This is also true for gastropods in general; there are ~40,000 marine gastropod species but only approximately 150 are pelagic (Lalli and Gilmer, 1989).

In addition to crawling, a limited number of benthic species can also swim (Farmer, 1970). We classify swimming in the Nudipleura into seven general categories: (*i*) left–right flexion (LR), (*ii*) dorsal–ventral flexion (DV), (*iii*) left–right undulation (LU), (*iv*) dorsal–ventral undulation (DU), (*v*) asymmetric undulation (AU), (*vi*) breaststroke (BS), and (*vii*) flapping (F) (Table 9.1).

LR swimming is characterized by the flattening of the body in the sagittal plane and repeated left–right bending near the midpoint of the body axis with the head and tail coming together laterally (Fig. 9.2*A*). This movement propels the animal through the water. Some animals, such as *Melibe leonina*, exhibit foot-first directionality, presumably because the dorsal cerata create drag. Other animals, such as *Tambja eliora*, proceed headfirst, with the tail lagging slightly, causing the body to take on an "S" form (Farmer, 1970). Animals in the genus *Plocamopherus* typically have a dorsal crest at the posterior end of the body that may act as a paddle and cause the head to proceed the tail (Rudman and Darvell, 1990).

FIGURE 9.1 An abbreviated phylogeny of the Nudipleura with reference to their behavior. Only the genera of the species listed in Table 9.1 are shown here unless species differences exist within the genus. The phylogenic relationships are based on Thollesson (1999), Waegele and Willan (2000), Wollscheid-Lengeling et al. (2001), Vonnemann et al. (2005), Göbbeler and Klussmann-Kolb (2010), and Pola and Gosliner (2010). The references for the behavior are listed in Table 9.1. Note that this figure represents all the known swimming species and only a tiny fraction of the more than 2,000 species that are not capable of swimming or for which there are no published reports of swimming. LR, left–right flexion; NS, nonswimmer; DV, dorsal–ventral flexion; LU, left–right undulation; BS, breaststroke; DU, dorsal–ventral undulation; AU, asymmetric undulation; F, flapping.

TABLE 9.1 Abbreviated Nudipleura Taxonomy with Reference to Swimming

Taxonomy	Swim Type	References
Nudibranchia		
Cladobranchia		
Aeolidida		
Aeolidioidea		
Aeolidiidae		
Aeolidiella alba	BS	Pruvot-Fol (1954), Farmer (1970)
Glaucidae		
Hermissenda crassicornis	LR	Lillvis et al. (2012)
Flabellinoidea		
Flabellinidae		
Flabellina cynara	BS	Marcus and Marcus (1967), Farmer (1970)
Flabellina iodinea	LR	MacFarland (1966), Farmer (1970)
Flabellina telja	LR	Marcus and Marcus (1967), Farmer (1970), Ferreira and Bertsch (1972)
Flabellina trophina	NS	[a]
Cumanotus beaumonti	BS	Picton and Morrow (1994)
Cumanotus cuenoti	BS	Tardy and Gantes (1980)
Arminoidea		
Armina californica	NS	[a]
Dendronotida[b]		
Bornellidae		
Bornella anguilla	LU	Johnson (1984)
Bornella calcarata	LR	Thompson (1980)
Bornella stellifer	LR	Risbec (1953), Farmer (1970), Willan and Coleman (1984)
Dendronotidae		
Dendronotus albopunctatus	LR	Robilliard (1972)
Dendronotus albus	LR	Farmer (1970), Robilliard (1970)
Dendronotus dalli	LR	Robilliard (1970)
Dendronotus diversicolor	LR	Robilliard (1970)
Dendronotus frondosus	LR	Farmer (1970), Robilliard (1970)
Dendronotus iris	LR	Kjerschow-Agersborg (1922), Haefelfinger and Kress (1967), Marcus and Marcus (1967), Farmer (1970), Robilliard (1970)

TABLE 9.1 Continued

Taxonomy	Swim Type	References
Dendronotus nanus	LR	Marcus and Marcus (1967), Farmer (1970), Robilliard (1972)
Dendronotus rufus	LR	Robilliard (1970)
Dendronotus subramosus	LR	Farmer (1970), Robilliard (1970)
Lomanotidae		
Lomanotus genei	LR	Garstang (1890), Thompson and Brown (1984)
Phylliroidae		
Phylliroë atlantica	LU	Lalli and Gilmer (1989)
Phylliroë bucephala	LU	Lalli and Gilmer (1989)
Cephalopyge trematoides	LU	Steinberg (1956), Lance (1968)
Scyllaeidae		
Notobryon wardi	LR	Thompson and Brown (1981)
Scyllaea pelagica	LR	Collingwood (1879), Pruvot-Fol (1954), Farmer (1970)
Tethydidae		
Melibe bucephala	LR	Schuhmacher (1973)
Melibe engeli	LR	Risbec (1937)
Melibe fimbriata	LR	Thompson and Crampton (1984)
Melibe japonica	LR	Willan and Coleman (1984)
Melibe leonina	LR	Kjerschow-Agersorg (1921), Hurst (1968), Farmer (1970), Lawrence and Watson (2002)
Melibe megaceras	LR	Gosliner (1987b)
Melibe pilosa	LR	Pease (1860), Farmer (1970), Ostergaard (1955)
Tethys fimbria	LR	Pruvot-Fol (1954), Farmer (1970)
Dironidae		
Dirona picta	NS	[a]
Dirona albolineata	NS	[a]
Tritoniidae		
Marionia blainvillea	DV[c]	Pontes (2002)
Marionia tethydes	DV[c]	Haefelfinger and Kress (1967)
Tritonia diomedea	DV	Willows (1967), Hume et al. (1982)
Tritonia festiva	DV	Birkeland (1974)
Tritonia hombergii	DV	Willows and Dorsett (1975)

TABLE 9.1 Continued

Taxonomy	Swim Type	References
Euctenidiacea		
Doridacea		
Doridoidea		
Dorididae		
Aphelodoris antillensis	DV	Quiroga et al. (2004)
Aphelodoris brunnea	DV	Gosliner (1987a)
Aphelodoris gigas	DV	Wilson (2003)
Aphelodoris karpa	DV	Wilson (2003)
Aphelodoris varia	NS	Wilson (2003)
Discodorididae		
Diaulula sandiegensis	NS	[a]
Discodoris evelinae	DV	Marcus (1955), Marcus and Marcus (1967)
Discodoris pusae	DV	Marcus (1955)
Sebadoris nubilosa	DV/DU[d]	Marcus and Marcus (1967), Farmer (1970)
Chromodoridae		
Archidoris odhneri	NS	[a]
Archidoris montereyensis	NS	[a]
Hypselodoris picta	NS	[a]
Cadlina luteomarginata	NS	[a]
Onchidoridoidea		
Goniodorididae		
Trapania velox	LR[f]	Cockerell (1901), Farmer (1970)
Polyceroidea		
Hexabranchidae		
Hexabranchus aureomarginatus	DV/DU[d]	Neu (1932), Ostergaard (1955), Farmer (1970)
Hexabranchus morsomus	DV/DU[d]	Risbec (1928), Marcus and Marcus (1962)
Hexabranchus sanguineus	DV/DU[d]	Risbec (1928), Gohar and Soliman (1963), Vincente (1963), Edmunds (1968), Farmer (1970)
Hexabranchus tinkeri	DV/DU[d]	Ostergaard (1955), Farmer (1970)
Polyceridae		
Nembrotha megalocera	LR	Yonow (1990)
Plocamopherus ceylonicus	LR	Willan and Coleman (1984), Rudman and Darvell (1990)

TABLE 9.1 Continued

Taxonomy	Swim Type	References
Plocamopherus imperialis	LR	Willan and Coleman (1984), Ellis (1999a), Marshall and Willan (1999)
Plocamopherus maculatus	LR	Pease (1860)
Plocamopherus maderae	LR	Lowe (1842)
Plocaompherus tilesii	LR	Rudman and Darvell (1990), Ellis (1999b)
Tambja blackii	LR	Pola et al. (2006)
Tambja eliora	LR	Lance (1968), Farmer (1970)
Tambja morose	LR	Marshall and Willan (1999)
Triopha fulgurans	LR	Risbec (1925), Farmer (1970)
Triopha catalinae	NS	[a]
Pleurobranchomorpha		
Pleurobranchidae		
Euselenops luniceps	F[e]	Pace (1901), Farmer (1970)
Pleurobranchaea californica	DV	Gillette et al. (1991), Davis and Mpitsos (1971)
Pleurobranchus membranaceus	AU	Thompson and Slinn (1959), Farmer (1970)

NOTE: This taxonomy is based upon that of Bouchet and Rocroi (2005). Abbreviations: AU = asymmetric undulation; BS = breaststroke; DU = dorsal–ventral undulation; DV = dorsal–ventral flexion; F = flapping; LR = left–right flexion; LU = left–right undulation; NS = nonswimmer.

[a]Tested with mechanical and salt stimuli in our laboratories.
[b]A paraphyletic group (Pola and Gosliner, 2010).
[c]Farmer (1970) reported that *Marionia* swim via left–right flexions and cited a German reference (Haefelfinger and Kress, 1967). However, a translation of this reference into English, by P. Katz, indicates that Haefelfinger and Kress reported dorsal–ventral flexions.
[d]Farmer (1970) categorized swimming in *Sebadoris* and *Hexabranchus* as "flapping." However, swimming in these species appears to include dorsal–ventral flexions of the body, in addition to undulations of the mantle.
[e]Video observation.
[f]Farmer (1970) classified *Trapania velox* as an LR swimmer. However, see text for additional discussion.

Plocamopherus ceylonicus (Rudman and Darvell, 1990; Marshall and Willan, 1999) and *Plocamopherus maderae* (Lowe, 1842) swim with LR flexions when dislodged from a substrate or disturbed in some way. *Tambja* appears to use LR swimming as an escape response; contact with the predacious nudibranch *Roboastra* will elicit swimming in *Tambja* (Farmer, 1970; Pola et al., 2006). LR swimming in *Melibe* and *Dendronotus iris* can be initiated in response to loss of contact with the substrate or in response to the touch of a predatory sea star (Lawrence and Watson, 2002; Sakurai et al., 2011).

FIGURE 9.2 Two examples of swimming behaviors. (*A*) LR swimming exhibited by *M. leonina*. The ventral side of the animal is shown with the mouth at the top of the image. During swimming, the foot is narrowed to a strip and the animal rhythmically flexes its body leftward and rightward, bending at a point midway along the body axis. (*B*) DV swimming exhibited by *T. diomedea*. The animal starts on the substrate, shown at the bottom with its head to the right. It launches with a ventral flexion, where the head and tail meet under the foot. Then, it flexes so that the head and tail meet above the dorsal body surface. The foot is flattened and expanded to the width of the body. A, anterior; P, posterior.
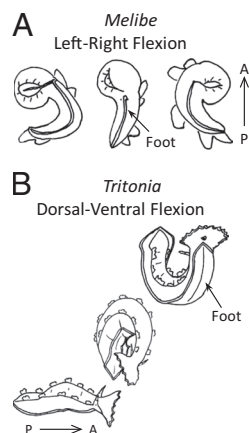
Melibe may also swim seasonally to disperse (Mills, 1994). The flexion cycle period for *Melibe* and *Dendronotus* is approximately 3 s, and swim bouts can last many minutes (Lawrence and Watson, 2002; Sakurai et al., 2011).

As its name suggests, *Bornella anguilla* swims with an eel-like movement caused by waves of muscular contraction (Johnson, 1984). Therefore, unlike other members of its genus, it is classified as an LU swimmer. LU swimming, which otherwise is found mostly in pelagic species, may be a further refinement of LR swimming.

DV swimming involves the animal flattening its body in the horizontal plane and repeatedly bending such that the tail and head meet in alternation above and below the midpoint of the body (Fig. 9.2*B*). *Tritonia diomedea* and *Pleurobranchaea californica* are two examples of DV swimmers that have been extensively studied (Willows, 1967; Davis and Mpitsos, 1971; Gillette and Jing, 2001; Katz, 2009). Swim bouts for *Tritonia* and *Pleurobranchaea* last less than 1 min and are triggered by contact with a predatory sea star or in the laboratory by high salt solutions or electric shock (Katz, 2010). The flexion cycle period under natural conditions is 5 to 10 s in *Tritonia* (Hume et al., 1982) and 3 to 6 s in *Pleurobranchaea* (Jing and Gillette, 1995).

DU swimming, like DV swimming, involves movement in dorsal and ventral directions, but here there are progressive symmetric waves of body wall or mantle muscular contraction. The Spanish dancer, *Hexabranchus sanguineus*, and other members of that genus are famous for their flamboyant swimming behavior (Gohar and Soliman, 1963; Edmunds, 1968; Farmer, 1970). *Hexabranchus* swimming differs in several ways from the DV swimming of *Tritonia* and *Pleurobranchaea*; in addition to the symmetrical undulation of the lateral fringes of the mantle, it has a shorter flexion

cycle period (2–4 s), swim bouts occur spontaneously, and swimming can last for long periods of time.

F swimming is similar to DV swimming in that the movement is bilaterally symmetric and dorsal–ventral in orientation, but instead of the head and tail meeting, the lateral edges of the mantle or foot rise and fall. F swimming is much more common in Opisthobranchia outside of the Nudipleura, such as *Clione limacina* (Arshavsky et al., 1986) and many species of *Aplysia* (Bebbington and Hughes, 1973; Donovan et al., 2006).

AU and BS are less common forms of locomotion. AU is characteristic of *Pleurobranchus membranaceus* (Thompson and Slinn, 1959) in which the animal swims upside down using its mantle as a passive keel while producing alternating muscular waves along its foot. BS involves the use of appendages including cerata and tentacles to stroke the water in a manner similar to a human swimmer's movements. Only four nudibranch species have been described as exhibiting this type of behavior (Table 9.1).

PHYLOGENETIC DISTRIBUTION OF SWIMMING BEHAVIORS

As noted earlier, we have been unable to find reports of swimming by about 97% of nudibranch species and approximately half the major subfamilies in the Pleurobranchomorpha clade. However, this does not mean they are not capable of swimming. Some species swim only as a high threshold escape response. Still, it is highly probable that the vast majority of the Nudipleura cannot and do not swim. This discussion is limited to species for which the type of swimming has been reported or for which swimming has been explicitly tested and shown not to occur.

LR swimming is by far the most prevalent of the six modes of swimming exhibited by nudibranchs: of the 60 nudibranch species documented to swim in the scientific literature, 40 species use LR or LU (Table 9.1). These 40 species are phylogenetically disparate, encompassing species in Doridacea and Cladobranchia (Fig. 9.1). Within the latter, there are LR swimmers in Aeolidoidea and Dendronotoidea. In Doridacea, all but one of the LR swimmers are in the family Polyceridae. There are no LR swimmers in the Pleurobranchomorpha or, to our knowledge, in any other Opisthobranch clade. This suggests that LR swimming is a derived characteristic of the nudibranch clade.

Unlike LR swimming, DV swimming is found in Nudibranchia and in Pleurobranchomorpha (Fig. 9.1). DV swimming is not present outside of Nudipleura and is therefore likely to be a synapomorphy of this clade. However, it is not widely displayed within Nudibranchia, appearing in just one family of Dendronotida (Tritoniidae) and in three families of Doridacea (Discodorididae, Dorididae, and Hexibranchidae). Discodorididae and Hexibranchidae also exhibit dorsal–ventral undulations (i.e., DU).

EVOLUTION OF SWIMMING BEHAVIORS

There are a number of possible scenarios that could account for the phylogenetic distribution of swimming behaviors among the Nudipleura. Considering the extreme rarity of swimming, it is possible, maybe even likely, that swimming evolved on multiple occasions from nonswimming species. The repeated gain of a function such as rhythmic movement could suggest that there is a predisposition toward these behaviors. The repeated appearance of LR and DV swimming may simply indicate that these two basic movements are the most likely to occur in a slug-shaped body with few appendages. When appendages such as moveable cerata are present, they have been repeatedly used for BS swimming. In the absence of such appendages, the only means of swimming are with LR-like or DV-like movements.

Given the presence of swimming across the phylogeny, it is possible that, rather than evolving independently many times from nonswimmers, swimming behaviors were repeatedly lost. Although this may lead to more transformations, it may be easier to lose a character than to gain one, as has been seen in other systems (Whiting et al., 2003; Moczek et al., 2006; Wiens et al., 2007; Harshman et al., 2008; Duboué et al., 2011).

For the moment, we will only consider the possible evolutionary scenarios that include transformations from one swimming state to another and ignore nonswimmers. It is generally the case that members of the same genus and often the same family exhibit the same form of swimming (Table 9.1), allowing us to group them together (Fig. 9.3). Here we will consider potential scenarios involving just the evolution of DV and LR swimming. It is possible that the ancestral species was able to swim using either DV or LR movements. However, this seems unlikely because there are no extant species that exhibit both of these behaviors. It is also unlikely that the ancestral state was LR swimming because of its absence in Pleurobranchomorpha.

Consider scenario 1 (Fig. 9.3*A*) in which DV swimming arose once at the base of the Nudipleura and LR swimming evolved independently several times. In this scenario, DV swimming behaviors in Pleurobranchomorpha, Doridacea, and Cladobranchia are homologous because they are shared by a common ancestor. Scenario 1 would also suggest that LR swimming evolved independently as many as seven times. Because of the unresolved branches in the phylogeny, there may be fewer switches in phenotype than this. In scenario 2 (Fig. 9.3*B*), LR swimming evolved once in the Nudibranchia, and DV swimming reevolved independently as many as four times. Again, the number of homoplastic events could be lower if the bifurcations in the phylogeny were better resolved.

The phylogenetic distribution of the swimming behavior suggests a resolution to the Dendronotida phylogeny, with Tritoniidae branching
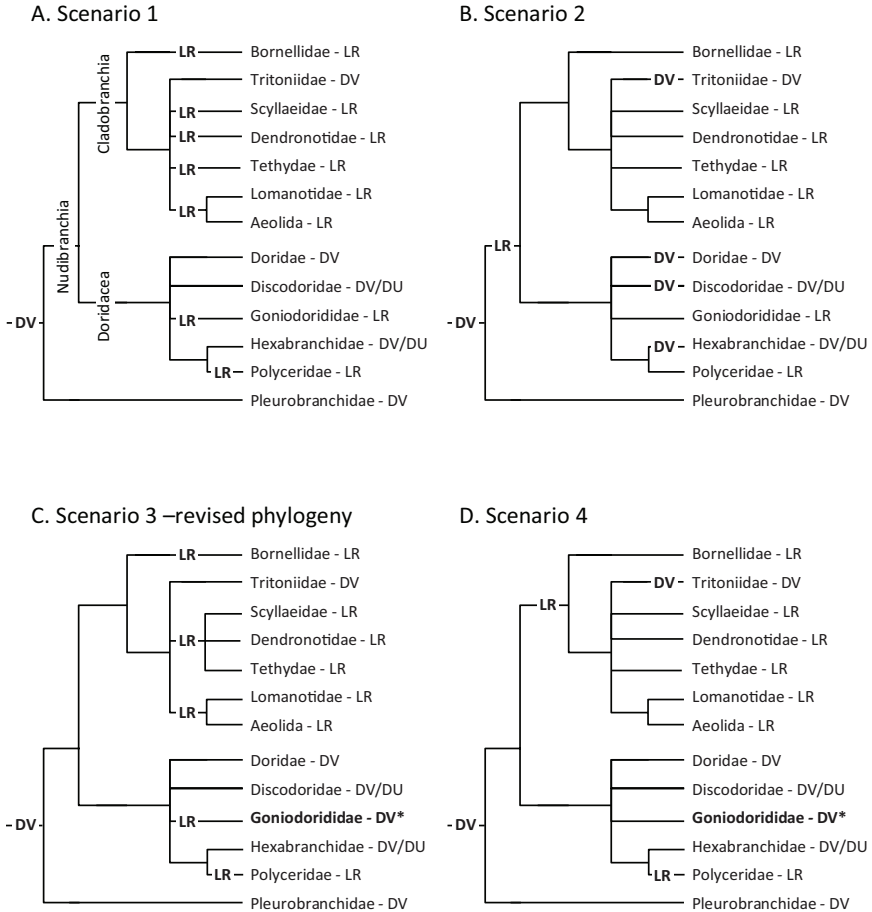
FIGURE 9.3 Possible evolutionary scenarios explaining the phylogenetic distribution of swimming behaviors. Just the families of the DV and LR swimming animals are shown. (*A*) In scenario 1, DV swimming is a synapomorphy of the Nudipleura that was lost and replaced six times by LR swimming. (*B*) In scenario 2, LR swimming is a synapomorphy of the Nudibranchia. DV swimming then reappears four times in different nudibranch lineages. (*C*) For scenario 3, the phylogenetic tree of Dendronotida is altered to group LR swimmers together. Goniodorididae (asterisk), which includes *T. velox*, is switched from LR to DV (as discussed in the text). This reduces the number of transitions to LR from six in scenario 1 to four. (*D*) Scenario 4 is similar to scenario 2, with Goniodorididae (asterisk) switched to DV. This represents the most parsimonious explanation if DV swimming is ancestral, with just three transitions from the basal DV state.

off separately from the LR swimmers. This would reduce the number of homoplastic events in Cladobranchia according to scenario 1 from five to three (scenario 3; Fig. 9.3*C*).

The phylogenetic distribution of the behavior also calls into question the accuracy of a report about the behavior of *Trapania velox*. Outside of the family Polyceridae, *T. velox* (family: Goniodorididae) is the only doridacean reported to swim with left–right flexions. Farmer (1970) categorized *T. velox* as an LR swimmer based on a previous report by Cockerell (1901), who described *T. velox* as being, "very active when swimming with an undulating motion on the surface of the water." However, there is no indication as to the plane of movement. Farmer (1970) reported working with this rare species and being unsuccessful at making it swim, and was thus unable to provide any additional information. We were unable to find any other reports of its behavior. If *T. velox* is reclassified as a DV swimmer, it would further decrease the number of homoplastic events in scenario 1 from seven to four (Fig. 9.3*C*). Thus, examining the phylogenetic distribution of behavior makes a prediction about the behavior of this rare species.

Redefining *T. velox* as a DV swimmer also suggests a fourth scenario (Fig. 9.3*D*), whereby LR swimming arose independently in Cladobranchia and Polyceridae. This would also involve reevolution of DV swimming in Tritoniidae. Scenario 4 would therefore be the most parsimonious explanation for the phylogenetic distribution of swimming behaviors if one does not take into account the hundreds of nonswimming species.

NEURAL CIRCUITS UNDERLYING SWIMMING

With our potential scenarios about the homology and homoplasy of swimming behaviors, it is now of interest to compare the neural mechanisms for these behaviors. The neural activity that underlies rhythmic DV and LR movements originates from central pattern generator (CPG) circuits (Delcomyn, 1980). These swim CPGs are composed of neurons whose anatomical and physiological properties allow them to be individually identifiable from animal to animal within a species. The same sets of characteristics can be used to identify homologous neurons in other species (Croll, 1987). This allows the composition of neural circuits and the roles of homologous neurons to be compared across species. The neural circuits underlying swimming have been determined in two DV swimmers [*T. diomedea* (Katz, 2009) and *P. californica* (Gillette and Jing, 2001; Jing and Gillette, 1999)] and two LR swimmers [*M. leonina* (Sakurai et al., 2011; Thompson and Watson, 2005) and *D. iris* (Sakurai et al., 2011)]. We can now begin to compare neural circuits underlying behaviors of animals to address phylogenetic and functional hypotheses.

DV Swim CPGs

The neural basis for DV swimming was first studied in *T. diomedea* (Willows, 1967; Dorsett et al., 1969; Getting et al., 1980; Getting, 1981, 1983). The swim CPG consists of just three neuron types (Fig. 9.4*A*). On each side of the brain, there are three dorsal swim interneurons (DSIs), one ventral swim interneuron (VSI), and one cerebral interneuron 2 (C2), for a total of 10 neurons (Katz, 2009, 2010). The DSIs initiate the dorsal flexion cycle in which C2 participates. C2 then excites VSI, which inhibits DSI and C2 and elicits the ventral phase of the movement. As would be expected for a DV swimmer, the contralateral counterparts for each neuron fire in relative synchrony (Fig. 9.4*B*).

The neurons comprising the CPG for DV swimming in *P. californica* include DSI and C2 homologues called As and A1, respectively (Jing and Gillette, 1995, 1999). The connectivity and activity of these homologues is similar in both species (Fig. 9.4*C* and *D*). The homologue of the *Tritonia* VSI has not been identified in *Pleurobranchaea*, although there is synaptic input to As and A1 during the ventral phase of the motor pattern that may arise from such a neuron (i.e., Ivs neuron) (Jing and Gillette, 1999). Alternatively, ventral-phase synaptic input may arise from a neuron that is not homologous to VSI, but serves a similar role.

There are also *Pleurobranchaea* swim CPG neurons (A3 and A10) that have not been identified in *Tritonia*. Despite more than 40 years of electrophysiological study concentrated in the area where the A3 and A10 somata would be, no neurons with equivalent synaptic connectivity or activity have been found in *Tritonia*. Thus, either these neurons do not exist in *Tritonia* or they cannot be recognized with electrophysiological criteria.

With the information available about the swim CPGs in *Tritonia* and *Pleurobranchaea*, we can currently say that some homologous neurons are used for similar functions in distantly related species. This result is compatible with any of the phylogenetic scenarios (Fig. 9.3). If DV swimming is homologous (scenarios 1 or 3; Fig. 9.3*A* and *C*), the similarities in the DV swim CPGs in *Tritonia* and *Pleurobranchaea* could be a result of their homology and the potential differences in the swim CPGs could represent divergence of the circuit architecture. The differences in the swim CPGs may just as readily reflect independent evolutionary paths (scenarios 2 or 4; Fig. 9.3*B* and *D*), which might suggest a predisposition to use certain neurons to produce these behaviors.

LR Swim CPGs

The LR swim CPG was first described in *M. leonina* (Watson et al., 2001; Thompson and Watson, 2005). The published circuit consists of a pair of bilaterally represented neurons: swim interneuron 1 (Si1) and swim
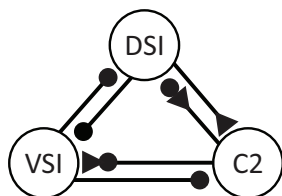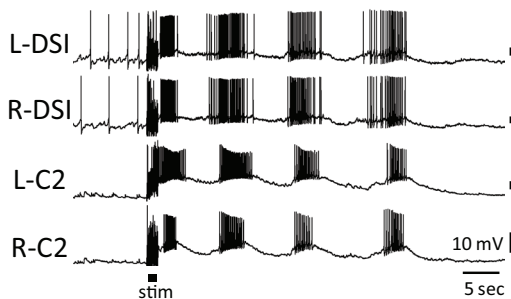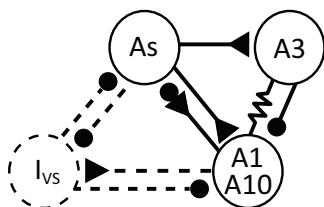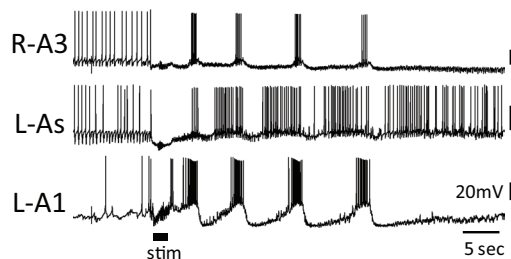
FIGURE 9.4 Neural circuits and swim motor patterns for the DV swimmers *Tritonia* and *Pleurobranchaea*. (*A*) The *Tritonia* swim CPG consists of three neuron types: DSI, C2, and VSI. (*B*) Simultaneous intracellular microelectrode recordings show that two contralateral DSIs fire bursts of action potentials in phase with each other and slightly ahead of the two C2s. VSI (not recorded here) fires action potentials in the interburst interval. The motor pattern is initiated by electrical stimulation of a body wall nerve (stim). (*C*) The *Pleurobranchaea* swim CPG contains five types of neurons (Jing and Gillette, 1999). The As neurons are homologues of the DSIs. A1 is homologous to C2. A10 is strongly electrically coupled to A1 and, for simplicity, is shown together with it. A3 is not found in *Tritonia*. The Ivs neuron has not been found, but has been postulated to exist based on recordings of inhibitory postsynaptic potentials in other neurons. (*D*) Simultaneous intracellular recordings from an A3, As, and A1. The As neuron leads the A1 neuron just as DSI leads C2. The swim motor pattern is initiated by electrical stimulation of a body wall nerve (stim). In *A* and *C*, the small filled circles represent inhibitory synapses, the triangles are excitatory synapses, and combinations are mixed inhibition and excitation. The resistor symbol represents electrical synapses.

interneuron 2 (Si2; Fig. 9.5*A*). Based on their anatomy and neurochemistry, these neurons are not homologous to any of the *Tritonia* or *Pleurobranchaea* swim CPG neurons.

In the *Melibe* swim CPG, each neuron reciprocally inhibits the two contralateral counterparts (Fig. 9.5*B*). There is also strong electrical coupling between the ipsilateral Si1 and Si2, causing them to fire in phase with each other and 180° out of phase with the contralateral pair (Fig. 9.5*C*). This bursting pattern drives the left–right alternations of the swimming behavior (Watson et al., 2002).

Homologues of the *Melibe* Si1 and Si2 were identified in *D. iris* based on anatomical, neurochemical, and electrophysiological features (Sakurai et al., 2011). However, there are important differences in the neural circuit formed by these neurons (Fig. 9.5*D*). Although the contralateral Si2 neurons reciprocally inhibit each other, Si1 does not inhibit or receive inhibition from either contralateral neuron. Instead, Si1 exhibits strong electrical coupling to its contralateral counterpart (Fig. 9.5*E*). During a swim motor pattern, the contralateral Si2 neurons fire bursts of action potentials in alternation, but the Si1 pair fire irregularly (Fig. 9.5*F*). Thus, whereas both Si1 and Si2 are members of the LR swim CPG in *Melibe*, only Si2 is in *Dendronotus*.

If LR swimming in *Melibe* and *Dendronotus* is homologous, as would be expected from scenarios 2, 3, or 4 (Fig. 9.3*B*–*D*), this would be an example in which the neural mechanisms diverged while the behavior stayed the same. However, it could be the case that the differences in neural mechanism reflect a different evolutionary origin for LR swimming in *Melibe* and *Dendronotus* as in scenario 1 (Fig. 9.3*A*).

FUNCTIONS OF DV SWIM CPG NEURONS IN OTHER SPECIES

DSI and C2 homologues can be recognized by using neuroanatomical and neurochemical criteria, allowing them to be identified in species that are not DV swimmers (Table 9.2). The DSIs are serotonergic (Katz et al., 1994; McClellan et al., 1994) and have a characteristic axon projection pattern (Getting et al., 1980). They have been identified in 10 different genera, including two opisthobranchs outside of the Nudipleura (Newcomb and Katz, 2007). Electrophysiological traits of the DSI homologues show little correlation with the type of behavior produced by the species (Newcomb and Katz, 2007). C2 has been identified based on peptide immunoreactivity and characteristic morphology in five genera within the Nudipleura (Lillvis et al., 2012). These DV swim CPG neurons are present regardless of the animal's mode of locomotion. This suggests that the swimming CPGs were built upon previously existing neural circuits, coopting existing neurons for new functions.

The DV swim CPG neurons are not members of the LR swim CPGs. The DSI and C2 homologs in *Melibe* are not rhythmically active in phase with the motor pattern (Fig. 9.6*A*), nor are the DSI homologues rhythmically active during the *Dendronotus* swim motor pattern (Fig. 9.6*B*). Thus,
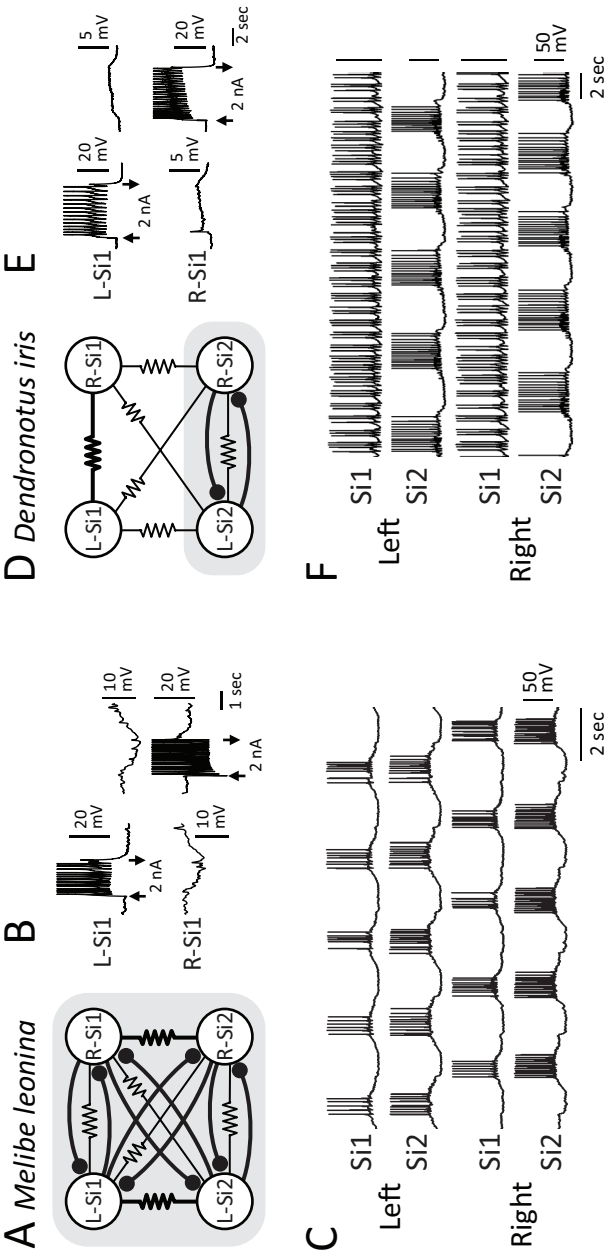
FIGURE 9.5 Neural circuitry and swim motor pattern for the LR swimmers *Melibe* and *Dendronotus*. (*A*) In the *Melibe* swim CPG (Thompson and Watson, 2005), there are two bilaterally represented neurons Si1 and Si2 that are mutually inhibitory across the midline and exhibit strong electrical coupling ipsilaterally (as indicated by thicker resistor symbol). (*B*) Depolarization of one Si1 by injecting 2 nA of current into it hyperpolarizes the contralateral counterpart. (*C*) The *Melibe* swim motor pattern consists of ipsilateral synchrony and alternation with the contralateral side. (*D*) In *Dendronotus*, the inhibitory connections to and from Si1 are absent, and the electrical coupling between the contralateral Si1 pair dominates (Sakurai et al., 2011). (*E*) Depolarization of an Si1 with 2-nA current injection depolarizes the contralateral counterpart. (*F*) In the *Dendronotus* swim motor pattern, the left and right Si2 fire alternating bursts of action potentials, but the Si1s fire irregularly. In *A* and *D*, the shaded boxes represent the functional CPGs.

TABLE 9.2 Homologous Neurons Identified in Different Species with Different Behaviors

	Nudipleura			Other Opisthobranchia
Neuron	DV swimmers	LR swimmers	Nonswimmers	
DSI	*Tritonia* (Getting, 1977)	*Melibe* (Newcomb and Katz, 2007)	*Armina* (Newcomb and Katz, 2007)	*Aplysia* (Mackey et al., 1989; Wright et al., 1995; Xin et al., 2001; Jing et al., 2008)
	Pleurobranchaea (Jing and Gillette, 1999)	*Dendronotus* (Newcomb and Katz, 2007)	*Triopha* (Newcomb and Katz, 2007)	*Clione* (Panchin et al., 1995; Satterlie and Norekian, 1995)
		Hermissenda (Tian et al., 2006)	*Tochina* (Newcomb and Katz, 2007)	
C2	*Tritonia* (Getting, 1977; Taghert and Willows, 1978)	*Melibe* (Lillvis et al., 2012)		
	Pleurobranchaea (Jing and Gillette, 1995)	*Hermissenda* (Lillvis et al., 2012)		
		Flabellina (Lillvis et al., 2012)		

categorically distinct behaviors are produced by CPGs containing non-overlapping sets of neurons.

It was shown that the DSI homologues in *Melibe* do have an effect on the production of the swim motor pattern; they can initiate a motor pattern in a quiescent preparation, and hyperpolarization can temporarily halt an ongoing motor pattern (Newcomb and Katz, 2009). In contrast to *Tritonia*, in which the DSIs are an integral part of the DV swim CPG, in *Melibe*, they act as extrinsic modulators. Thus, the functions of homologous neurons differ in species with different behaviors.

The DSIs are not dedicated to one function even within a species. In *Pleurobranchaea*, the DSI homologues synapse onto serotonergic neurons that increase ciliary beating and thereby increase the speed of crawling (Jing and Gillette, 2000). In *Tritonia*, DSI accelerates crawling through synapses onto the efferent peptidergic pedal neuron Pd5, which in turn increases cilia beat frequency (Popescu and Frost, 2002). DSI homologues in the nonswimming *Tochuina tetraquetra* and *Triopha catalinae* also mono-synaptically excite homologues of Pd5 and presumably increase the speed of crawling (Newcomb and Katz, 2007). In *Hermissenda*, which produces LR flexions, the DSI homologues do not increase ciliary beating, but
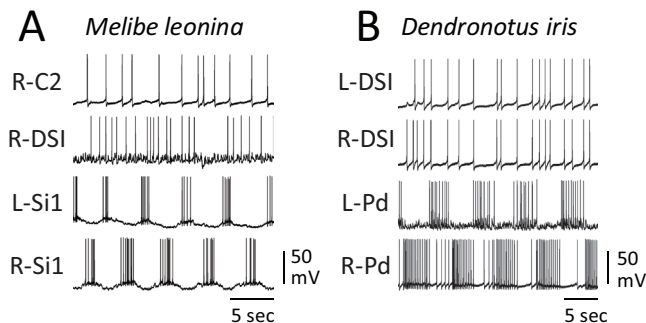
FIGURE 9.6 Homologues of the *Tritonia* DV swim CPG neurons are not rhythmically active during LR swim motor patterns. (*A*) In *Melibe*, the C2 and DSI homologues do not display any rhythmic bursting in phase with the swim motor pattern reflected in the alternating firing pattern of the left and right Si. (*B*) In *Dendronotus*, a contralateral pair of DSI homologues exhibit synchronous irregular spiking that shows no relation to the ongoing LR swim motor pattern displayed by two contralateral pedal motor neurons (L-Pd and R-Pd).

instead excite motor neurons that cause contraction of the anterior foot (Tian et al., 2006). In the more distantly related opisthobranch, *Aplysia californica*, DSI homologues also initiate muscular crawling (Jing et al., 2008). Whereas, in the pelagic opisthobranch, *C. limacina*, the DSI homologues increase the frequency of parapodial "wing" flapping and excite motor neurons that innervate the wings (Arshavsky et al., 1992; Satterlie and Norekian, 1995). Thus, the DSI homologues share common functions in controlling the foot and/or locomotion.

The C2 and DSI homologues have additional roles outside of locomotion. In *Pleurobranchaea*, the C2 homologue (A1) suppresses feeding through its connections to feeding-related interneurons (Jing and Gillette, 1995). In contrast, the DSI homologues (As) have the opposite effect by exciting a number of feeding interneurons (Jing and Gillette, 2000). This is a shared function with other opisthobranchs such as *A. californica*, in which the DSI homologues (CC9-10) help excite one of the same feeding interneurons as in *Pleurobranchaea*, the metacerebral cell (Jing et al., 2008). Thus, individual neurons are multifunctional. Some functions are shared across species, whereas other functions are particular to some species.

CONCLUSIONS

A phylogenetic analysis of the neural basis for swimming in the Nudipleura has revealed several interesting aspects about the evolution

of behavior. First, the basic building blocks of neural circuits, namely the neurons, are shared across diverse species. For example, DSI homologues are found across Opisthobranchia. Second, neurons, which are multifunctional within a species, appear to take on additional functions over the course of evolution. For instance, the DSI homologues are involved in several behaviors in various species, including generating DV swimming or enhancing other types of locomotion such as enhancing LR swimming or wing flapping. They also accelerate crawling and promote feeding. It is reasonable to expect that highly interconnected interneurons would not be dedicated to a single function, but would dynamically interact with many neurons involved in a variety of different behaviors.

This comparative analysis has also revealed that species with categorically similar behaviors such as the two DV swimmers, *Tritonia* and *Pleurobranchaea*, or the two LR swimmers, *Melibe* and *Dendronotus*, have overlapping sets of neurons in the swim CPG circuits. In contrast, the CPGs underlying categorically distinct behaviors consist of nonoverlapping sets of neurons. However, even in species that exhibit similar behaviors such as *Melibe* and *Dendronotus*, the CPG circuits can differ in neuronal and synaptic composition. Thus, although behavior itself is not a predictor of its underlying neural mechanism, it is a good first approximation.

We do not understand why the circuits in *Melibe* and *Dendronotus* differ. There could be functional reasons; perhaps Si1, which is not rhythmically active in *Dendronotus*, has an additional function that is incompatible with swimming in that species. There may also be phylogenetic reasons; perhaps *Melibe* and *Dendronotus* independently evolved swim CPGs and came up with different circuit organizations. Whatever the reason, the results show that analogous behaviors can be generated by circuits with different circuit architectures. Recent work in invertebrates has shown that there can be variability in neural circuits that is not reflected in the performance of the behavior even across individuals within a species (Goaillard et al., 2009; Roffman et al., 2011).

There is a great degree of behavioral homoplasy. Although scenario 4 (Fig. 9.3*D*) may be the most parsimonious explanation for the phylogenetic distribution of the swimming behaviors, it should be kept in mind that only approximately 2% to 3% of nudibranch species have been reported to swim. Therefore, there is probably even more behavioral homoplasy than any of the scenarios in Fig. 9.3 indicate. It is conceivable that swimming arose independently in each family where it is found, 16 times in all (Fig. 9.1 and Table 9.1).

Given that *Tritonia* and *Pleurobranchaea* are very distantly related within the Nudipleura clade, it is even more likely that they independently evolved DV swim CPGs. If so, the incorporation of DSI and C2 homologues into such a circuit represents parallel evolution, whereby

homologous structures independently came to have similar functions (Sanderson and Hufford, 1996; Hoekstra and Price, 2004; Scotland, 2011; Wake et al., 2011). This has been suggested for other systems as well. For example, homologous brain nuclei appear to be involved in vocal learning in lineages of birds that evolved song independently (Feenders et al., 2008; Hara et al., 2012). Similarly, interaural coincidence detection circuits arose independently in the brainstem nuclei of birds and mammals (Schnupp and Carr, 2009). Finally, the appearance of similar cortical areas are correlates with the independent evolution of precision hand control in primates (Padberg et al., 2007), suggesting that constraints in cortical organization led to the evolution of similar neural mechanisms underlying dexterity (Krubitzer, 2009).

If homologous neurons are repeatedly incorporated into neural circuits for analogous behaviors, it suggests that these neurons may be part of a more readily achievable state for swimming. Thus, the nervous system may affect the evolvability of behavior because some configurations of existing neurons could be more robust than others. The concept of evolvability first arose from genetics (Kirschner and Gerhart, 1998; Masel and Trotter, 2010), but has since been applied to nervous systems (Airey et al., 2000; Bendesky and Bargmann, 2011; Katz, 2011; Yamamoto and Vernier, 2011). Exploring the aspects of neural organization that lead to repeated evolution of particular behaviors will point to the factors that are most important for behavioral output.

ACKNOWLEDGMENTS

10

Shared Developmental and Evolutionary Origins for Neural Basis of Vocal–Acoustic and Pectoral–Gestural Signaling

ANDREW H. BASS*‡ AND BORIS P. CHAGNAUD†

Acoustic signaling behaviors are widespread among bony vertebrates, which include the majority of living fishes and tetrapods. Developmental studies in sound-producing fishes and tetrapods indicate that central pattern-generating networks dedicated to vocalization originate from the same caudal hindbrain rhombomere (rh) 8-spinal compartment. Together, the evidence suggests that vocalization and its morphophysiological basis, including mechanisms of vocal–respiratory coupling that are widespread among tetrapods, are ancestral characters for bony vertebrates. Premotor-motor circuitry for pectoral appendages that function in locomotion and acoustic signaling develops in the same rh8-spinal compartment. Hence, vocal and pectoral phenotypes in fishes share both developmental origins and roles in acoustic communication. These findings lead to the proposal that the coupling of more highly derived vocal and pectoral mechanisms among tetrapods, including those adapted for nonvocal acoustic and gestural signaling, originated in fishes. Comparative studies further show that rh8 premotor populations have distinct neurophysiological properties coding for equally distinct behavioral attributes such as call duration. We conclude that neural network innovations in the spatiotemporal patterning of vocal and pectoral mechanisms of social communication, including forelimb gestural signaling, have their evolutionary origins in the caudal hindbrain of fishes.

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E arly hindbrain development in all major vertebrate lineages exhibits a shared anatomical blueprint of cranial motor nuclei and nerves originating in one or more serially arranged segments or rhombomeres [rhs; e.g., Lumsden and Keynes (1989), Gilland and Baker (2005)]. Here, we consider the development and evolution of hindbrain circuitry leading to novel innovations in social signaling, integrating information across behavioral, neurophysiological, and morphological levels of analysis. Two neural networks are the focus: the sonic–vocal basis of acoustic signaling (Fig. 10.1*A*) and pectoral control of anterior appendages, fins, and forelimbs (Fig. 10.1*B*). For context, we first briefly review vertebrate phylogeny and the ancestral "blueprint" for hindbrain motor phenotypes.

VERTEBRATE PHYLOGENY

Living craniates include jawless vertebrates or agnathans and jawed vertebrates or gnathostomes [Fig. 10.1*B*; reviewed in Nelson (2006)]. Fossil evidence indicates several lineages of extinct agnathans [e.g., Osteostracans; Fig. 10.1*B*; e.g., Forey and Janvier (1993)]. Chondrichthyes (i.e., cartilaginous fishes) are the most basal group of jawed vertebrates and include two subclasses, Elasmobranchii (i.e., sharks, skates, and rays) and Holocephali or chimaeras. Bony vertebrates, the sister group to Chondrichthyes, include Actinopterygii or rayfinned fishes and the Sarcopterygii or lobe-finned fishes. Sarcopterygians include the coelacanth (*Latimeria*), lungfish (Dipnoi), and tetrapods.

Here, we mainly review recent evidence showing that a caudal hindbrain (rh8)-spinal cord compartment is the developmental origin of premotor-motor circuitry for sonic–vocal and pectoral behavioral phenotypes. Actinopterygians, which include nearly half of living vertebrate species, were the focus of these studies. By integrating these new findings into a single framework, we aim to achieve a more complete understanding of the evolutionary origins of vocal and pectoral motor systems among vertebrates in general, including the more highly derived pectoral systems of tetrapods that serve a range of functions including nonvocal sonic and forelimb gestural signaling.

HINDBRAIN SEGMENTAL BLUEPRINT

Vertebrates have two functional series of hindbrain motor nuclei, somatic and branchiomeric (Lumsden and Keynes, 1989; Gilland and Baker, 2005), that were likely present in the earliest, pregnathostome vertebrates (Northcutt, 1985). Somatic nuclei innervate head muscle derived from unsegmented (i.e., prechordal plate) and segmented paraxial mesoderm (i.e., occipital somites); branchiomeric nuclei target derivatives of
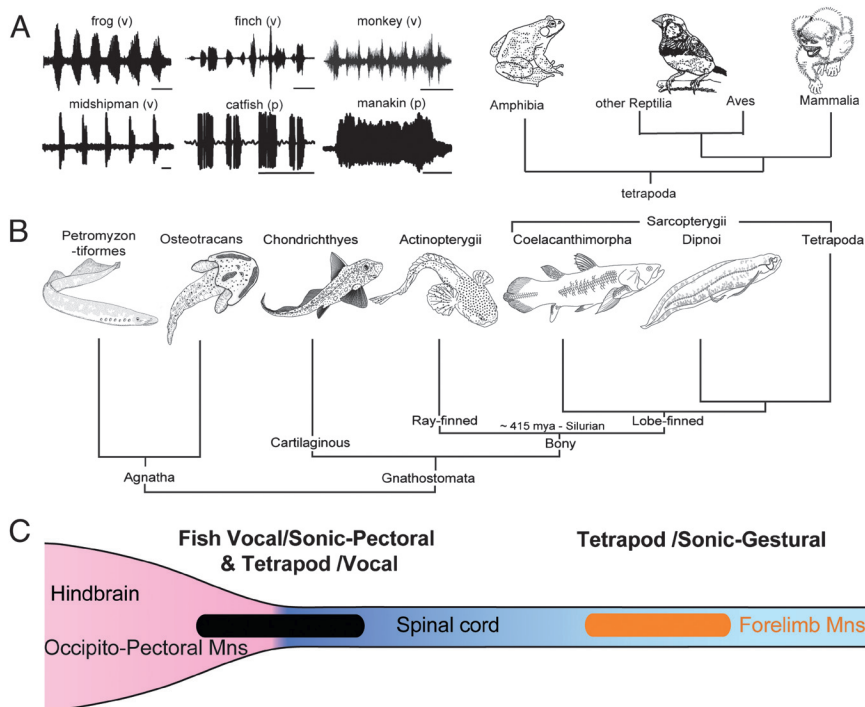
FIGURE 10.1 Evolution of vocal–pectoral motor systems in fishes and tetrapods. (*A*) Waveforms of representative social vocalizations of bullfrog (time base 1 s), zebra finch (250 ms), squirrel monkey (200 ms), midshipman fish (500 ms), catfish (250 ms), and club-winged manakin (100 ms). Vocal (v) and nonvocal pectoral (p) basis is indicated. (*B*) Cladogram of vertebrates, including jawless (agnatha) and jawed (gnathostome) radiations (Osteostracans represent an extinct agnathan group with pectoral fins). (*C*) Summary of location of vocal and sonic motor neurons. Among fishes, the occipitospinal motor column (black) gives rise to motor neurons innervating muscles of vocal organs dedicated to sonic functions (e.g., swim bladder) and pectoral fins that can also serve a sonic function. This same column gives rise to vocal motor neurons in tetrapods. Among tetrapods, forelimb motor neurons (orange) that function in both sonic and gestural signaling are located in the spinal cord. [*A* adapted from Bass et al. (2008); *B* and *C* adapted from Ma et al. (2010).] [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

paraxial mesoderm that migrate into the pharyngeal arches (Nieuwenhuys et al., 1998; Gilland and Baker, 2005; Noden and Francis-West, 2006). Comparative studies delineate a conserved pattern of hindbrain somatic and branchiomeric motor nuclei spatially segregated along the rostral–

caudal axis across eight rhs (Murakami et al., 2004; Gilland and Baker, 2005). Most nuclei originate in one or two rhs with little variation in extent or location across taxa (Gilland and Baker, 2005). Of particular interest for this review is rh8, which has two to three times the longitudinal extent of more anterior segments and can be subdivided into at least two to three subdivisions in teleost fishes and birds (Hanneman et al., 1988; Cambronero and Puelles, 2000; Bass et al., 2008). Additional evidence for hindbrain segmentation, including a distinct rh8-spinal boundary, comes from rh-specific patterns of gene expression [e.g., Prince et al. (1998), Tümpel et al. (2009), Ma et al. (2010)].

EVOLUTIONARY DEVELOPMENTAL "HOTSPOTS" FOR NOVEL PATTERN GENERATORS

Caudal hindbrain rhs are a developmental and evolutionary "hotspot" [*sensu* Myers et al. (2000)] for innovations in neural networks controlling complex motor function. Bass and Baker (1997) hypothesized that the appearance of novel respiratory and cardiovascular pumps during the protochordate–vertebrate transition (Gans and Northcutt, 1983; Northcutt and Gans, 1983) depended upon the evolution of equally novel, genetically specified pattern-generating circuits developing in rhs 7 and 8. Rhombomeres 7 and 8 were also proposed as the source of more recently derived premotor-motor networks unique to jawed vertebrates, such as those controlling sound production, that have social signaling functions (Bass and Baker, 1997). The development of precerebellar climbing fibers from a distinct rh8 nucleus, the inferior olive (Cambronero and Puelles, 2000), underscored a preeminent role for caudal hindbrain nuclei in the spatiotemporal patterning of complex motor behaviors such as vocalization and eye movement.

SONIC–VOCAL PATTERN GENERATOR

Sonic motor systems in fishes provide excellent models for directly linking neural mechanisms to behavioral outcomes, in part, because the physical attributes of acoustic signals (e.g., interpulse and intercall intervals, duration, amplitude), like their underlying neural activity, are easily quantified (Bradbury and Vehrencamp, 2011). Sonic mechanisms vary within and between fish lineages (Fine and Ladich, 2003; Ladich and Fine, 2006; Bass and Ladich, 2008). Although most species studied so far generate acoustic signals by vibrating the swim bladder, a second well-known set of mechanisms depends on pectoral appendage vibration (Kratochvil, 1978; Bass and Baker, 1991; Fine et al., 1997). Neuronal patterning of sound production has been most extensively investigated in species using a sonic

swim bladder; hence, we first discuss these species. We will then turn our attention to pectoral-dependent mechanisms in the broader context of the motor control of pectoral appendages.

Swim bladder vibration is driven by the contraction of a single pair of muscles attached directly or indirectly to the swim bladder. This biomechanical simplicity has provided a unique opportunity to show how acoustic characters are directly determined by the intrinsic and network properties of a hindbrain central pattern generator controlling one pair of muscles. Toadfishes, a single order and family (Batrachoidiformes, Batrachoididae) of teleost fishes commonly known as toadfish and midshipman fish, have been widely studied as neurobehavioral models for acoustic communication (Bass and McKibben, 2003; Bass and Remage-Healey, 2008; Greenfield et al., 2008). Among toadfishes, sonic muscles directly attached to the swim bladder are innervated by paired occipital nerve roots exiting the caudal hindbrain (Ladich and Fine, 2006; Bass and Ladich, 2008). The temporal properties of occipital nerve motor volleys directly set pulse repetition rate (equivalent to fundamental frequency of harmonic calls for fish), duration, and complex patterns of frequency and amplitude modulation of entire calls (Bass and Baker, 1990; Remage-Healey and Bass, 2004, 2006; Rubow and Bass, 2009). Individual sound pulses are matched 1:1 with each spike-like, occipital nerve potential (Fig. 10.2*A* and *B*) that results from the synchronous activity of an expansive vocal motor nucleus (VMN) extending from the caudal hindbrain into the rostral spinal cord (Fig. 10.2*C* and *D*) (Bass and Baker, 1990). Paired midline VMNs fire in synchrony (Bass and Baker, 1990), with bilaterally synchronous occipital spikes leading to simultaneous contraction of both vocal muscles and one sound pulse (Cohen and Winn, 1967).

A descending vocal motor pathway in toadfishes extends from forebrain preoptic-anterior hypothalamic to midbrain and caudal hindbrain levels (Bass et al., 1994; Goodson and Bass, 2002; Kittelberger et al., 2006). Premotor vocal pacemaker neurons (VPNs) densely innervate VMNs and receive input from a more rostral, anatomically separate prepacemaker [vocal prepacemaker (VPP)] nucleus (Fig. 10.2*C* and *D*) (Bass and Baker, 1990; Bass et al., 1994; Chagnaud et al., 2011). In an in vivo preparation, surgical isolation of the hindbrain-spinal region including the VPP–VPN–VMN network shows this region alone can produce a patterned output matching call temporal properties (Remage-Healey and Bass, 2004, 2006).

Investigations of a toadfish known as midshipman, using in vivo intracellular recording and staining, show how the VPP–VPN–VMN network determines natural vocal attributes. Chagnaud et al. (2011, 2012) demonstrate that precise temporal patterning of natural vocalization (Fig. 10.2*A* and *B*) depends on extreme networkwide synchrony and distinct intrinsic properties for each vocal nucleus. Sustained depolarizations in VPP, sub-
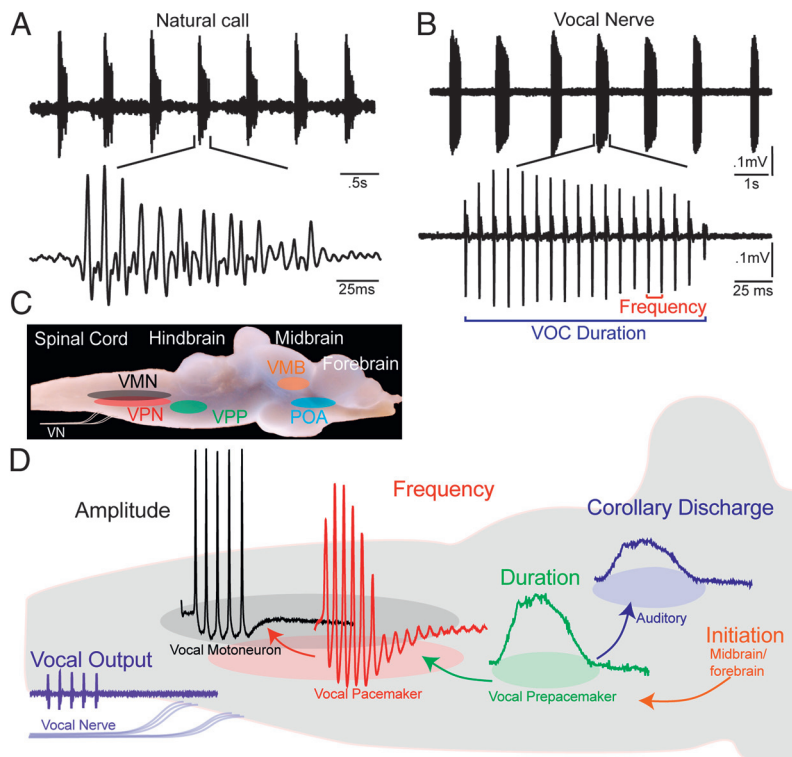
FIGURE 10.2 Vocal behavior and neural network of plainfin midshipman fish. (*A*) Oscillogram record of repetitive series of natural calls ("grunt train") recorded with hydrophone; lower trace shows one call. (*B*) Spontaneous vocal motor volley recorded from vocal occipital nerve (VOC) with temporal properties like those of natural vocalization; lower trace shows one VOC. VOC duration is time between first and last pulses; frequency is pulse repetition rate. (*C*) Vocal motor nuclei superimposed on lateral view of intact brain. Indicated are VPN, VPP, and VMN nuclei and vocal nerve (VN). Vocal midbrain (VMB) and forebrain preoptic area (POA) are vocally active sites. (*D*) Premotor compartmentaliza-tion of neurons code for distinct acoustic attributes. Representative intracellular records from vocal nuclei and vocal nerve superimposed on background sagittal image of caudal hindbrain. Descending input from vocal midbrain/forebrain neurons activates vocal hindbrain. Vocal prepacemaker nucleus is source of known corollary discharge informing auditory nuclei about a vocalization's temporal properties. [Adapted from Chagnaud et al. (2011).] [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

threshold membrane oscillations in VPN, and a combination of differential recruitment and low excitability in VMN directly code natural call duration, frequency, and amplitude, respectively (Chagnaud et al., 2011, 2012) (Fig. 10.2*D*). In addition to coding duration, prepacemaker neurons are the source of input to a rostral hindbrain nucleus directly innervating the inner ear and lateral line organs that is the anatomical basis for a vocal corollary discharge (Fig. 10.2*D*) (Weeg et al., 2005). These new results for fishes, together with nerve recordings and more limited single-neuron recordings in tetrapods, led to the proposal that anatomically separate hindbrain populations code distinct call attributes in fishes and tetrapods (Chagnaud et al., 2011).

Among tetrapods, the coupling of sound production and respiration leads to airflow-dependent vibration of sonic laryngeal and syringeal membranes [e.g., Gans (1973), Gans and Maderson (1973), Bradbury and Vehrencamp (2011)]. Despite the close connection between vocal and respiratory pattern generators in tetrapods, evidence for a vocal–respiratory pattern generator in more basal vertebrates such as fishes has been missing. Video and sound analysis of advertisement calling ("humming") by midshipman fish (Brantley and Bass, 1994) reveals a strong rhythmic correlation between vocal, respiratory, and postural (i.e., pectoral) systems. Vocal–respiratory coupling in this case likely reflects the increased oxygen demands of repetitive muscle contractions during the unusually long duration (from minutes to 1 h) hum vocalizations. Pectoral fin motion may stabilize the body during prolonged calling and/or aid in the increased movement of oxygenated water across the gills during humming [e.g., Peterson (1975)].

We propose that vocal–respiratory coupling originated in fishes and was subsequently adopted by tetrapods. Neurophysiological support for this hypothesis comes from studies of the vocal pattern generator in fully aquatic frogs that produce sound independent of airflow and yet exhibit vocal–respiratory coupling in the caudal hindbrain (Zornik and Kelley, 2007). In birds and mammals, nuclei integrating vocal (i.e., laryngeal and syringeal) and respiratory activity are also positioned in the caudal hindbrain, adjacent to vocal motor neurons (Holstege, 1989; Zhang et al., 1995; Wild et al., 2009; Schmidt et al., 2012). Although a vocal–respiratory integration site has yet to be identified in fishes, it will likely be in the caudal hindbrain as in tetrapods.

EVOLUTIONARY DEVELOPMENT OF SONIC–VOCAL PATTERN GENERATOR

We originally adopted the term "vocal" to describe occipital-innervated sonic systems in fishes, like the toadfishes discussed earlier, because of multiple characters they share with tetrapods (Bass et al., 1994): (*i*) pro-

duction of social context-dependent signals, for example, agonistic vs. advertisement; (*ii*) dedication of muscles, like those of the tetrapod syrinx and larynx, to sound production; (*iii*) shared origins of sonic muscles in fishes and tetrapods from occipital somites; (*iv*) likely homology of occipital nerve roots innervating fish sonic muscles and hypoglossal nerve roots innervating avian syringeal muscles; and (*v*) the same location in caudal hindbrain of fish sonic motor nucleus and avian tracheosyringeal division of hypoglossal motor nucleus innervating syringeal muscles (Nottebohm et al., 1976).

Developmental studies in fishes now support the hypothesis that hindbrain pattern generators for vocalization in the two main clades of bony vertebrates, Actinopterygii and Sarcopterygii (Fig. 10.1*B*), share developmental and evolutionary origins. Fluorescent dextran-amine injections into the developing vocal muscle of newly hatched midshipman and toadfish larvae showed a cigar-shaped VMN extending from caudal rh8 into the rostral spinal cord, a longitudinal extent more than twice that of the more anterior rhs 2 to 6 (Fig. 10.3*A*). Experimental mapping of VMN relative to highly conserved neuronal landmarks in vertebrates showed, for example, rostral VMN coincident with the caudal pole of the vagal motor column and the caudal pole of the precerebellar inferior olive, both of which originate from rh8 in tetrapods (Cambronero and Puelles, 2000). Transneuronal neurobiotin labeling in larvae also showed vocal premotor neurons positioned in caudal rh8 immediately lateral and rostral to VMN (Fig. 10.3*B* and *C*), matching the locations of VPN and VPP, respectively, in adults (e.g., Fig. 10.3*D*) (Bass et al., 2008).

Taxonomic analysis, based on developing and adult hindbrain organization, next showed vocal premotor-motor circuitry (including sites of vocal–respiratory coupling, as detailed earlier) in amphibians, birds, and mammals mapping to the same rh8-spinal compartment as the developing VPP–VPN–VMN network of fish [reviewed in Bass et al. (2008); also see Nottebohm et al. (1976), Holstege (1989), Zhang et al. (1995), Straka et al. (2006), Jürgens and Hage (2007), Zornik and Kelley (2007), Wild et al. (2009), Schmidt et al. (2012); Fig. 10.3*E*). Together, the evidence led to the proposal that an rh8-spinal compartment is the developmental and evolutionary origin of hindbrain vocal pattern-generating circuitry among all the major lineages of vocal vertebrates.

SHARED ORIGINS OF SONIC–VOCAL MUSCULATURE AND CENTRAL MECHANISMS

Peripheral sonic mechanisms vary between and even within fish lineages (Fine and Ladich, 2003; Ladich and Fine, 2006; Bass and Ladich, 2008; Parmentier et al., 2011). For example, sculpin (Scorpaeniformes,
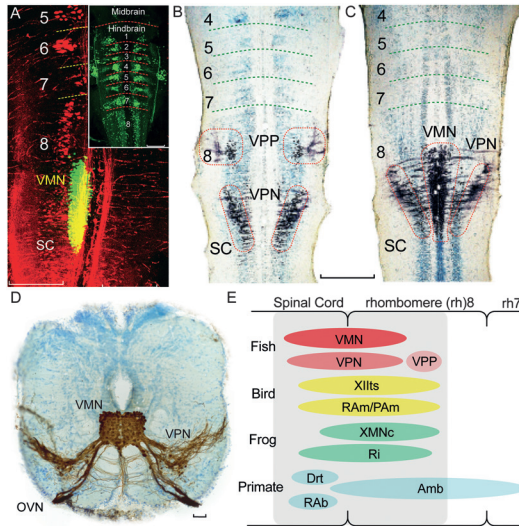
FIGURE 10.3 Map of developing vocal pattern generator in rh8-spinal compartment. (*A*) Fluorescently labeled neurons in plainfin midshipman fish larvae visualized with laser scanning confocal microscopy (horizontal plane). Simultaneous visualization of reticulospinal neurons labeled via retrograde transport from the spinal cord (Alexa 546 dextran-amine, red) and VMN (Alexa 488 dextran-amine, green) labeled via the developing vocal muscle. Yellow is composite overlap and does not indicate double labeling. *Inset*: Clusters of reticulospinal neurons (Alexa biocytin 488, green) in each rh, from 1 to 8. (Scale bars: 0.2 mm.) (*B* and *C*) Mapping in horizontal plane of VPP, VPN, and VMN neurons (black) in Gulf toadfish larvae; labeling via transneuronal transport of neurobiotin from developing vocal muscle. Cresyl violet counterstain reveals segmental, reticulospinal clusters. (Scale bar: 0.2 mm.) (*D*) Transverse section in caudal hindbrain of toadfish showing transneuronal neurobiotin labeling (brown) of paired midline VMN and adjacent VPNs; VMNs and VPNs have extensive dendritic and axonal branching. VMN axons exit via occipital vocal nerve root (OVN; cresyl violet counterstain). (Scale bar: 100 μm.) (*E*) Sagittal view summarizing relative positions of hindbrain vocal premotor-motor networks in rh8-spinal compartment of fish, birds, frogs, and mammals including primates, based on early-stage and adult phenotypes [see Bass et al. (2008) for details]. Most laryngeal motor neurons that shape the temporal envelope of mammalian calls originate from caudal nucleus ambiguus (Amb). Drt, dorsal reticular nucleus; PAm, nucleus parambigualis; RAb, nucleus retroambiguus; RAm, nucleus retroambigualis; Ri, inferior reticular formation; XIIts, tracheosyringeal division of hypoglossal motor nucleus; XMNc, caudal XMN. [Adapted from Bass et al. (2008).] [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

Cottidae) lack a swim bladder and instead vibrate a single pair of muscles attached to the pectoral girdle (Bass and Baker, 1991). Closely related sea robins (Scorpaeniformes, Triglidae), like distantly related midshipman and other toadfishes, have a pair of vocal muscles that are completely attached to the swim bladder (Bass and Baker, 1991). Important to the current discussion is that sound-generating muscles in sculpin and sea robins, like toadfishes and other families of sonic fishes, are innervated by occipital nerve roots (Bass and Baker, 1991). This suggests that vocal muscles among fishes share developmental origins from occipital somites (Tracy, 1959, 1961), irrespective of skeletal mechanics and degree of taxonomic relatedness.

Occipital innervation of vocal muscles originating from a VMN at the same hindbrain (rh8)-spinal level is now documented for nine families of closely and distantly related teleost taxa (Bass and Baker, 1991; Ladich and Bass, 2005; Onuki and Somiya, 2007; Bass and Ladich, 2008). Piranhas (Characiformes) are an exception to the pattern, with spinal-only innervation and a spinal-positioned VMN [Ladich and Bass (2005), Onuki and Somiya (2007); Onuki and Somiya (2007) describe other likely examples]. However, brain stimulation indicates vocal premotor, pattern-generating circuitry in piranhas in the same caudal hindbrain region as the VPP–VPN circuit in toadfishes (Kastberger, 1981).

Like the sonic organs of fishes, the nonavian larynx and avian syrinx have lineage-specific skeletal characters [e.g., Bradbury and Vehrencamp (2011)], but share vocal muscle origins from occipital somites [see Huang et al. (1999) and Noden and Francis-West (2006) for tetrapods]. Laryngeal and syringeal premotor-motor networks are also located in the same rh8-spinal compartment as the vocal network in fishes (Fig. 10.3*E*). Hence, vocal premotor-motor circuitry, like vocal muscles, shares developmental and evolutionary origins among vertebrates. Studies of frogs further show that laryngeal nerve output resembles occipital nerve activity in fishes. Like the occipital motor volley in vocal fish, the laryngeal motor volley of frogs matches the temporal properties of natural calls (Schmidt, 1992; Yamaguchi and Kelley, 2000). These findings, together with those discussed earlier for piranhas, direct our attention to the conserved nature of vocal premotor mechanisms, regardless of motor neuron targets [also see Zornik et al. (2010)].

Among fishes, acoustic communication is widespread and best known for the highly speciose teleosts [e.g., Ladich et al. (2006), Malavasi et al. (2008), Lobel et al. (2010), Parmentier et al. (2011)]. There is also well-documented behavioral evidence for acoustic signaling in more basal actinopterygians [see Nelson (2006) for phylogeny] including sturgeon (Acipenseriformes) (Johnston and Phillips, 2003), bichir (Polypteriformes) (Ladich and Tadler, 1988), and bowfin (Amiiformes) (Fülleborn, 1894).

Among basal sarcopterygians, sound production ("grunting") is noted for lungfish (Dipnoi) (Günther, 1870; Thomson, 1968). A critical test of the hypothesis that occipital somite-derived vocal muscle and rh8/occipital-spinal-derived vocal networks are ancestral characters for both major clades of bony vertebrates awaits the demonstration of these characters in one of the more basal (i.e., nonteleost) actinopterygians and a basal sarcopterygian (i.e., nontetrapod). If evidence from one of these more basal groups does not support the hypothesis, we would conclude that the observed vocal characters have independently evolved among actinopterygian and sarcopterygian lineages.

EVOLUTIONARY DEVELOPMENT OF PECTORAL APPENDAGE CIRCUITRY

Developmental mapping of the VMN in toadfishes relative to other hindbrain landmarks showed VMN coextensive with the rostral pole of a pectoral motor nucleus (Cambronero and Puelles, 2000). This finding led to the suggestion that the rh8-spinal compartment may be the source of neuronal innovations in the central patterning of nonvocal, pectoral-dependent function such as forelimb movement (Cambronero and Puelles, 2000). Since that time, a pectoral motor nucleus in basal and derived groups of bony vertebrates has been shown conclusively to develop in the same rh8-spinal compartment as the vocal system. By using multiple neuronal markers and alignment of the neuroepithelium with myotomes during the pectoral fin bud stage, Ma et al. (2010) precisely mapped the entire extent of the pectoral column along with the cranial-vertebral and rh8-spinal boundaries in representative species from three orders of teleosts used extensively as neurobehavioral models: midshipman (Batrachoidiformes), salmon (Salmoniformes), and zebrafish (Cypriniformes). The results for teleosts were compared with those for paddlefish (Acipenseriformes), a more basal order of actinopterygians (Fig. 10.4*A*–*C*).

Pectoral muscles were innervated by paired occipital (Oc1, Oc2) and anterior spinal (Sp1, Sp2) nerves (Fig. 10.4*C*, *Left*). Pectoral motor neurons, identified following retrograde transport of fluorescent dye from fin buds, were concurrently mapped with other neuronal landmarks including (*i*) foramina where occipital nerve roots exit the embryonic skull, (*ii*) genetic markers (hoxb4 expression) for rh8-spinal boundary (Ma et al., 2009), (*iii*) nonpectoral motor nuclei including an islet1-GFP line labeling cranial motor nuclei (Higashijima et al., 2000), and (*iv*) cerebellar input from the rh8-derived inferior olive nucleus (Cambronero and Puelles, 2000). Pectoral motor neurons extended between myotomes 2–3 and 5–6, with axons exiting via paired occipital roots (Oc1, Oc2) through a single foramen rostral to the cranial-vertebral boundary; axons also exited via the
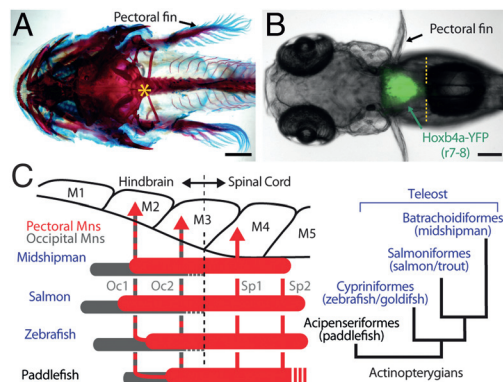
FIGURE 10.4 Map of developing pectoral motor nucleus in rh8-spinal compartment of basal and derived groups of actinopterygian fish (*A* and *B*, dorsal views). (*A*) Craniovertebral junction (asterisk) in postlarval, juvenile midshipman fish cleared and stained with alcian blue and alizarin red. (*B*) Demarcation of rh8-spinal boundary (yellow hatching) in zebrafish hoxb4a enhancer trap line. (*C*) Alignment of myotomes ("M"), occipital (Oc1, Oc2), and spinal (Sp1, Sp2) nerves and pectoral (red) and occipital (gray) motor neurons. Phylogeny of study species is also shown (*Right*). [Adapted from Ma et al. (2010).] [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

first one or two spinal roots (Sp1, Sp2) (Ma et al., 2010) (Oc1 and Oc2 also innervate vocal muscles; Fig. 10.4C, *Left*). Given the genetic mapping of cranial-vertebral (Fig. 10.4A) (Morin-Kensicki et al., 2002) and hindbrain-spinal (Ma et al., 2009) (Fig. 10.4B) boundaries between myotomes 3 and 4, the results demonstrated an rh8/occipital-spinal column innervating pectoral muscles in basal and derived actinopterygian species.

To extend the conclusions more broadly, the actinopterygian innervation pattern was compared with that of more basal cartilaginous fishes (Chondrichthyes, Chimaeriformes/ratfish), and representative fish species in the other major clade of bony vertebrates, Sarcopterygii (Dipnoi, lungfish), that includes tetrapods (Fig. 10.1B). Together with published accounts for a more basal sarcopterygian, the coelacanth (*Latimeria*) (Northcutt and Bemis, 1993; Millot and Anthony, 1965) (Fig. 10.1B), the results showed that occipital and spinal nerve innervation of pectoral muscles was a consistent character across all the investigated lineages of vertebrates.

In sum, precise mapping in pre- and postlarval stages of development showed that the ancestral pattern for pectoral appendage innervation

in bony vertebrates is from the rh8/occipital-spinal compartment (Fig. 10.1*C*). Pectoral fins, considered more ancient than pelvic fins (Coates, 2003), were previously assumed to receive innervation only from the spinal cord, like the pectoral forelimbs of tetrapods [reviewed in Ma et al. (2010)]. However, the new results in fishes indicate that spinal-only pectoral innervation is a shared derived character (i.e., synapomorphy), along with decoupling of pectoral appendages from the skull and evolution of a neck (Daeschler et al., 2006), only for tetrapod forelimbs (Fig. 10.1*C*). Despite the change in motor neuron location, premotor pectoral circuitry may be present in the caudal hindbrain of tetrapods, as it is in fishes (Auerbach and Bennett, 1969; Koyama et al., 2011). Although direct evidence is lacking, brain stimulation suggests that caudal hindbrain circuits configure pectoral/forelimb motor neuron activity in mammals [e.g., Drew et al. (1986)]; single neuron recordings like those in fishes (Auerbach and Bennett, 1969; Koyama et al., 2011) are needed to more rigorously test this hypothesis.

SHARED ORIGINS OF VOCAL AND PECTORAL CIRCUITRY

Additional evidence from the studies reviewed here of pectoral motor development suggests that each functional segment of a myotome (e.g., vocal or pectoral) has an rh8/occipitospinal complement. Fluorescent dye labeling of occipital myotomes in midshipman, salmon, and zebrafish embryos showed an occipital motor column, inclusive of pectoral motor neurons, extending approximately one myotome anterior to the rostral pole of the pectoral column with axons exiting via Oc1 and Oc2 (Fig. 10.4*C*, *Left*). As vocal muscle develops from myotome 2 (Tracy, 1959, 1961), this more complete labeling of the developing occipital motor column would also include the vocal motor complement. Vocal motor neurons likely come from a vocal "segment" of the occipitospinal column, separate from a pectoral segment innervating pectoral muscle that is also derived, in part, from myotome 2 (Tracy, 1959, 1961). Together, the results indicate that vocal and pectoral motor systems in fishes share developmental origins from the rh8/occipitospinal compartment. As discussed later, this would also include pectoral-dependent mechanisms of acoustic signaling.

Sonic mechanisms engaging the pectoral skeleton range from tendon snapping in croaking gouramis (Kratochvil, 1978) to pectoral spine vibration in catfish (Fig. 10.1*A*) (Fine et al., 1997) and pectoral girdle vibration in sculpin that lack a swim bladder (Bass and Baker, 1991). Despite these divergent mechanisms, sonic motor neurons are positioned in the same hindbrain-spinal region of the pectoral motor column in sculpin (Bass and Baker, 1991; Ladich and Bass, 2005), catfish (Ladich and Fine, 1994; Ladich and Bass, 1998), and gouramis (Ladich and Fine, 1992). Sonic neurons

map to the same location in sea robins, close relatives of sculpin that have sonic muscles completely attached to the swim bladder as in distantly related toadfishes (Bass and Baker, 1991). Some species of catfish exhibit both swim bladder and pectoral-dependent sonic phenotypes (Ladich and Fine, 1994). These results highlight the developmental and evolutionary coupling of pectoral motor systems that are multifunctional (locomotion and sound production) with vocal systems that are dedicated to acoustic signaling (e.g., in toadfish and sea robins; Fig. 10.1C), a character that is observed among tetrapods as well (as detailed later).

SHARED INTRINSIC AND NETWORK PROPERTIES FOR rh8

Precise temporal patterning of motor output and hence behavior, like that exemplified by vocalization, requires a suite of intrinsic and network properties to synchronize population-level activity [e.g., Van Vreeswijk et al. (1994), Perez Velazquez and Carlen (2000), Uhlhaas and Singer (2010)] including (*i*) repolarization conductances underlying oscillatory activity of premotor neurons; (*ii*) electrotonic coupling, within and between premotor and motor populations; (*iii*) widespread premotor excitatory input to target neurons; (*iv*) rhythmic firing of premotor and target population; (*v*) synchronous premotor firing; and (*vi*) inhibitory input to premotor neurons. Rhombomere 8/occipital premotor populations showing combinations of these characters include the VPP–VPN network (Chagnaud et al., 2011, 2012), pacemaker neurons in electromotor systems (Bennett, 1971), area I neurons of the occulomotor system (Pastor et al., 1994), T-reticular neurons (Kimmel et al., 1985; Koyama et al., 2011) driving pectoral motor neurons during the escape response (Auerbach and Bennett, 1969), and the inferior olive [e.g., Urbano et al. (2006); Llinás and Yarom (1981)].

Each rh8 premotor population has a distinct electroresponsive "signature" coding for an equally distinct behavioral attribute. Pacemaker (i.e., VPN) membrane oscillations directly code vocal frequency, whereas VPP-sustained depolarizations code vocal duration (Fig. 10.5A and B) (Chagnaud et al., 2011). Oscillatory pacemaker neurons in weakly electric fish, a VPN analogue, directly set electric organ discharge frequency (a VPP analogue is likely missing given the constant electric organ discharge) (Bennett, 1971). Area I in fish codes for eye position (Fig. 10.5C) (Pastor et al., 1994; Aksay et al., 2000, 2003). Like the vocal system, inhibitory coupling and synchronous firing, in this case shown by paired recordings, shape area I firing patterns (Aksay et al., 2001, 2003). Like VPN, rh8-derived inferior olive neurons (Cambronero and Puelles, 2000) tend to fire synchronously in an oscillatory fashion (Fig. 10.5D) (Llinás et al., 1974; Llinás and Yarom, 1986). In addition to voltage-dependent conductances underlying this rhythmicity, inferior olive neurons show strong gap junc-
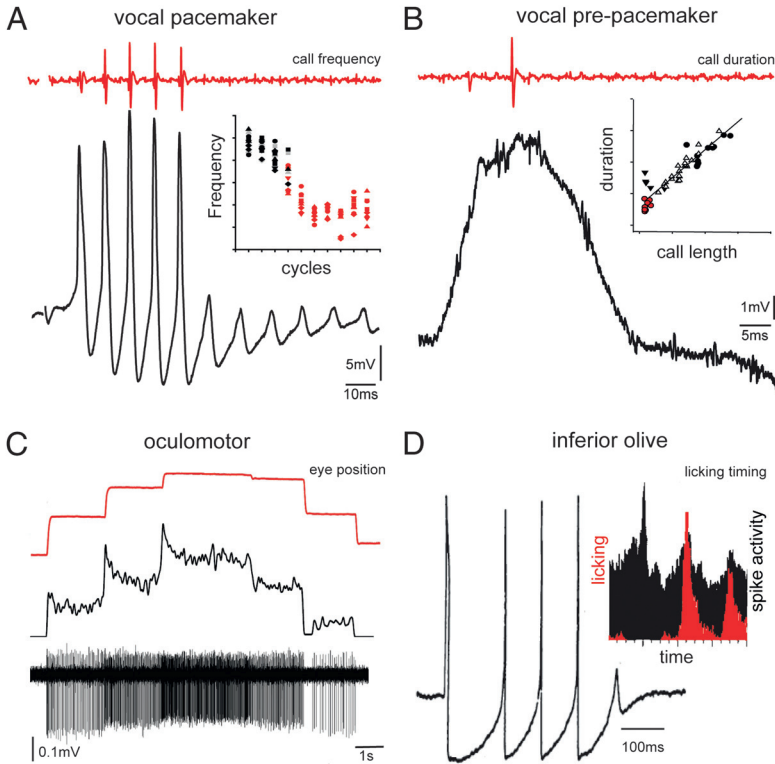
FIGURE 10.5 Spatiotemporal coding of behavioral attributes by rh8 premotor nuclei. Shown are single traces of neuronal activity (black) of midshipman fish vocal pacemaker (*A*) and VPP (*B*), goldfish oculomotor (*C*) and guinea pig inferior olive (*D*), and corresponding behavioral readout (red). (*A*) *Inset*: Dependency of membrane oscillations (i.e., cycles) and pulse repetition rate/frequency of vocal output. (*B*) *Inset*: Dependency of duration of membrane-sustained depolarization and call duration (i.e., length). (*D*) *Inset*: Correlation in rats between tongue licking behavior (red) and cerebellar complex spike activity (black) that directly reflects levels of inferior olive activity. [*A* and *B* adapted from Chagnaud et al. (2011); *C* reprinted by permission from Macmillan Publishers Ltd: *Nature Neuroscience* (Aksay et al., 2001), copyright 2001; *D* reproduced with permission from John Wiley & Sons (Llinás and Yarom, 1986); *D* (*Inset*) reprinted by permission from Macmillan Publishers Ltd: *Nature* (Welsh et al., 1995), copyright 1995.] [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

tional coupling that synchronizes functional groups or patches of neurons involved in complex tasks such as tongue licking (Fig. 10.5*D*) (Llinás and Paré, 1994; Welsh et al., 1995; Urbano et al., 2006).

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Although comparable investigations of intrinsic and network properties are currently lacking for the pectoral motor system, the available neurophysiological data on the neural basis of escape behavior in hatchetfish (Auerbach and Bennett, 1969) are consistent with the view that rh8 premotor populations provide a coherent timing signal synchronizing the activity of one or more neuromuscular compartments determining a behavior [*sensu* Llinás and Paré (1994)]. Recent studies of hindbrain circuitry in zebrafish, including the pectoral network, have begun to identify developmental events establishing the neuronal complement of rh- and neurobehavioral-specific nuclei (Kinkhabwala et al., 2011; Koyama et al., 2011).

COUPLING OF VOCAL AND PECTORAL–GESTURAL CIRCUITRY

There has been much discussion regarding the vocal vs. gestural origins of speech and language [e.g., Gentilucci et al. (2008), Liebal and Call (2012); also see Lieberman (2006), MacNeilage (2008)]. The comparison may, however, be a false dichotomy when we consider the shared developmental origins and social signaling functions of vocal and pectoral systems. Birds and mammals, like fishes [as detailed earlier; also see Ladich et al. (1992)], exhibit vocal and pectoral-dependent mechanisms of acoustic communication. For example, various bird species that use the syrinx to vocalize also use pectoral wings innervated by forelimb spinal motor neurons (Fig. 10.1*C*) to generate nonvocal, sonic signals important for communication (e.g., manakin; Fig. 10.1*A*) (Prum, 1998; Hingee and Magrath, 2009; Miller and Baker, 2009; Bostwick et al., 2010; Barske et al., 2011). Examples of nonvocal, sonic pectoral signaling among mammals that use the larynx to vocalize include drumming by macaque monkeys and gorillas and acoustic gesturing by humans (Reynolds, 1965; Remedios et al., 2009). More generally, temporal coupling between vocalization and pectoral forelimb movement in humans has led to the hypothesis that "tasks requiring precisely timed movements of the vocal tract and hands and arms appear to share common brain mechanisms" [Iverson and Thelen (1999); also see Gentilucci et al. (2008)]. Vocal–gestural coupling is largely considered to depend on forebrain (e.g., premotor/motor cortex, Broca area) and cerebellar (Iverson and Thelen, 1999; Iverson and Fagan, 2004) mechanisms, with essentially no consideration of the potential role of hindbrain premotor circuitry. Collectively, the available developmental and behavioral evidence discussed here and in previous sections suggests that the neural basis for vocal and pectoral coupling observed among tetrapods, including nonvocal sonic and gestural signaling, has ancient origins among fishes at the most fundamental level of hindbrain pattern generators (Fig. 10.1*C*).

CONCLUDING COMMENTS

Pattern-generating circuitry underlying the vocal basis for acoustic communication in fishes and tetrapods evolved from an ancestrally shared hindbrain, rh8-spinal compartment. This compartment also gave rise to premotor-motor circuitry for pectoral appendages that serve locomotion and nonvocal, sonic–acoustic signaling functions in fishes. These shared developmental origins suggest that the functional coupling between more highly derived vocal and pectoral mechanisms that have evolved for acoustic and gestural signaling in tetrapods originated in fishes.

More broadly, we propose that, among vertebrates in general, rh8-spinal networks include anatomically separate premotor nuclei, each of which has a distinct suite of intrinsic and network properties determining specific behavioral attributes (Fig. 10.5). Each network's ensemble of premotor nuclei configures the spatiotemporal activity of one or more neuromuscular systems underlying entire behaviors such as vocalization [also see Llinás and Paré (1994)]. By comparing rh8-spinal networks across vertebrate lineages, we can identify ancestral characters contributing to evolutionarily derived networks, for example, the anatomical and neurophysiological properties of sonic–vocal networks in fishes found in the sonic–vocal networks of birds and mammals. This includes phylogenetically deep homologies, that is, "molecular and cellular components . . . contributing to phenotypic novelties" that "enable us to reconstruct how a phenotype was built over evolutionary time" (McCune and Schimenti, 2012).

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To Flock or Fight: Neurochemical Signatures of Divergent Life Histories in Sparrows

JAMES L. GOODSON,* LEAH C. WILSON,
AND SARA E. SCHROCK

Many bird species exhibit dramatic seasonal switches between territoriality and flocking, but whereas neuroendocrine mechanisms of territorial aggression have been extensively studied, those of seasonal flocking are unknown. We collected brains in spring and winter from male field sparrows (*Spizella pusilla*), which seasonally flock, and male song sparrows (*Melospiza melodia*), which are territorial year-round in much of their range. Spring collections were preceded by field-based assessments of aggression. Tissue series were immunofluorescently multilabeled for vasotocin, mesotocin (MT), corticotropin-releasing hormone (CRH), vasoactive intestinal polypeptide, tyrosine hydroxylase, and aromatase, and labeling densities were measured in many socially relevant brain areas. Extensive seasonal differences are shared by both species. Many measures correlate significantly with both individual and species differences in aggression, likely reflecting evolved mechanisms that differentiate the less aggressive field sparrow from the more aggressive song sparrow. Winter-specific species differences include a substantial increase of MT and CRH immunoreactivity in the dorsal lateral septum (LS) and medial amygdala of field sparrows, but not song sparrows. These species differences likely relate to flocking rather than the suppression of winter aggression in field sparrows, because similar winter differences were found for two other emberizids that are not territorial in winter—

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dark-eyed juncos (*Junco hyemalis*), which seasonally flock, and eastern towhees (*Pipilo erythropthalmus*), which do not flock. MT signaling in the dorsal LS is also associated with year-round species differences in grouping in estrildid finches, suggesting that common mechanisms are targeted during the evolution of different life histories.

A t the termination of the breeding season, many bird species leave their exclusive territories and join flocks that range from small parties to thousands of individuals. This dramatic seasonal shift in behavioral phenotype undoubtedly has profound fitness implications, but to our knowledge, no studies have addressed the neural or endocrine mechanisms that promote seasonal flocking. In contrast, mechanistic studies of avian territorial aggression are relatively extensive and have inarguably revolutionized the field of behavioral endocrinology (Wingfield, 2005; Soma, 2006). However, few of these studies explore the *brain* mechanisms of territoriality (Soma, 2006; Maney and Goodson, 2011). Using four emberizid songbird species that have evolved divergent life-history strategies, we here examine seasonal variation and evolutionary diversity in six neurochemical systems and demonstrate links of those systems to both winter flocking and territorial aggression.

On the basis of the immediate early gene responses of (*i*) male rodents to resident–intruder encounters, and (*ii*) male song sparrows (*Melospiza melodia*) to simulated territorial intrusion (playback of song and presentation of a caged male decoy), it seems that the neural substrates of territorial aggression are extensively comparable in birds and mammals. Thus, in both taxa significant activation is observed in the medial bed nucleus of the stria terminalis (BSTm), lateral septum (LS), paraventricular nucleus of the hypothalamus (PVN), anterior hypothalamus (AH), lateral portion of the ventromedial hypothalamus (VMH), and midbrain central gray [Kollack-Walker et al. (1997), Maney and Ball (2003), Goodson and Evans (2004), Goodson et al. (2005); also see Kingsbury et al. (2011)]. For the year-round territorial song sparrow, immediate early gene results are largely comparable in winter and summer (Goodson and Evans, 2004; Goodson et al., 2005), although microarray data suggest that hypothalamic responses to simulated intrusion are very different in winter and summer, perhaps reflecting the fact that luteinizing hormone is released during territorial challenges only in the breeding season (Mukai et al., 2009). Conversely, neurons that produce steroidogenic enzymes such as aromatase (ARO) may show greater activity in winter, given that territoriality in song sparrows shifts from reliance on gonadal steroids during the breeding season to nongonadal hormone production during the fall and winter (Wingfield, 2005; Soma, 2006).

Remarkably, neural mechanisms that influence group-size decisions have received very little attention, although recent studies have begun to address this topic using five estrildid finch species that exhibit relatively stable group sizes year-round. These studies show that multiple neurochemical systems have evolved in relation to grouping behavior, particularly within the LS and associated subnuclei of the posterior septum. Receptor densities for vasotocin (VT; homolog of the mammalian nonapeptide vasopressin), mesotocin (MT; homolog of the mammalian nonapeptide oxytocin), corticotropin-releasing hormone (CRH), and vasoactive intestinal polypeptide (VIP) all exhibit patterns of parallel and divergent evolution that closely track species-typical group size (Goodson et al., 2006, 2009b). Furthermore, VT neurons in the BSTm that project to the LS are sensitive to social valence and exhibit differential Fos responses in territorial and flocking species (Goodson and Wang, 2006). Antisense knockdown of VT production in those cells potently reduces gregariousness in the highly social zebra finch (*Taeniopygia guttata*) (Kelly et al., 2011), and antagonism of V_{1a}-like and oxytocic receptors in the septum likewise reduces preferred group sizes (Goodson et al., 2009b; Kelly et al., 2011). The relative distribution of nonapeptide receptors across LS subnuclei may also be relevant to species differences in grouping, because flocking species have proportionally higher receptor binding in the dorsal (pallial) LS, whereas territorial species exhibit proportionally more binding in the subpallial LS (Goodson et al., 2006, 2009b). Consistent with these findings, septal VT infusions reduce territorial aggression in emberizid sparrows and estrildid finches (Goodson, 1998a,b). Finally, dopamine circuits are likely also relevant to grouping behavior, as gregarious finch species exhibit significantly more tyrosine hydroxylase-immunoreactive (TH-ir) neurons in the caudal ventral tegmental area (VTA) than do territorial species (Goodson et al., 2009a). The activity of these neurons is tightly coupled to courtship behavior, and perhaps to other aspects of affiliation as well (Goodson et al., 2009a).

These prior studies of avian sociality have focused exclusively on species that exhibit stable, year-round variation in species-typical group sizes (Goodson and Kingsbury, 2011). We hypothesize that the same neurochemical systems have evolved to mediate seasonal transitions between territoriality and flocking, but this remains to be determined. As a first approach to this hypothesis, we here quantify the neurochemical innervation of numerous brain areas in emberizid species that (*i*) alternate between gregarious and territorial phenotypes (field sparrow, *Spizella pusilla*, and dark-eyed junco, *Junco hyemalis*) (Carey et al., 1994; Nolan et al., 2002), (*ii*) are territorial year-round in much of their range (song sparrow) (Arcese et al., 2002), or (*iii*) switch from breeding territoriality to loose distributions in fall and winter, without flocking (eastern towhee, *Pipilo*

erythropthalmus) (Greenlaw, 1996). The four clades giving rise to these species diverged at approximately the same time, relatively early in emberizid phylogeny (Carson and Spicer, 2003). Our focus is on males, given that breeding territoriality is typically most intense in males. Complete datasets from spring and winter birds are reported for song and field sparrows, including correlations with spring aggression. Winter differences that may reflect flocking in field sparrows were further explored in comparisons of winter juncos and towhees. Given that winter differences in neurochemistry between field and song sparrows potentially reflect differences in either winter aggression or winter flocking, the junco-towhee comparison is particularly useful. Specifically, we hypothesize that if winter differences between field and song sparrows reflect flocking, then juncos and towhees should exhibit a comparable winter difference. If winter differences between field and song sparrows reflect a lack of aggression in field sparrows, then juncos and towhees should not differ, because neither is territorial in winter.

We hypothesized that flocking-related changes in neurochemistry would be evidenced in one of two ways. Most obvious would be a winter *increase* in field sparrows (which flock in winter) that is not exhibited by song sparrows (which are territorial year-round). Alternatively, given that neurochemical circuits that promote winter flocking may also be involved in other affiliation behaviors that are expressed in the breeding season, such as pair bonding and caring for young, we hypothesized that field sparrows may *maintain* some neuroendocrine systems year-round that show a winter collapse in song sparrows. Both patterns are observed and are strongly supported by follow-up comparisons of juncos and towhees.

Finally, all of the substances examined here are made in multiple cell groups in the brain and may be relevant to a wide variety of behaviors, including both flocking and territoriality, dependent upon the brain area. For instance, whereas VT neurons in the BSTm respond primarily to affiliation-related social stimuli, those in the PVN are responsive to a diversity of stressors (Goodson and Kingsbury, 2011). TH cell groups likewise show great variation in response profiles (Charlier et al., 2005; Bharati and Goodson, 2006; Goodson et al., 2009a). We therefore do not combine analyses across all brain areas for each neurochemical, given that each neurochemical is not a unitary "system."

RESULTS

General Approach

Tissue from field and song sparrows ($n = 6$ males per species and season; 24 total) was immunofluorescently multilabeled for VT, VIP, and

TH (series 1), and MT, CRH, and ARO (series 2). We were not uniformly satisfied with the quality of TH labeling in series 1, and therefore labeled a third series for TH using an antibody that yielded robust labeling in all subjects (*Methods*; a third series was not available for two spring subjects, one field and one song sparrow, because of earlier processing errors). We followed up on significant winter differences by labeling a single series of junco and towhee tissue for MT, CRH, and TH; and labeled a limited amount of tissue from a second junco–towhee series for VT and VIP. Note that for logistical purposes related to antibody lineups, most antigens were labeled using different fluorophores in the field–song and junco–towhee datasets, and thus labeling densities can only be compared within each species pair, not across.

Optical densities (ODs) of immunolabeling were measured in the medial preoptic nucleus, several hypothalamic areas (PVN, AH, and lateral and medial divisions of the VMH); anterior and posterior medial amygdala (MeA); BSTm; lateral BST; central gray; nucleus intercollicularis; rostral and caudal VTA; and nucleus accumbens. In addition, we quantified labeling in subnuclei of the septal complex that are differentiated on the basis of chemoarchitecture, peptide receptor distributions, and/or transcriptional responses to social stimuli (Goodson and Evans, 2004; Goodson et al., 2004, 2006, 2009b; Leung et al., 2011). These are the nucleus of the pallial commissure; caudocentral septum (CcS); rostral LS subdivision (LSr); and both pallial and subpallial portions of the caudal LS subdivision, which are denoted here as LSc.d and subpallial LSc (includes both ventral and ventrolateral subnuclei). The LSc.d and subpallial LSc were analyzed at rostral and caudal levels. In addition to OD, we conducted counts of TH-ir) cells in the VTA (A10 cell group), central gray (A11), dorsolateral tuberomammillary area (external mammillary nucleus; A12), and subparaventricular area (A14). VIP-ir cells were counted in the tuberal hypothalamus, and CRH, VT, and MT cells were counted in the PVN. Alpha values after Benjamini-Hochberg corrections for the false discovery rate (Benjamini and Hochberg, 1995) are reported in the figure captions and tables for the field and song sparrows, for which we collected full datasets (*Methods*). Results of Species × Season ANOVAs and within-species regressions with aggression are reported in the SI Appendix of Goodson et al. (2012b).

Neurochemical Signatures of Seasonal Flocking

As described in the Introduction, we hypothesized that flocking-related changes in neurochemistry would take the form of either (*i*) a winter *increase* in flocking field sparrows that is not exhibited by song sparrows, or (*ii*) the *maintenance* of some neuroendocrine systems year-round in field sparrows that show a winter collapse in song sparrows.

The first pattern is observed for both MT-ir and CRH-ir fiber densities in the anterior and posterior MeA ("nucleus taeniae"), and the rostral LSc.d [SI Appendix, Tables S1 and S2, of Goodson et al. (2012b)]. CRH is additionally increased in the LSr. The LS innervation consists of extremely fine-caliber processes that arborize most extensively in the pallial LS. In winter field sparrows, MT-ir processes form numerous light pericellular baskets. Similarly fine processes are observed in the MeA.

ANOVA results for the LSc.d are shown in Fig. 11.1*A* and *B*. Importantly, both MT-ir and CRH-ir fiber densities in the rostral LSc.d and LSr correlate negatively with multiple measures of aggression (Fig. 11.1*C–F*), and thus the increased densities in winter field sparrows may serve to suppress aggression rather than promote flocking. To address this issue, we quantified MT and CRH immunolabeling in wintering dark-eyed juncos, which flock, and eastern towhees, which loosely distribute in winter and do not flock. This comparison reveals significantly higher MT-ir and CRH-ir fiber densities in the rostral LSc.d of juncos relative to towhees (Fig. 11.1*G* and *H*) but no differences in CRH OD in the LSr ($P = 0.07$). A parallel set of results is obtained for MT and CRH OD in the anterior MeA (field > song; junco > towhee; Fig. 11.2), but juncos and towhees do not differ in the posterior MeA (MT, $P = 0.28$; CRH, $P = 0.71$). Notably, colocalization of CRH and MT in PVN neurons is significantly greater in winter field sparrows than song sparrows (Fig. 11.3*A*), and winter juncos likewise tend to show more colocalization than towhees ($P < 0.06$; Fig. 11.3*B*). Double-labeling does not correlate with measures of aggression (all $P > 0.10$).

The second pattern described above, in which field sparrows *maintain* circuitry year-round that collapses during winter in song sparrows, is observed for VT-ir cell number in the PVN; and VIP OD in the PVN, AH, rostral subpallial LSc, CcS, and BSTm (in some cases field sparrows maintain relatively more but show a slight decline from spring). As shown in Fig. 11.4*A* and *B*, the field–song difference in VT neurons is matched by a similar difference between winter juncos and towhees, indicating a relationship to flocking. However, with the exception of VIP OD in the BSTm, the Species × Season effects for VIP are complex, with species differences in both winter *and* spring, but in different directions. That is, spring VIP OD measures in the PVN, AH, and septal areas are actually higher in song than in field sparrows. Furthermore, as described in the following section, AH and CcS measures correlate *positively* with spring aggression, which we did not anticipate for variables that promote flocking. Despite these complexities, we conducted follow-up comparisons in juncos and towhees, and although no differences are observed for VIP OD in the AH ($P = 0.14$) or CcS ($P = 0.85$; areas where VIP immunolabeling correlates positively with aggression), juncos do show greater VIP OD in the PVN and BSTm,
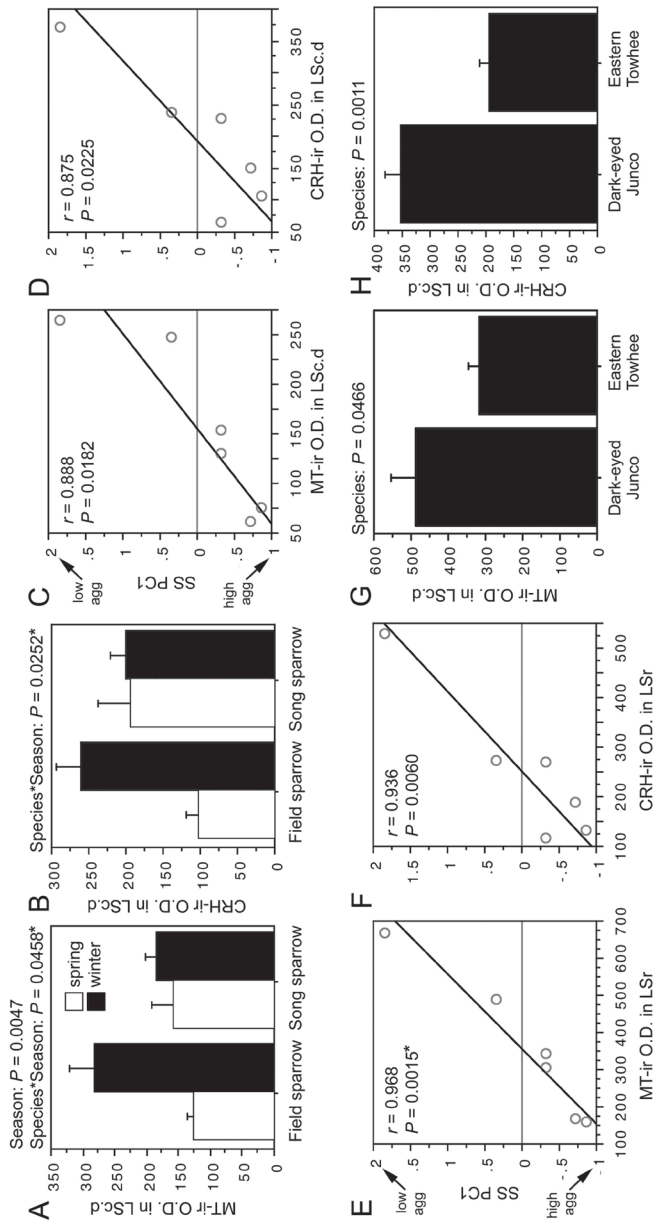
FIGURE 11.1 OD (in arbitrary units) of (*A*) MT-ir fibers and (*B*) CRH-ir fibers in the LSc.d of field and song sparrows collected in spring and winter, showing increased innervation density in winter field sparrows. (*C–F*) MT-ir and CRH-ir fiber densities correlate negatively with song sparrow aggression (SS PC1) in both the LSc.d (*C* and *D*) and LSr (*E* and *F*), suggesting that the increased innervation in winter field sparrows may suppress aggression rather than promote flocking. (*G* and *H*) However, comparisons of two species that are not territorial in winter show that MT-ir and CRH-ir fiber densities are greater in the flocking species (dark-eyed junco) than in the nonflocking species (eastern towhee). Data are shown as means ± SEM. *Significant after Benjamini-Hochberg corrections (sparrows).
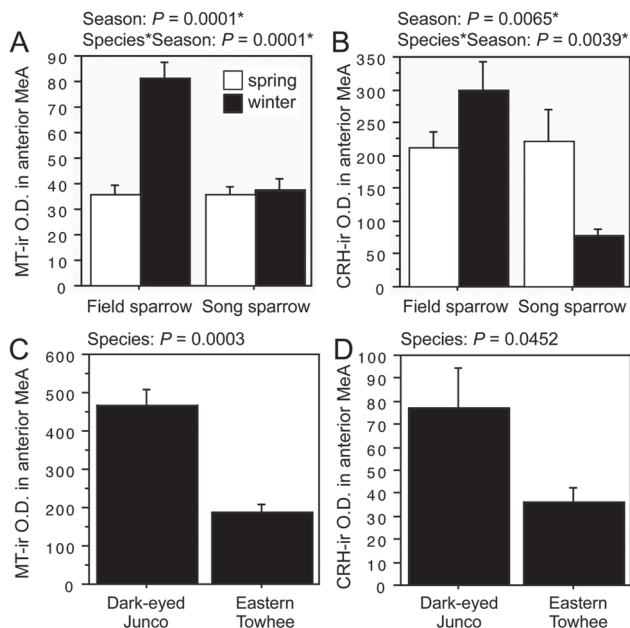
FIGURE 11.2 OD (in arbitrary units) of (*A*) MT-ir fibers and (*B*) CRH-ir fibers in the anterior MeA of field and song sparrows collected in spring and winter, showing increased innervation density in winter field sparrows. (*C* and *D*) MT-ir and CRH-ir fiber densities are greater in the flocking dark-eyed junco than in the nonflocking eastern towhee. Data are shown as means ± SEM. *Significant after Benjamini-Hochberg corrections (sparrows).

following the pattern of higher fiber density in winter field sparrows relative to song sparrows. Relevant data are shown in Fig. 11.4C–F.

In addition to the patterns described above, one other finding initially suggested a possible relationship to flocking. This is a main effect of Species for TH immunolabeling in the rostral and caudal VTA, where field sparrows exhibit significantly higher TH-ir cell numbers and OD year-round relative to song sparrows [SI Appendix, Table S3, of Goodson et al. (2012b)]. Cell numbers also correlate negatively with aggression (next section). However, comparable differences are not exhibited by winter juncos and towhees, suggesting that the year-round difference between field and song sparrows reflects their year-round differences in aggression, as presented below.

Finally, no winter differences are exhibited for VT OD in the BSTm (as would be predicted from estrildids), although VT-ir fiber density in spring is significantly higher in field sparrows than in song sparrows [SI
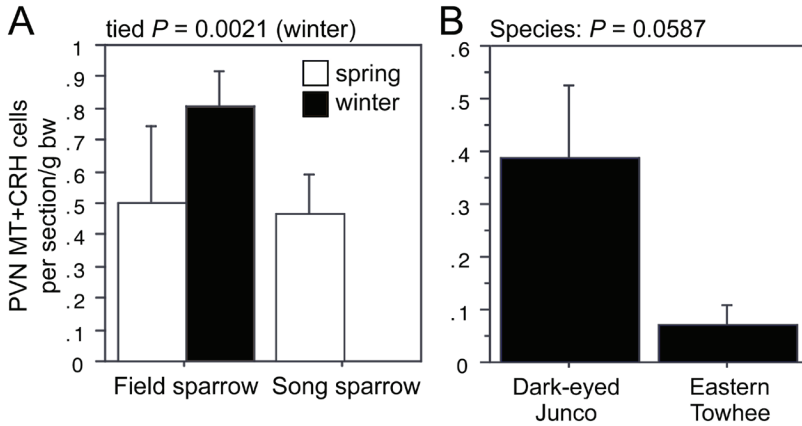
FIGURE 11.3 (*A*) Number of PVN neurons double-labeled for MT and CRH in field and song sparrows. Because of a lack of variance in winter song sparrows, winter data were analyzed using Mann-Whitney tests. (*B*) A similar trend is observed for winter juncos and towhees.

Appendix, Fig. S1, of Goodson et al. (2012b)]. Again, as described in the next section, this is associated with species differences in aggression.

Neurochemical Signatures of Species-Specific Territorial Behavior

Before collections in the breeding season, we took three measures of territorial behavior during 3 min of song playback: latency to respond (by song, fly-by, or flyover), flights (defined as close fly-bys and flyovers), and songs. We then erected a mist net, began another round of playback, and took a second measure of response latency. Many measures of neurochemistry correlate significantly with these behavioral measures on a within-species level (next section). However, relevant to our focus on divergent life histories, we were particularly interested in determining whether measures of neurochemistry predicted *species differences* in aggression, given that that field sparrows are substantially less aggressive during the breeding season than are song sparrows.

To quantify the species differences in aggression, we conducted a principal component (PC) analysis of the four behavioral measures, combining data for both species ($P = 0.0029$). This yields a single component (PC1) that strongly loads all four measures (Fig. 11.5) and explains 68% of the behavioral variance. A *t* test of PC scores confirms that song sparrows are more aggressive than field sparrows during the breeding season (Fig. 11.5), and more striking, PC scores for the two species are nonoverlapping.

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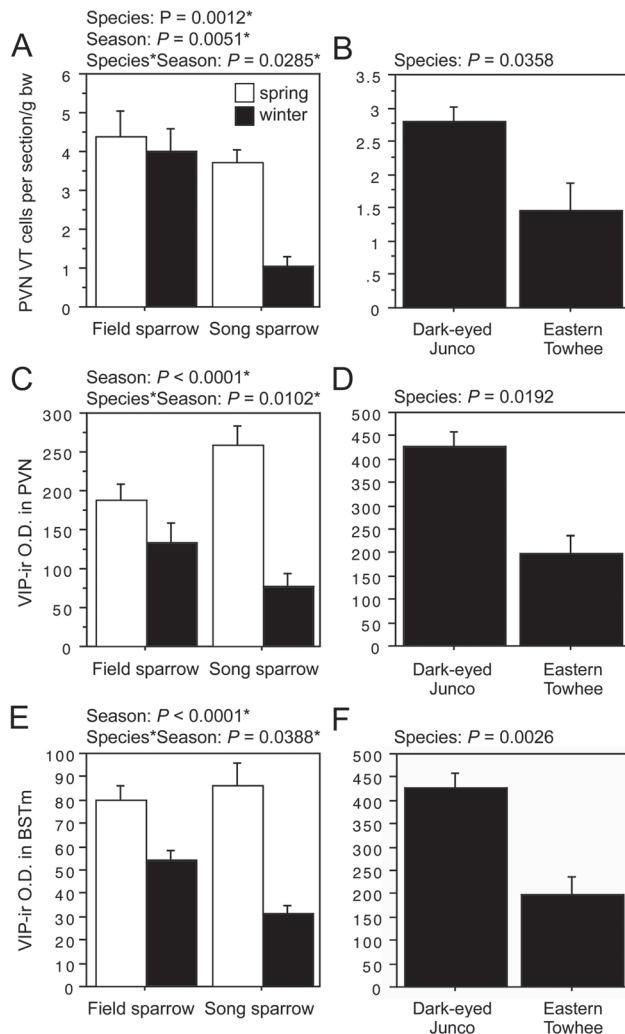
FIGURE 11.4 (*A–F*) Left panels show VT-ir cell number in the PVN, VIP-ir OD (in arbitrary units) in the PVN, and VIP-ir OD in the BSTm of field and song sparrows. Right panels show corresponding data for juncos and towhees. Data are shown as means ± SEM. *Significant after Benjamini-Hochberg corrections (sparrows).

Thus, neurochemical measures that correlate with PC1 are strong candidates as mechanisms underlying evolutionary divergence in territoriality (although experience of aggression may also be a factor; see *Discussion*).
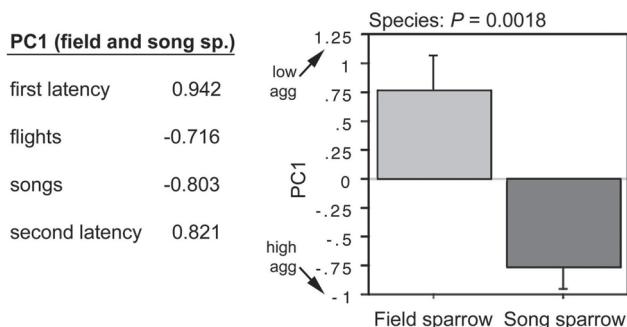
FIGURE 11.5 PC loadings from a combined analysis of field and song sparrow aggression (*Left*) and a comparison of PC scores by species (*Right*). PC1 explains 68% of the variance and yields non-overlapping values for field and song sparrows. Data are shown as means ± SEM.

Note that because of the strong loadings of latency measures, the direction of PC1 values is counterintuitive (i.e., higher PC scores reflect lower aggression). The PC1 score for one of the field sparrows was 2.8 standard deviations above the mean and thus this subject was excluded from the regressions.

Regression analyses reveal significant negative correlations with PC1 (and thus positive correlations with aggression) for VIP OD in the AH and CcS; ARO OD in the posterior MeA (with a strong trend in the anterior MeA, as well); CRH OD in the posterior MeA and nucleus accumbens; and MT OD in the caudal subpallial LSc. In contrast, regression analyses reveal positive correlations with PC1 (and thus negative correlations with aggression) for VIP OD in the medial and lateral VMH; VT OD in the BSTm, central gray, and nucleus intercollicularis; CRH OD in the CcS; and TH OD in the medial preoptic nucleus, AH, LSr, and nucleus intercollicularis. In addition, TH-ir cell numbers in the rostral VTA, tuberomammillary hypothalamus, and subparaventricular area correlate positively with PC1. Ten of the strongest correlations are shown in Fig. 11.6. Note that significance is not obtained solely on the basis of large species differences, because data points within each species tend to follow the overall slope.

Individual Differences in Aggression

As just described, many neurochemical measures correlate with both individual and species differences in aggression. However, neurochemical variables may relate to individual differences within a given species without also relating to differences in aggression across species. We
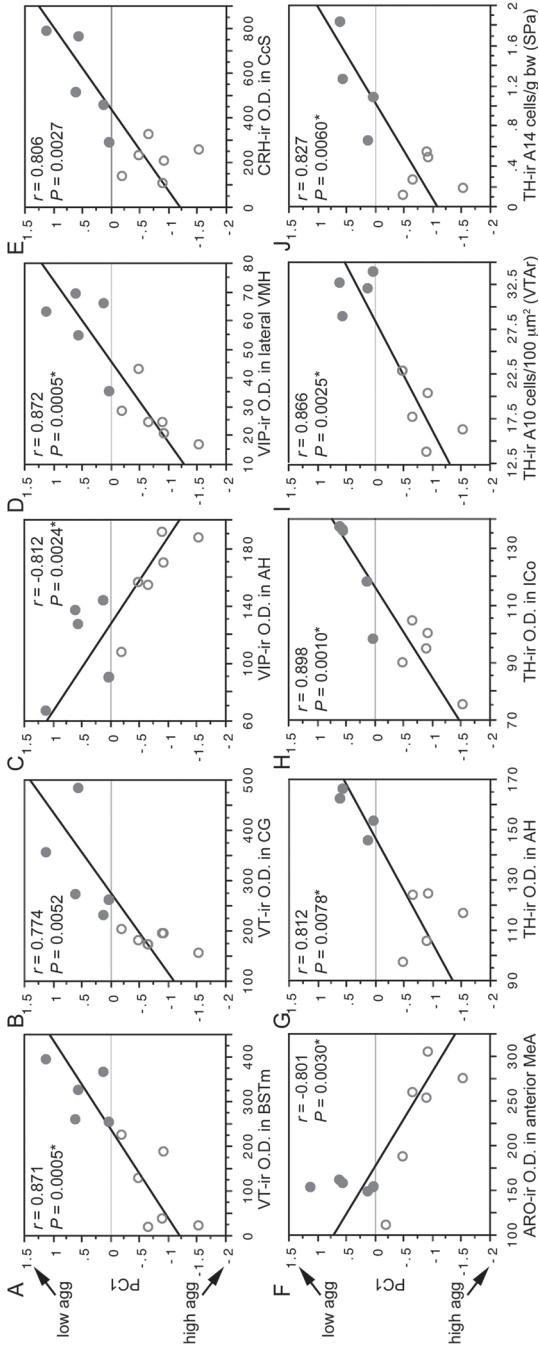
FIGURE 11.6 (*A–J*) Regressions of neurochemical measures (OD, *A–H*; cell counts, *I* and *J*) and an index of aggression (PC1; Fig. 11.5) in field and song sparrows (closed and open circles, respectively). See *x*-axes for neurochemical variable and brain area. *Significant after Benjamini-Hochberg corrections (sparrows). CG, central gray; ICo, nucleus intercollicularis; SPa, subparaventricular area.

therefore conducted behavioral PC analyses for field and song sparrows independently. However, whereas a significant matrix is obtained for song sparrows ($P = 0.0318$), this is not the case for field sparrows ($P = 0.60$), likely because the field sparrows displayed few flights and songs, and little variation in those measures. Thus, we conducted regressions for field sparrows based on the average of their two latency measures, and for song sparrows based on a single-species PC (SS PC1), that explains 64% of the variance and exhibits strong loadings for flights (-0.913) and both latencies (0.901 and 0.928, respectively), but a weak loading for songs (-0.234). Results of these analyses are reported in the SI Appendix, Tables S7–S12, of Goodson et al. (2012b).

DISCUSSION

Although neuroendocrine mechanisms of seasonal territoriality have been extensively described (Wingfield, 2005; Soma, 2006; Maney and Goodson, 2011), those of seasonal flocking have not, and brain mechanisms that evolve in relation to species differences in the intensity of territorial aggression are likewise unknown. We now show that in emberizid songbirds, several neurochemical variables reflect seasonal shifts from territoriality to flocking, whereas numerous other variables correlate with both individual and species differences in territorial aggression. Given that the relevant neurochemical systems may be influenced by social interactions (e.g., via altered hormone levels), we must be cautious in our interpretations, because neurochemical variation may be the *product* of species differences in behavior rather than the drivers of it. However, as expounded upon in the following sections, other relevant findings suggest that many of the species differences are indeed products of evolution and mechanistic drivers of behavioral variation. Finally, our results reveal a remarkable degree of seasonal, neurochemical plasticity within socially relevant brain areas that is far more extensive than previously appreciated.

Neurochemical Profiles of Seasonal Flockers

Estrildid finches that are gregarious year-round exhibit nonapeptide binding sites in the rostral LSc.d (pallial LS) at much higher densities than do territorial estrildids (Goodson et al., 2006, 2009b). The relevance of these binding sites to flocking is supported by the demonstrations that intraventricular and intraseptal infusions of nonapeptide receptor antagonists (V_{1a} and oxytocin receptor antagonists) reduce preferences for larger groups in the highly gregarious zebra finch (Goodson et al., 2009b; Kelly et al., 2011), as does antisense knockdown of VT-ir neurons in the BSTm (Kelly et al., 2011)—neurons that seem to provide the majority of VT-ir innervation to

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the LS (De Vries and Buijs, 1983; De Vries and Panzica, 2006). Conversely, preferences for larger groups are facilitated by intraventricular infusions of MT (Goodson et al., 2009b). The present findings are strongly consistent with those in estrildids: field sparrows show a significant increase in MT-ir fiber density in the LSc.d during winter, when they form flocks, whereas the year-round territorial song sparrow does not. Flocking dark-eyed juncos likewise show a higher MT-ir fiber density in the LSc.d during winter than do nonflocking, nonterritorial eastern towhees. This pattern of MT results is replicated in the anterior MeA, and a very similar pattern of CRH innervation is observed in both the rostral LSc.d and anterior MeA.

Social affiliation in rodents is also linked to nonapeptide signaling in the LS. For instance, nonapeptide receptor densities in the LS increase in response to communal rearing (Curley et al., 2009), promote pair bonding (Liu Y et al., 2001), and correlate positively with both social investigation (Ophir et al., 2009) and maternal behaviors [and in the pallial LS specifically (Curley et al., 2012)]. Although the specific significance of peptide action in the pallial LS remains to be directly demonstrated, recent findings in mice demonstrate that the pallial LS plays an important role in linking contextual stimulus information to the activation of the mesolimbic dopamine system, which influences incentive motivational processes and reward (Luo et al., 2011). The functional properties of the anterior MeA are relatively less clear. In mammals, the posterior subnuclei have been far more extensively studied, although Newman (1999) has suggested that the anterior MeA exerts broad effects on social arousal. Homology of MeA subnuclei in birds and mammals remains to be demonstrated.

The finding that CRH innervation paralleled the MT innervation was unexpected, but is consistent with the fact that these two peptides are produced in many of the same neurons in the PVN and that colocalization is greater in winter flockers (Fig. 11.3). CRH is generally linked to anxiety-like processes and stress (Lovejoy and Balment, 1999), which may be the connection to flocking, given that thermoregulatory and foraging challenges lead to facultative grouping in many vertebrate species (Davies, 1976; Gilbert et al., 2010). Thus, we might hypothesize that winter flockers are in some sense hyperresponsive to the challenges of winter. This hypothesis also fits well with the observation that flocking birds exhibit significantly greater numbers of VT-ir PVN neurons in the winter than do nonflocking birds. Given that VT-ir fiber density collapses during winter in almost every brain area that we examined, it seems likely that these "extra" PVN neurons in flocking species project to the anterior pituitary, where VT acts as a secretagogue for adrenocorticopin hormone (Goodson and Bass, 2001) and thereby contribute to a higher glucocorticoid tone.

Finally, we observed complex patterns of VIP-ir fiber densities, some of which correlate positively with aggression (next section). How-

ever, winter flocking (and not aggression) is associated with higher densities of VIP-ir fibers in the PVN and BSTm. Similarly, gregarious finch species exhibit higher densities of VIP binding sites in the BSTm than do territorial species (Goodson et al., 2006), providing additional evidence that VIP signaling in the BSTm promotes grouping.

Species Differences in Territorial Aggression

As shown here, field sparrows are significantly less aggressive than are song sparrows. Thus, the present dataset allows us to identify neurochemical mechanisms that may have evolved in relation to territorial behavior, because we are able to correlate measures of neurochemistry with aggressive behavior across both individuals and species. As a caveat to this approach, we observed widespread winter decreases in immunolabeling, suggesting the likelihood of positive relationships between gonadal hormones and labeling density. Thus, because male–male interactions typically elevate levels of testosterone (Wingfield, 2005), we must consider that any positive correlations between neurochemistry and behavior may be the product of male–male interactions and not the cause of it. For instance, ARO gene expression correlates positively with both aggression and plasma T in juncos (Rosvall et al., 2012). Nonetheless, most of the strongest relationships described here for neurochemistry and aggression are *negative*.

For instance, VT-ir fiber density in the BSTm collapses in winter, yet we also see that it correlates negatively with individual and species differences in aggression. This observation is consistent with the findings that (*i*) gregarious estrildids exhibit relatively more VT-ir neurons in the BSTm than do territorial species (Goodson and Wang, 2006), (*ii*) those neurons respond selectively to affiliation-related stimuli (Goodson and Wang, 2006), and (*iii*) infusions of VT into the septum (a major recipient of BSTm VT projections) reduce overt territorial aggression in both field sparrows and territorial finches (Goodson, 1998a,b).

Similarly, VIP immunolabeling correlates negatively with sparrow aggression in the lateral VMH and tuberal hypothalamus, but also positively in the AH and caudal septum. These results are strongly consistent with a variety of findings in territorial finches. For instance, intraseptal VIP infusions facilitate offensive aggression (Goodson, 1998b), whereas antisense knockdown of VIP production in the AH virtually abolishes it (Goodson et al., 2012a) (note that VIP-ir cells in the AH are only detectable after colchicine pretreatment and were thus not examined here). VIP-ir cell numbers in the AH of control finches correlate positively with aggression, but consistent with our present findings, VIP-ir cell numbers relate negatively to aggression in the tuberal hypothalamus [SI Appendix

in Goodson et al. (2012a)]. These finch data were obtained from birds in nonbreeding condition, suggesting that the positive relationship between AH VIP and aggression is not dependent upon gonadal steroids. Hence, VIP circuitries in the AH-CcS and mediobasal hypothalamus, which bear positive and negative relationships to aggression, respectively, are likely both relevant to behavioral evolution in sparrows.

We observed many other correlations across species that cannot be as readily interpreted because of a lack of direct functional data, but those findings nonetheless provide the basis for many hypothesis-driven experiments on the evolution of aggression.

Widespread Seasonal Plasticity

Although the present study was designed to focus on aggression and flocking, the analyses in field and song sparrows reveal a remarkable and unanticipated amount of seasonal plasticity, including all six neurochemical systems and 21 brain areas that we examined. Most remarkable are CRH and VIP. Seasonal plasticity has been shown for VIP within the septum and infundibulum (Kosonsiriluk et al., 2008; Wacker et al., 2008), but to our knowledge no such plasticity has been shown for the CRH innervation of the brain. However, we observed significant seasonal variation in 13 of the sampling areas for CRH, and 11 of the sampling areas for VIP. Seasonal plasticity for both peptides is exhibited in the MeA, BST, septal complex, medial preoptic nucleus, hypothalamic nuclei, and midbrain. Even in the case of VT, for which extensive seasonal and hormone-mediated plasticity is already known (as with VP in mammals) (Goodson and Bass, 2001; De Vries and Panzica, 2006), the extent of seasonal remodeling came as a surprise. Interestingly, the most extensive plasticity known for mammals comes from jerboas (*Jaculus orientalis*) that were collected in the field (Lakhdar-Ghazal et al., 1995), as were the animals in the present study, suggesting that exposure to a full range of seasonal cues is necessary to reveal the natural extent of seasonal plasticity.

CONCLUSIONS

We here hypothesized that flocking-related changes in neurochemistry take the form of either (*i*) a winter *increase* in flockers that is not exhibited by nonflocking species, or (*ii*) the *maintenance* of some neuroendocrine systems year-round in flockers that show a winter collapse in nonflockers. The first pattern is exhibited in the MT and CRH innervation of the pallial LS and anterior MeA, and in the colocalization of MT and CRH in the PVN. The second pattern is observed for VT-ir cell numbers in the PVN, and VIP innervation of the PVN and BSTm. A much larger number of neu-

rochemical variables seem to evolve in relation to territorial aggression, and all neurochemicals and brain areas examined here exhibit remarkable seasonal plasticity.

METHODS

Animals

Spring field and song sparrows were caught April thru May 2009 in the vicinity of Bloomington, IN. Wintering sparrows were caught in the vicinity of Bloomington, IN, and in Davidson County, TN, between December 2008 and February 2009. Juncos and towhees were collected in the vicinity of Bloomington, IN, in January 2010. Collections were made under applicable state and federal permits, and all procedures were in accordance with guidelines established by the National Institutes of Health for the ethical treatment of animals.

Tissue Processing and Image Analysis

Subjects were euthanized within 30 min of capture. Perfusions, tissue processing, and immunofluorescent labeling followed standard protocols (Goodson et al., 2004, 2009a; Kabelik et al., 2010). All Alexa Fluor (A.F.) conjugates were purchased from Invitrogen. Secondaries were raised in donkey. Sparrow series 1 was labeled using sheep anti-TH (Novus Biologicals), guinea pig anti-VP (Bachem), and rabbit anti-VIP (Bachem), with A.F. 488, biotin followed with streptavidin-A.F. 594, and A.F. 680 secondaries, respectively. Sparrow series 2 was labeled using custom sheep anti-ARO, rabbit anti-MT (VA10; a kind gift of H. Gainer, National Institute of Neurological Disorders and Stroke, Bethesda, MD), and guinea pig anti-CRH (Bachem), using A.F. 488, 594, and 680 secondaries, respectively. Sparrow series 3 was labeled using mouse anti-TH (Immunostar) and A.F. 594 secondary. The specificity of all antibodies has been addressed [Goodson et al. (2004), Kabelik et al. (2010); see company datasheets for TH]. Each processing run contained a mixture of species and seasons. Junco and towhee series 1 was labeled using rabbit anti-MT, mouse anti-TH, and guinea pig anti-CRH, with A.F. 488, 594, and 680 secondaries, respectively. Additional junco and towhee tissue was labeled using guinea pig anti-VP and rabbit anti-VIP, with A.F. 594 and 680 secondaries, respectively.

Although some larger areas with robust labeling were captured at 5×, most photomicrographs were obtained at 10× using a Zeiss AxioImager microscope outfitted with a Z-drive and optical dissector (Apotome; Carl Zeiss). OD of label and background was measured in Adobe Photoshop CS5 (Adobe Systems, Seattle, WA) from monochrome images, and back-

ground values were subtracted for statistical analysis. Cell counts were conducted as previously described (Goodson and Wang, 2006; Goodson et al., 2009a). All cells were counted in each relevant section for smaller cell groups and are represented as number of cells per section/gram body weight. TH-ir cells in the VTA were counted within a standardized box and are represented as number of cells per 100 μm^2.

Statistics

All ANOVAs, regressions, and PC analyses described in the *Results* were conducted using Statview 5.0 for Macintosh. Given the large number of analyses, some concern arises with regard to type I error, although all brain areas and neurochemicals examined here are known a priori to be relevant to social behavior (although not in all possible combinations). Corrections for multiple comparisons in such instances are usually too conservative and not appropriate (Rothman, 1990), and we therefore do not emphasize them in our interpretations. However, they may still provide a useful metric for evaluation; thus each of our data tables and figure panels provides information on significance relative to Benjamini-Hochberg corrections for the false discovery rate (Benjamini and Hochberg, 1995). Corrections were applied to each set of ANOVAs (e.g., for VT measures across all brain areas) and to each corresponding set of regressions. Again, though not emphasized in the *Results,* the robustness of our findings is notable; for example, 73 of 78 ANOVAs that yield *P* values < 0.05 were significant following corrections. Note that although the Benjamini-Hochberg correction initially applies a Bonferroni criterion, it adjusts α in a stepwise manner for remaining tests as long as *P* values continue to be significant at each step.

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From Chemotaxis to the Cognitive Map: The Function of Olfaction

LUCIA F. JACOBS

A paradox of vertebrate brain evolution is the unexplained variability in the size of the olfactory bulb (OB), in contrast to other brain regions, which scale predictably with brain size. Such variability appears to be the result of selection for olfactory function, yet there is no obvious concordance that would predict the causal relationship between OB size and behavior. This discordance may derive from assuming the primary function of olfaction is odorant discrimination and acuity. If instead the primary function of olfaction is navigation, that is, predicting odorant distributions in time and space, variability in absolute OB size could be ascribed and explained by variability in navigational demand. This olfactory spatial hypothesis offers a single functional explanation to account for patterns of olfactory system scaling in vertebrates, the primacy of olfaction in spatial navigation, even in visual specialists, and proposes an evolutionary scenario to account for the convergence in olfactory structure and function across protostomes and deuterostomes. In addition, the unique percepts of olfaction may organize odorant information in a parallel map structure. This could have served as a scaffold for the evolution of the parallel map structure of the mammalian hippocampus, and possibly the arthropod mushroom body, and offers an explanation for similar flexible spatial navigation strategies in arthropods and vertebrates.

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WHY IS THE SIZE OF THE OLFACTORY BULB SO VARIABLE?

In 1995, Barbara Finlay and Richard Darlington launched a series of studies that supplied an answer to the fundamental question of why sizes of brain regions vary (Finlay and Darlington, 1995). Proposed initially for mammals but extended to basal vertebrates (e.g., sharks) and evolution by artificial selection (e.g., domestication), it supplied the missing link between the constraints of development and allometry. The "late equals large" principle has one important exception: the olfactory bulb (OB). The size of this forebrain structure, within species, order, or class, does not scale with the rest, and indeed the entire olfactory limbic system (LI), including the hippocampus and amygdala, does not conform to this otherwise universal scaling law (Reep et al., 2007; Yopak et al., 2010; Finlay et al., 2011).

Why this should be the case is not yet clear. In their most recent analysis, Finlay et al. (2011) suggest: "we speculate that the independent variation of olfactory bulb from the rest of the brain may be not so much selection for olfactory variability, but rather selection for tighter coupling of the other sensory systems that must share thalamic projections and neocortical representations." I would like to propose instead that such selection for olfactory variability exists. The commonly conceived function for olfaction is the ability to detect and discriminate odorants (Bargmann, 2006; Arzi and Sobel, 2011; Murthy, 2011). A second function, spatial orientation to odorants, is seen as an application of olfactory discrimination. Reversing the primacy of these two functions turns many assumptions and interpretations of olfaction on their heads. What I will call the olfactory spatial (OS) hypothesis offers a unique explanation for the independent scaling of the vertebrate OB: that the scaling reflects directional selection on animals to decode and map patterns of odorants for the purpose of spatial navigation.

CONVERGENCE IN OLFACTORY SYSTEM STRUCTURE AND FUNCTION

The need to orient in space to maximize fitness by acquiring resources and avoiding competition and predation is universal. Indeed it is a defining archetype of what it means to be an animal, most of which are mobile. Olfaction is also universal: "chemicals are probably the original stimuli, since they can participate directly in biochemical reactions without needing a sensory transduction step. This may be the reason that chemicals seem to be the most universal of stimuli. Indeed, it is possible that all organisms make use of chemical stimuli" (Dusenbery, 1992).

Not only do all animals use chemical stimuli, but they do so by using similar mechanisms (Ache and Young, 2005; Bargmann, 2006; Jacobs, 2012, Fig. S1). Eisthen documents four convergences in the olfactory system in insects, crustaceans, nematodes, mollusks, and vertebrates: odorant binding proteins in the fluid overlying olfactory receptor (OR) neurons, G protein-coupled receptors as odorant receptors, a two-step pathway in the transduction of odorant signals, and the presence of glomerular neuropils in the first central target of the axons of OR cells (Eisthen, 2002).

Such structural similarities in olfactory systems remain a remarkable and somewhat mysterious phenomenon. The olfactory system presents other problems: OR projections segregate and project to receptor-specific glomeruli, but beyond the glomerulus, there is no obvious topography (Sosulski et al., 2011). The unpredictable variation in the number of OR genes across species is also mysterious. The numbers must be significant, as OR genes represent the largest multigene family in mammals, representing 4% to 5% of the entire proteome (Niimura, 2009). At present, there is no accepted hypothesis to explain this variation, which can range from 1,500 chemosensory receptors in the nematode worm (*Caenorhabditis elegans*), 130 in *Drosophila melanogaster*, 900 in the laboratory mouse, to 350 in humans (Bargmann, 2006).

Thus, the study of olfaction is a world of paradoxes: the independent scaling of the OB, the function of convergent neuro-architectures, and the diversity of OR genes. However, perhaps these paradoxes arise from the assumption that the primary function is discrimination. If instead the OS hypothesis is correct, the structural similarities may be explained by convergent cognitive processes for spatial navigation. Likewise, variability in OB size and OR gene number could reflect the species' use of odorants in spatial navigation. To explore this proposal, first it is necessary to consider how olfaction differs from other senses.

THE PECULIAR CASE OF OLFACTORY PERCEPTION

By its physical properties, the chemical world must be encoded differently. As Bargmann (2006) concluded, "the visual system and auditory system are stable because light and sound are immutable physical entities. By contrast, the olfactory system, like the immune system, tracks a moving world of cues generated by other organisms, and must constantly generate, test, and discard receptor genes and coding strategies over evolutionary time." Olfaction's genius for tracking moving targets has important implications. As Osorio et al. (1994) concluded: "the mammalian neocortex with its protean powers has evolved from the olfactory forebrain of primitive vertebrates [Sarnat and Netsky, 1981]. Perhaps

because olfaction demands a neural architecture preadapted to learning complex input patterns."

There is a rich literature on olfactory perception in humans and other animals, including insects, crustaceans, and rodents (Wilson and Stevenson, 2006). A primary finding is that the percept of an odorant is nonlinearly intensity dependent. Low and high concentrations of the same odorant can be perceived as dissimilar and unrelated (Wilson and Stevenson, 2006, table 4.1). A second finding is that an odorant mixture can be perceived as a mixture of its elemental components (i.e., individual odorants) or as a synthetic odor object, which cannot be decomposed. Studies pitting different histories and rewards for different configurations, both in invertebrate and vertebrate taxa, demonstrate that the ability to switch from the elemental to the synthetic percept is widespread (Wilson and Stevenson, 2006). The mechanism for this allocation of perception and attention is not yet understood, however (Kay et al., 2005; Frederick et al., 2009).

Nonetheless, these observations have implications for the problem of higher-level organization in the olfactory system, as it may be possible to construct a spatial logic from these rules. As seen in Fig. 12.1, if the percept changes abruptly with intensity, a uniform intensity gradient acquires demarcations. A navigator could use this pattern to confirm its direction or speed of movement along the gradient. If two demarcated
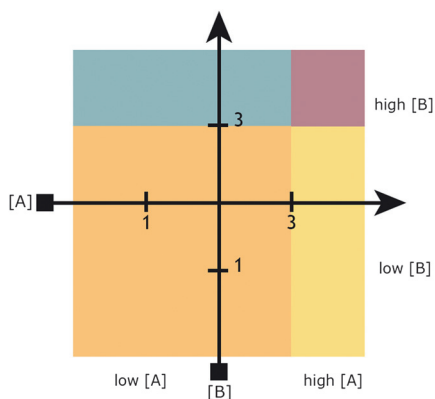
FIGURE 12.1 Schematic predictions of the spatial olfaction hypothesis. A hypothetical orthogonal grid created by plumes from two odorants, A and B, which increase in concentration from one to three arbitrary units. With increasing intensity, there is a qualitative shift in percept (indicated by shading). This further divides the hypothetical olfactory space into subregions known as neighborhoods (see text).

gradients intersect, their conjunction could be organized by this principle into local areas of odorant mixtures, which herein will be called neighborhoods. A neighborhood organization could be used to learn the geometrical relationships among odorants, that is, the olfactory space, which is a mental map of the spatial relationships among odorant distributions in the physical world.

The addition of synthetic odor objects would increase the spatial resolution of the olfactory space (Fig. 12.2). Now, in addition to the low-resolution neighborhoods, the olfactory space could also have high-resolution locations. These synthetic object landmarks could be associated with a neighborhood as well as with other objects in the same neighborhood.

Such an olfactory space would allow a navigator to extract new information from learned odorants. Knowing its speed and rate of sampling, a navigator could extrapolate into the future, predicting the percept farther up the gradient, that is, both in space and time. If the prediction was correct, the navigator would have confirmed its location in olfactory space. If wrong, the navigator could recalibrate its position by searching for
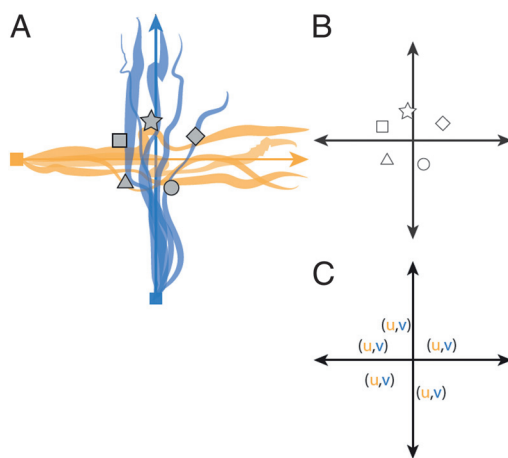
FIGURE 12.2 Schematic predictions of the spatial olfaction hypothesis. The distributions of synthetic odor objects are landmarks in a dynamic olfactory space. (*A*) Encoding of odorant ratios as synthetic odor object percepts. (*B*) Synthetic objects occur at known locations, as defined by odorant ratios, and therefore are landmarks in olfactory space. (*C*) The coordinate of a synthetic object can therefore be computed from its elemental components. The coordinate system variables (u, v) are adopted from meteorology, where u designates streamwise direction and v crosswind direction (Conover, 2007).

neighborhoods and/or synthetic objects. These two mapping systems for olfactory space would differ in other ways as well. The neighborhood system could be used to quickly form a low-resolution map, on which the navigator deduces direction and general location from changes in intensity and the order of neighborhoods. The synthetic object map would have higher spatial resolution but would also be slower to construct, with the navigator having to learn the location of unique synthetic objects. However, by encoding an odorant ratio in two ways, a navigator could use this information to shortcut between synthetic object locations along elemental gradients (Fig. 12.2C). By such novel mapping, the navigator could deduce new relationships among these synthetic objects. These new relationships could be used to simulate trajectories in physical space linking two locations and they could also be used to create higher-level categorizations of the original synthetic objects.

Obviously, the question of turbulence looms large, yet animals are highly adapted to decode turbulence (Atema, 1996; Koehl, 2006; Gardiner and Atema, 2007), and odorant distributions may be stable, even in air (Wallraff, 2004). Olfactory systems are also notably integrated with mechanosensory systems to measure turbulence, such as vibrissae (mammals), antennae (insects), antennules (crustaceans), and lateral lines (fish) (Dehnhardt and Mauck, 2008; Thewissen and Nummela, 2008). Thus, theoretically animals could collect the necessary mechanosensory data to decode the spatial relationships of odorants suspended in a dynamic medium (i.e., air or water).

PARALLEL MAP SOLUTION

If the primary function of olfaction is navigation, the parallel function hypothesis proposed earlier is one solution to this problem, although not the only one. I propose it for two reasons: first, it is a hypothesis that incorporates the known oddities of olfactory perception. Second, Françoise Schenk and I have proposed a similar parallel structure for the hippocampal cognitive map (Jacobs and Schenk, 2003). If the OS hypothesis is correct, it suggests that the hippocampal parallel map evolved from the olfactory parallel map, as the mammalian instantiation of a bilaterian cognitive architecture, as discussed later.

The parallel map theory (PMT), illustrated in Fig. 12.3, was first proposed as a cognitive mechanism for true navigation in vertebrates, and second, to explain the evolution and function of the mammalian hippocampus (Jacobs, 2003, 2006; Jacobs and Schenk, 2003). In PMT, the bearing map (BE) is analogous to the olfactory elemental map, whereas the sketch map (SK) is analogous to the olfactory synthetic object map. The BE (Fig. 12.3A) is constructed by the navigator as it actively moves in
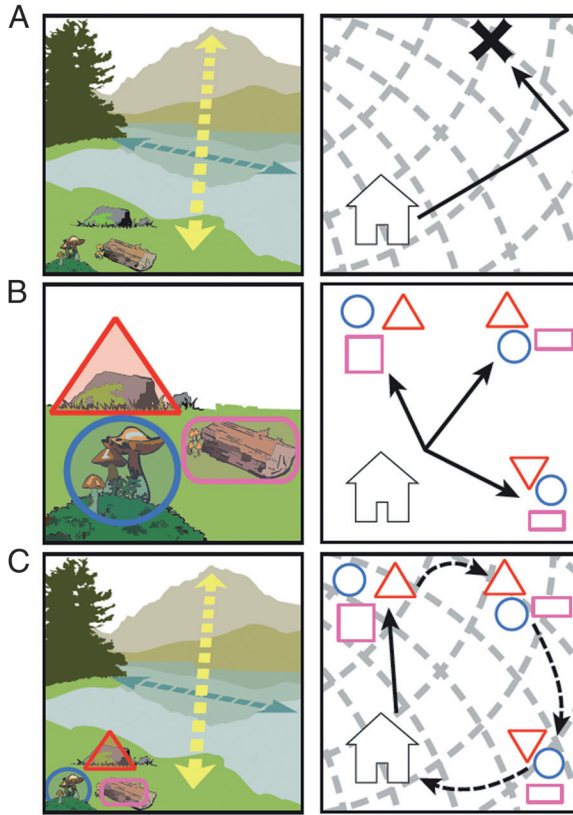
FIGURE 12.3 The parallel map theory of navigation, illustrated with real-world examples and with abstract schematics. (*A*) BE: arrows indicate the vector information extracted from two directional cues, a distant mountain and the polarized shape of an oblong body of water. The schematic shows the abstract bicoordinate map and movements of a navigator. (*B*) SKs: shapes outline three unique positional cues. The schematic represents three SKs near the home base of the navigator, with each SK differing not in the number or characteristics of the cues but in the topology of the array. (*C*) Integrated map: by encoding the location of positional cues (i.e., SKs) on a bicoordinate map (i.e., BE), the navigator can compute novel vectors between two known points, that is, cognitively map.

space, comparing successive samples along gradients of graded stimuli, that is, directional cues. With just a BE, a navigator can extrapolate and predict a future location, even in unexplored territory. In mammals, the proposed neural substrate of the BE is the dentate gyrus. In contrast, the SK encodes constellations of memorized positional cues (i.e., local

landmarks; Fig. 12.3*B*). The SK encodes the topological arrangement of positional cues to derive relational and temporal order information, and its proposed substrate is the CA1 subfield of Ammon's horn. The BE and SK are brought into register on the integrated map, subserved by subfield CA3, in which objects on the SK are recoded in BE coordinates (Fig. 12.3*C*). In concordance with PMT predictions, Manahan-Vaughn and coworkers have recently shown that directional cues facilitate long-term depression (LTD) in the dentate gyrus whereas positional cues facilitate LTD in CA1, and both cue types facilitate LTD in CA3 (Kemp and Manahan-Vaughan, 2008; Hagena and Manahan-Vaughan, 2011).

As with olfactory space, the hippocampal parallel map provides a powerful tool for mapping spatial relations, with global generalization (i.e., BE) and local specificity (i.e., SK), and the ability to move between these representations in the fully encoded integrated map. In olfactory space, the map is based on chemosensory and mechanosensory inputs. In the BE, chemosensory, mechanosensory inputs as well as other sensory (e.g., visual, auditory, electrosensory) inputs are integrated to create a robust, multisensory representation of space. Such multimodal integration allows information from multiple directional cues to be calibrated. This calibration is critical to spatial navigation under natural conditions (Freake et al., 2006).

The close relationship between the olfactory system and the hippocampus in mammals has long been recognized; indeed, olfaction was once believed to be the primary function of the hippocampus (Sarnat and Netsky, 1981). Thus, the OS hypothesis is not necessarily radical or new, but is instead the revisiting of an old idea in light of new evidence about olfaction and new insights from evolutionary neuroscience.

PREDICTIONS OF THE OS HYPOTHESIS

If the function of olfaction is navigation, perhaps using a parallel map geometry, olfactory structure size should scale with navigational demand. At the same time, the impairment of olfactory structures should impair olfactory discrimination and olfactory navigation. Discrimination of odorants is a separate function of the olfactory system and a component of navigation. It is possible and even likely that these two functions, discrimination and navigation, will be found to segregate in olfactory systems by anatomical locus, physiological mechanism, and/or genetic encoding. However, at present, the genetic code for olfactory perception remains unbroken, and most olfaction research focuses on the discrimination of static odorants, not spatial orientation to changing odorant distributions (Arzi and Sobel, 2011; Murthy, 2011). What is needed to test the OS hypothesis are behavioral and physiological disassociations of the two functions

in animals navigating under natural conditions, or laboratory conditions designed to simulate the natural complexity of odorant distributions.

With the exception of studies on homing pigeons, such data are mostly lacking. There is not sufficient space here to review the pertinent scientific literatures (e.g., physiology of animal olfaction, the hippocampus and spatial navigation). Instead, the studies most relevant to the question of the scaling of the OB in vertebrates are mentioned. Even in vertebrates, scaling of the vomeronasal and accessory olfactory systems, or the question of patterns in OR gene number, cannot be assessed here, although an OS-based analysis of these structures and gene families is under way.

If the olfactory system encodes spatial maps of odorants, the absolute size of the OB should covary with the need to make maps of high spatial resolution. It should not scale with demand for the fine discrimination of odorants, for example, those used in social interactions or discriminating foods by taste. Such discrimination should be accomplished via physiological plasticity in response to the experiences of the individual (Beshel et al., 2007; Kay et al., 2009). Therefore, absolute OB size should be predicted by navigational demand. Further, it should be that form of navigation subserved by the BE: first creating vectors from graded stimuli, then combining these into bicoordinate maps for short-cutting and extrapolation (Fig. 12.3). Thus, the OS hypothesis also predicts that olfactory impairment should impair the BE, and thereby the integrated map and cognitive mapping. Evidence across vertebrates is reviewed later, with a short foray into arthropods, and the chapter concludes with a proposed scenario for the evolution of the OS system.

MAMMALS

Although the primacy of olfactory inputs for mammals is widely accepted (Davis and Eichenbaum, 1991), there are surprisingly few experimental studies of the use of air- or waterborne odorants for navigation. Studies of olfactory search by rescue dogs are one exception but are few in number (Hepper and Wells, 2005). Most studies are those of laboratory rats orienting to discrete sources of odors in a laboratory maze. Under these conditions, rats will track an odor trail to a goal (Wallace et al., 2002), even underwater (Means et al., 1992). They can also orient to an array of odorant sources and will do so in the absence of visual cues (Lavenex and Schenk, 1996). As they mature, however, rats require visual cues to orient in a lighted maze, even in the presence of learned olfactory cues. This accords with PMT, which predicts an ontogenetic change from the gradient-based BE to the object-based SK (Jacobs and Schenk, 2003; Rossier and Schenk, 2003). In the laboratory, such effects might be stronger if the static atmospheric conditions could be redesigned to capture the complex-

ity of a natural windscape, the evolved context for olfactory navigation (Conover, 2007).

Nonetheless, impairment of the OB in laboratory rats orienting in the Morris water maze suggests that the OB is necessary for navigation, even in the presence of visual cues. Rats deprived of olfaction via peripheral anosmia showed no impairment, relying instead on visual cues. In contrast, rats with olfactory bulbectomy showed a severe and long-lasting (6 wk) impairment (van Rijzingen et al., 1995). This suggests that the olfactory system acts as a necessary scaffold for visual navigation, that is, the same scaffolding function originally proposed for the BE (Jacobs and Schenk, 2003). It illustrates a basic tenet of the OS hypothesis: that the function of the OB is spatial navigation, not simply odorant discrimination, as the lesion of the olfactory epithelium impaired discrimination but not navigation.

Comparative studies pointing to the navigational function of the OB in mammals began with a study of terrestrial carnivores by Gittleman (1991), which showed that relative OB size increased with home range size. More recently, Reep et al. (2007) examined the relationship between isocortex (IS) and the LI (OB, olfactory cortex, subicular cortices, hippocampus, septum) in diverse mammalian groups (carnivores, ungulates, xenarthrans, and sirenians). Overall, they found the absolute size of the OB covaried with that of the hippocampus, but was inversely related to the absolute size of the IS, as was the size of the LI to the IS. However, when comparing LI and IS in relation to "brain core" volume [defined as striatum, diencephalon, medulla, and mesencephalon (Finlay et al., 2001)], different patterns emerged. These included high IS plus high LI in carnivores, high IS plus low LI in simians, low IS plus low LI in microbats, and low IS plus high LI in insectivores. Megabats (pteropids) had intermediate IS plus intermediate LI, and ungulates and marine mammals had intermediate IS and low LI (Jacobs, 2012, Fig. S2). The authors made the case that such patterns emerged from developmental constraints (Reep et al., 2007).

EFFECTS OF PREDATORY STRATEGY

The OS hypothesis would predict that the size of the LI should increase in predators whose prey are predictable in time and space and who can be tracked by their odorants. Likewise, the size of the multisensory IS might be related to planning ability, with an IS increasing in size if prey are predictable but wily and difficult to capture. To apply this corollary of the OS hypothesis, I divide the world into foragers that are "detectors" or "predictors." Detectors eat prey that are easy to find (e.g., grasses) or impossible to find (e.g., aerial insect clouds) and should thus not invest in brain space for a spatial tracking system. Predictors eat prey the locations

of which can be predicted with sufficient data and should therefore invest as needed in a spatial tracking system, whether olfactory (i.e., LI) or not.

Such predictions are confirmed in the results of Reep et al. (2007): low LI plus low IS should be found in detectors. Indeed, this is the pattern for grazing ungulates and sirenians and the echolocating microbats, many of which feed on aerial insects (Jacobs, 2012, Fig. S2). In contrast, the ancestral mammal was probably an olfactory predator eating small prey, such as invertebrates. Less encephalized prey should engage in fewer spatial counterploys to thwart an olfactory predator (Conover, 2007). This should be reflected in a predictor pattern of high LI plus low IS. This pattern is indeed seen in insectivores and prosimians (Jacobs, 2012, Fig. S2). If, however, predictors also face the challenge of eating prey that can map and avoid their movements (Conover, 2007), they must not only invest heavily in LI for mapping odorants in space but also in IS for predicting prey movements. This high LI/high IS pattern is found in terrestrial carnivores. Finally, among predictors, if prey are best detected by using a nonolfactory modality (e.g., vision), investment should decrease in LI but increase in IS; this pattern is seen in the low LI/high IS in simians (Jacobs, 2012, Fig. S2).

The pinnipeds present a quandary at first, as they are carnivores, and therefore should be predictors, with a high IS, whereas theirs is only intermediate. Olfaction must be jettisoned, however, in terrestrial species that return to the water, because of its incompatibility with respiration (Thewissen and Nummela, 2008). However, as Reep et al. (2007) conclude, "the reduction of volume in the hippocampus, which gets only a minor olfactory projection compared to other sources of input, is suspiciously high for an explanation based on denervation."

An alternative hypothesis is that pinnipeds are detectors, not predictors. Such a hypothesis is surprisingly tenable: unlike odontocetes such as dolphins, pinnipeds do not echolocate. Instead, they detect prey with specialized underwater visual systems and mechanoreception by using specialized vibrissae. Some pinnipeds use their mobile vibrissae to haptically search the benthic sea floor for stationary prey, and others use the vibrissae to track the hydrodynamic trails of prey such as fish (Dehnhardt and Mauck, 2008). Schools of highly mobile prey may represent an ephemeral food source that is easier to find than predict in the absence of olfaction, the main sensory modality of other marine carnivores, such as sharks (Gardiner and Atema, 2010), and even aerial marine piscivores, such as albatrosses (Nevitt, 2008). The pinniped loss of olfaction, combined with low predictability in prey movements, would decrease selection for spatial tracking (Stephens, 1991) and pinnipeds may have deinvested in predicting and reinvested in detecting. Again, this is highly speculative but offers a possible explanation for the data.

Chiropterans are interesting because of the divergence in predatory behavior between the microbats, specialized for echolocation, and megabats (pteropids), who use simple or no echolocation, relying on vision and olfaction to detect prey, for example, fruit. As predicted by the OS hypothesis, microbats show the low LI/low IS pattern. In contrast, megabats show an intermediate LI/intermediate IS pattern (Jacobs, 2012, Fig. S2), which is consistent with their use of olfaction to find their prey.

Hippocampal plasticity, which should also reflect OS function, also differs between microbats and megabats. Adult neurogenesis is found widely in animals but in vertebrates it is always found in the OB and the medial pallium (hippocampus in mammals) (Lledo et al., 2006; Derby, 2007). Thus, the two structures necessary for the OS system are also the only locations in which adult neurogenesis is found in all vertebrates, including mammals. OB neurogenesis increases with new odorant presentation (Mouret et al., 2009), whereas hippocampal neurogenesis increases with spatial exploration (Lledo et al., 2006). This vertebrate pattern of neurogenesis suggests its ancestral function was related to mapping and encoding the spatial distributions of novel odorants (Jacobs and Schenk, 2003).

However, microbats present the exception to this vertebrate rule, despite showing normal hippocampal function, including hippocampal place cells (Ulanovsky and Moss, 2007). A study of 12 microbat species found no hippocampal neurogenesis in nine species and greatly reduced levels in the others; measures of neurogenesis even varied among species in a genus (Amrein et al., 2007). The OS interpretation of this labile pattern is that detector microbats, relying heavily on spatial audition, have fundamentally replaced their OS system and now require less plasticity in BE components (e.g., OB, dentate gyrus). This hypothesis is supported by new data from the same group on megabats, which show a much higher level of hippocampal neurogenesis than microbats, but lower than that seen in laboratory rodents (Gatome et al., 2010). This, too, would be predicted by the OS hypothesis, as megabats appear to be the predictors of the chiropterans. As with fruit-eating simians, these bats forage for a food resource that can be tracked in space and time. Cognitive mapping has also been demonstrated in a wild megabat, the Egyptian fruit bat (Tsoar et al., 2011), as have medial entorhinal grid cells (Yartsev et al., 2011). Concordant with this proposed predictor status, megabats show an intermediate LI/intermediate IS pattern (Reep et al., 2007). Further evidence comes from a comparative study of relative OB size, hippocampal size, and wing size in bats (Safi and Dechmann, 2005), in which wing size is a proxy for navigational ability, increasing in cluttered environments. Wing size increased with relative hippocampal size in microbats, but was unrelated to relative OB size. In contrast, relative OB size and wing size

were positively correlated in megabats (Safi and Dechmann, 2005), again supporting the hypothesis that megabats are olfactory predictors whereas microbats are auditory detectors.

In summary, scaling analyses of mammalian LI and IS show distinct patterns of covariation (Reep et al., 2007). The OS hypothesis offers a unified explanation for these patterns, by proposing an increase in OS structures in predictors and a decrease in detectors. Decreases in LI size occur with shifts in sensory ecology (e.g., pinniped return to water, primate shift to diurnal frugivory, microchiropteran shift to aerial echolocator). Likewise, when prey are mobile and encephalized, the predator's need to predict their movements drives an increased investment in LI and IS.

Such processes, hypothesized for extant mammals, may also shed light on macroevolutionary patterns in mammalian brain evolution. A recent study that used high-resolution X-ray computed tomography was able to identify three transitions in which early Jurassic mammals showed a significant and sudden increase in absolute brain size (Rowe et al., 2011). At all three transitions, the increase in brain size could be ascribed primarily to increases in absolute OB and olfactory cortex size. The authors conclude, "but at its start, the brain in the ancestral mammal differed from even its closest extinct relatives specifically in its degree of high-resolution olfaction, as it exploited a world of information dominated to an unprecedented degree by odors and scents" (Rowe et al., 2011). The alternative OS explanation is that this is evidence of mammals evolving more sophisticated spatial cognitive abilities, with increases in OB size accompanied by increases in hippocampal size and olfactory cortex size with eventual increases in IS. The mammalian brain may thus have evolved first via mosaic evolution for olfaction, then via concerted isocortical evolution.

BIRDS

New imaging studies of the relatives of modern birds, the theropod dinosaurs, have shown that OB size was larger in active predators, relative to cerebral size and corrected for phylogenetic independence. Moreover, an analysis of phylogenetic trends showed that the direct ancestors of modern birds did not show the modern bird's reduction in relative OB size, which must therefore be a secondary adaptation (Zelenitsky et al., 2011). This implies that carnivorous predators, whether diurnal theropods or nocturnal terrestrial mammals (Gittleman, 1991), are olfactory predictors, and require an enhanced OS system to track mobile, dispersed prey.

Finding this pattern in the diurnal ancestor of modern birds is concordant with the observation that despite their visual acuity, many bird

species still require olfaction for spatial navigation (DeBose and Nevitt, 2008). For example, procellariform (tube-nosed) seabirds, the "fishes of the air," use olfaction to track unpredictable distributions of prey-related odors (Nevitt, 2008). When vision is reduced, however, as in secondarily nocturnal species, there is an increase in relative OB size in birds; this has evolved independently multiple times in modern birds (Healy and Guilford, 1990).

The strongest evidence among vertebrates, however, for the OS hypothesis comes from the homing pigeon. This domesticated strain of the rock dove has been artificially selected for its ability to home from unknown locales for many centuries. Compared with nonhoming strains, the homing pigeon has in absolute size both a larger OB and a larger hippocampus (Rehkämper et al., 1988). Originally proposed by Papi and later developed by Wallraff, it has now been well established that homing pigeons rely heavily on olfaction for navigation. As reviewed by Wallraff (2005), the olfactory navigation hypothesis has been widely tested, across different laboratories and continents, by using a variety of behavioral and physiological manipulations. Physiological impairments have included blocking nostrils, anesthetizing the olfactory epithelium, transecting the olfactory nerve, and ablating the piriform cortex. Such procedures impair navigation even when visual cues are available (Wallraff, 2005). Although homing pigeons also orient by using geomagnetic fields (Wiltschko and Wiltschko, 2005), this input appears to be weighted less heavily than olfaction in experimentally displaced homing pigeons (Gagliardo et al., 2006) and in migrating songbirds (Holland et al., 2009). Such experimental evidence for the primacy of olfactory inputs in navigation, across multiple diurnal bird orders, lends strong credence to the OS hypothesis.

REPTILES

Chemical stimuli play a pivotal role in the behavior of reptiles, but we lack studies addressing the covariation of absolute OB size and navigational ability. There is a correlation, however, between relative medial cortex (medial pallium homologue) size and active predation, whereby medial cortex size is larger in active than in sit-and-wait lizards (Day et al., 1999). In snakes, rattlesnakes forced to navigate after experimental displacement have an increased volume of medial, but not dorsal or lateral, cortex (Holding et al., 2012).

Spatial orientation has been well studied in several species of turtles. The semiaquatic red slider turtle can orient by using true spatial strategies in the laboratory, and this ability is impaired after lesions of the medial cortex (López et al., 2003). Sea turtles orient to magnetic fields and to a

map-like representation of such fields, adjusting their heading in response to simulated ocean locations in the laboratory (Lohmann and Lohmann, 1996; Putman et al., 2011). In the field, sea turtles may also use windborne odorants to locate their natal beach by orienting upwind (Hays et al., 2003), but as secondarily aquatic vertebrates, sea turtles have a smaller relative OB size and fewer OR genes than land turtles (Vieyra, 2011). Thus, living and extinct reptiles appear to show predictable heterogeneity and plasticity in the components of the OS system, in concordance with the OS hypothesis.

FISH

Chemical stimuli are a primary source of information for spatial orientation in fish, from short reorientations to long-distance homing of salmon. Across all spatial scales, fish orient to odorants by calibrating odor sampling to their lateral line perception of hydrodynamic trails (DeBose and Nevitt, 2008). The smooth dogfish not only requires intact lateral lines to use odorant sources for orientation, but uses the internostril time delay to determine its location relative to the plume (Gardiner and Atema, 2010). Experimental studies of navigation in goldfish demonstrate that it is mediated by the medial pallium homologue in teleosts, the dorsolateral ventral region of the telencephalon (Salas et al., 2006). As in birds and mammals (Jacobs, 2009), mating system predicts sex differences in the relative size of this region (Costa et al., 2011).

A recent analysis of brain scaling in cartilaginous fish has shown that, as in mammals, OB size variance is unrelated to phylogeny. Instead, as in the analysis of LI and IS in mammals (Reep et al., 2007), the patterns of absolute telencephalon and OB size admitted of no ready explanation (Yopak et al., 2010). However, some of the observed patterns may be addressed with the OS hypothesis. For example, telencephalon and OB absolute size are larger in deep-water than reef-associated species. The shark in deep water may face the same challenge as a nocturnal carnivore on land. In both cases, the predator must predict prey movements and locations by using an olfactory BE, as the positional cues for the SK are absent (deep water) or ambiguous (low light). Therefore, sharks in deep water, but not in reefs, may orient to prey as olfactory predictors. If so, the OS hypothesis may offer insights about basal vertebrate clades as well as tetrapods.

ARTHROPODS

It may be possible to apply the implications of the OS hypothesis even further back in evolutionary time. Tomer et al. (2010) have reported that

similar highly conserved gene networks are found in the vertebrate pallium and the mushroom body of a marine annelid. They conclude that this ancestral gene network could underlie the evolution and development of complex brains in vertebrates and annelids (Tomer et al., 2010).

This result is particularly timely in light of new studies showing arthropod species, lacking a hippocampus, can demonstrate cognitive mapping. Orienting to laboratory simulations of local geomagnetic fields, Caribbean spiny lobsters can accurately orient toward their home den (Boles and Lohmann, 2003). Studies of cognitive mapping in honeybees by Menzel et al. (2005, 2012) have shown that displaced honeybees can initiate homing flights from any location within the explored area along novel shortcuts and can choose among at least three goals. Honeybees can also shortcut between vectors learned from exploration and those learned from the waggle dance (Menzel et al., 2011).

Applying the same OS logic to arthropods, navigational demand should predict larger investment in the olfactory glomerular structure (i.e., OB in vertebrates) and the multisensory associational structure (i.e., hippocampus). In insects, this is the antennal lobe and mushroom body (Strausfeld et al., 2009; Strausfeld, 2012). Antennal lobe size should covary with the use of olfaction in navigation, whereas the multisensory mushroom body, encoding visual, mechanosensory, and olfactory information, should covary with antennal lobe size when navigation is primarily in relation to odorants. There are some indications that this could be the case. As in pinnipeds and sea turtles, secondarily aquatic insects, such as hemipteran water striders, have reduced antennal lobes but large mushroom bodies. Like audition in microbats, the olfactory inputs may have been replaced by mechanosensory encoding of surface ripples. The question of "what the lobes do that causes them to be retained when olfaction is lost" (Strausfeld et al., 2009) may therefore have the same answer as in mammals. To understand these potential adaptive radiations in olfactory systems across such diverse taxa, I next consider how the OS system might have evolved in their common ancestor.

EVOLUTION OF OLFACTION AND EVOLUTION OF NAVIGATION

Molecular clock and geological evidence agree that the history of bilateria began in the Ediacaran Period, 635 to 542 Myr ago (Peterson et al., 2008). This fauna lived on or just below the tough, erosion-resistant biomat surface, supporting lifestyles such as mat encrusters, mat scratchers, mat stickers, and undermat miners (Seilacher, 1999). There was no evidence for spatial sensory organs, such as paired eyes for spatial vision,

or paired antennae for spatial olfaction (Plotnick et al., 2010). The situation changed dramatically as 2D Precambrian matgrounds transformed to 3D Phanerozoic mixgrounds (Seilacher, 1999). The increasing energy content of prey could have fueled the Cambrian arms race, resulting in ever bigger and more complex predators (Plotnick et al., 2010) and associative learning (Ginsburg and Jablonka, 2010). Nonassociative learning processes, such as habituation, were likely present before the evolution of the brain, even of neurons (Moroz, 2009; Corning et al., 1973). However, it was the challenge of the transition from the peaceful "Garden of Edicara" (Seilacher, 1999) to the Cambrian bloodbath of predator eating predator that probably supplied the selective force necessary for the evolution of the first brains.

In a highly competitive regime, active prey demand active predators. It is possible that the Cambrian arms race began with the evolution of spatial olfaction and the selective advantage this would give mobile predators. Spatial representation therefore would have evolved as a concrete and specific adaptation for this purpose, exapted from the primitive building blocks of chemotaxis and chemoreception. It would function to encode, organize, and predict the locations of prey, first in olfactory space. As the arms race accelerated, predators with new sensory modalities, such as vision, could detect prey hiding in olfactory refugia, such as turbulent eddies (Conover, 2007). Adding visual cues to the olfactory space would create a robust, multisensory BE. This could then be calibrated and anchored to other reliable environmental features, such as benthic algal mats, rock formations, and magnetic fields. At this point in time, the ancestors of deuterostomes and protostomes, using the common genetic toolkit (Tomer et al., 2010), could have diverged in the details of their OS system, according to developmental constraints. However, all would retain the primacy of olfaction, that is, olfactory-guided navigation, as the ancestral function of the forebrain (Jacobs, 2012, Fig. S3), and they would for this reason eventually converge on a similar neuroarchitecture and similar cognitive mechanisms, such as cognitive mapping.

Built on the olfactory integrated map, this forebrain could encode inputs and memories at both global (i.e., BE) and local (i.e., SK) frames of reference. These frames could be used to organize new data by their similarity to old data and to make supracategorical concepts, by linking local neighborhoods via common vectors. Now the forebrain would not only encode and recall data, it could also extract new relationships de novo—relationships, like the cognitive map shortcut, that had not yet been experienced. By making this construction first in olfactory space, then in a multisensory BE, olfaction may have laid the foundation for the evolution of memory organization in the bilaterian brain.

CONCLUSIONS

The OB is a troublesome structure, one that does not scale predictably with the rest of the brain, regardless of taxonomic level of analysis, whether order, family, species, or even individual (Finlay et al., 2011). At present, there is no accepted functional hypothesis to explain this pattern of variation. The OS hypothesis offers a possible solution to this problem by proposing that olfaction evolved for the primary purpose of navigating in a chemical world. From this beginning, I propose that it developed specializations not just for the discrimination of odorants but for organizing the stimuli into functional associative memory structures. I suggest that olfactory percepts may bear evidence that this organization is a parallel map structure.

If the OS hypothesis is correct, the implications are profound. First, the primary function of olfaction would be navigation and its organization explained not by its ability to discriminate but to map odorants in space. Second, the OS system would represent the first and primary driving force in the evolution of associative learning, instantiated by the hippocampus in vertebrates and the mushroom body in arthropods and other protostomes. Not least, the hypothesis lays out a broad research program in "cognitive evo devo," an enterprise to identify the primitives of cognition hand-in-hand with the primitives of the nervous system (Jacobs, 2012, Fig. S3). The peculiar properties of olfaction, as an optimal substrate for combinatorial associative learning, may supply a foundation for this enterprise and thereby inform our understanding not just of the limbic system but of the isocortex as well.

13

Evolution of Brains and Behavior for Optimal Foraging: A Tale of Two Predators

KENNETH C. CATANIA

Star-nosed moles and tentacled snakes have exceptional mechanosensory systems that illustrate a number of general features of nervous system organization and evolution. Star-nosed moles use the star for active touch—rapidly scanning the environment with the nasal rays. The star has the densest concentration of mechanoreceptors described for any mammal, with a central tactile fovea magnified in anatomically visible neocortical modules. The somatosensory system parallels visual system organization, illustrating general features of high-resolution sensory representations. Star-nosed moles are the fastest mammalian foragers, able to identify and eat small prey in 120 ms. Optimal foraging theory suggests that the star evolved for profitably exploiting small invertebrates in a competitive wetland environment. The tentacled snake's facial appendages are superficially similar to the mole's nasal rays, but they have a very different function. These snakes are fully aquatic and use tentacles for passive detection of nearby fish. Trigeminal afferents respond to water movements and project tentacle information to the tectum in alignment with vision, illustrating a general theme for the integration of different sensory modalities. Tentacled snakes act as rare enemies, taking advantage of fish C-start escape responses by startling fish toward their strike—often aiming for the future location of escaping fish. By turning

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fish escapes to their advantage, snakes increase strike success and reduce handling time with head-first captures. The latter may, in turn, prevent snakes from becoming prey when feeding. Findings in these two unusual predators emphasize the importance of a multidisciplinary approach for understanding the evolution of brains and behavior.

S tar-nosed moles and tentacled snakes each have novel sensory appendages protruding from their faces. These appendages give both animals a unique appearance unparalleled among their peers—no other mammal or snake has comparable appendages (Fig. 13.1). However, there is more than the bizarre appearance of these animals to attract our attention. Extreme sensory specializations often reveal general principles of nervous system function and organization that are less obvious in other species (Hodgkin and Huxley, 1952; Carr and Konishi, 1990; Heiligenberg, 1991; Bass and Zakon, 2005; Kawasaki, 2009; Konishi, 2010; Nottebohm and Liu, 2010). More generally, extremes in morphology provide informative case studies in evolutionary biology. Indeed, Darwin (1859) devoted a special section of *On the Origin of Species by Means of Natural Selection* to "Organs of extreme perfection and complication." One can argue whether these unusual species seem in some way perfected, but surprisingly, the complexity of the mole's star has been cited as evidence of a divine creator (Weston and Wieland, 2003).

My goal is to review recent studies of these two species beginning with star-nosed moles, the species for which we have the most information from many years of study. The mole's nose is exceptional not only in appearance but also in the high density of mechanoreceptors that covers the nasal rays and the complexity of the modular neocortical network that processes touch information from the star. These findings make the question of how and why the star evolved even more mysterious. However, expanding studies to include the mole's habitat and behavior in the context of optimal foraging theory (Stephens and Krebs, 1986) strongly suggests a selective advantage (the ability to specialize on very small prey) that led to the evolution of the star as the highest resolution touch organ among mammals. Another extension of the research to include comparative and developmental studies provides compelling evidence for how the star evolved (Gould, 1977; Catania et al., 1999).

Recent investigations of aquatic tentacled snakes reveal a very different use for sensory appendages (Catania et al., 2010). Rather than serving active touch, the snake's tentacles seem to act as fish-detecting motion sensors. However, the most interesting finding from the tentacled snake is its remarkable ability to use fish escape responses to its advantage (Catania, 2009, 2010).
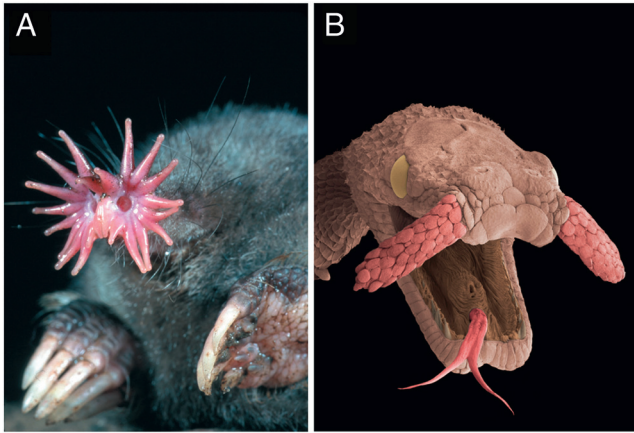
FIGURE 13.1 A star-nosed mole (*Condylura cristata*) and tentacled snake (*Erpeton tentaculatus*). (*A*) Star-nosed moles have large forelimbs, small eyes, and a nose ringed by 22 appendages or rays. (*B*) A colorized scanning electron micrograph shows the snake's scaled tentacles. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

The details of how and why each species evolved appendages are very different, but the lessons from investigating their biology are similar. In each case, an integrative approach combining neurobiological, behavioral, and ecological facets is necessary to best understand the sensory system. In the spirit of such an approach, it is hoped that the reader will view Movies S1, S2, S3, S4, S5, and S6 of Supporting Information in Catania (2012) when reading the descriptions of behavior.

SENSORY ORGANS AND INNERVATION OF THE STAR

The star is a little over 1 cm across and composed of 22 epidermal appendages or rays. Thus, it is a skin surface and not a specialization for olfaction. The rays are numbered from 1 to 11, starting with the dorsal-most ray and ending ventrally with a small ray in front of the mouth (Fig. 13.2*A*). Each ray is covered with small domes called Eimer's organs (Eimer, 1871; Van Vleck, 1965) (Fig. 13.2*B*). Such mechanosensory organs are found on the noses of most moles (Quilliam, 1966; Shibanai, 1988; Catania, 2000b) and are anatomically similar to small, domed push rods found on the snout of distantly related monotremes (Andres et al., 1991; Iggo et al., 1996; Manger and Pettigrew, 1996; Proske et al., 1998). In star-nosed moles, each organ is about 40–60 μm in diameter and has a small
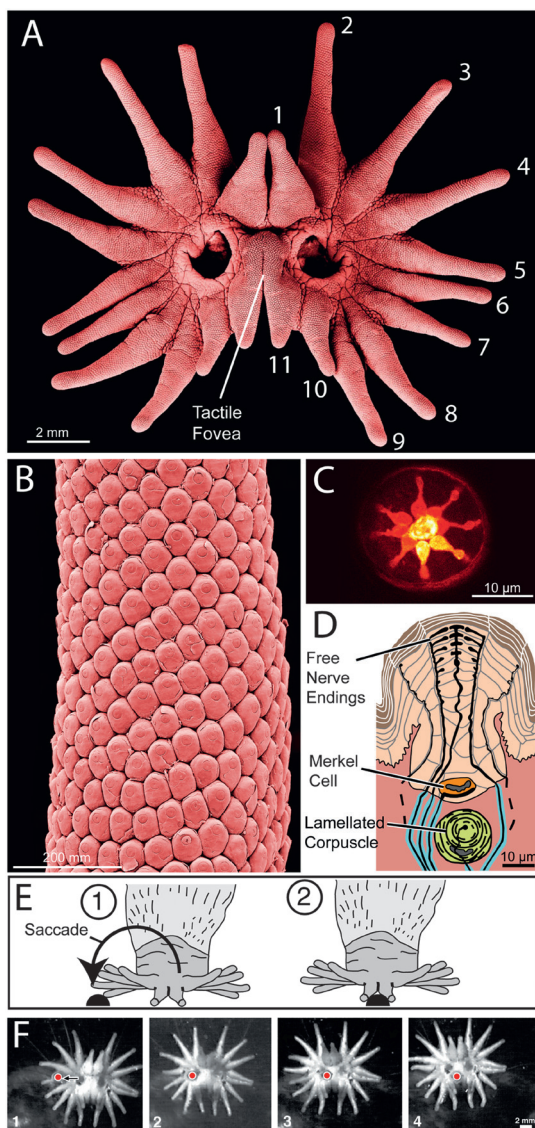
FIGURE 13.2 The epidermis of the star. (*A*) A star under the scanning electron microscope showing the 22 rays. (*B*) Higher magnification showing Eimer's organs covering a single ray. (*C*) Nerve endings labeled with DiI at the apex of an Eimer's organ (confocal microscopy). (*D*) The internal organization of a single Eimer's organ. (*E*) Schematic illustration of a saccadic star movement. (*F*) Frames from high-speed video illustrate a saccadic star movement to a small prey item (outlined in red).[Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

(15–20 μm) central disk on the outer surface. The disk is a single epidermal cell marking the top of a stack of cells that runs through the center. Each central cell column is associated with a Merkel cell–neurite complex at its base and a series of free nerve endings that travel through the column in a precise geometric ring pattern with a single nerve ending in the center (Fig. 13.2*C* and *D*). Directly below the cell column, a single lamellated corpuscle is located in the dermis.

This sensory unit is repeated 25,000 times on a typical star, providing a high concentration of mechanoreceptors. The mechanoreceptors are innervated by over 100,000 myelinated fibers (Catania and Kaas, 1997) carried by massive trigeminal nerves. The star has five times more mechanosensory afferents than the entire human hand (Vallbo and Johansson, 1984). Electrophysiological recordings from the nerves reveal tiny receptive fields on the star and show that Eimer's organs are directionally sensitive and respond to the slightest deflection (Marasco and Catania, 2007).

BEHAVIOR REVEALS A HIGH-SPEED TACTILE FOVEA

Star-nosed moles repeatedly touch the star to objects and tunnel walls as they explore their underground habitat. This behavior is very rapid; a mole may touch the star to 10–13 different places per second as it searches for food (Catania and Remple, 2004; Catania, 2012, Supporting Information, Movie S1). Despite the extreme speed of these exploratory movements, slow-motion analysis of foraging behavior reveals a functional subdivision of the star into peripheral and central touch, much like visual systems with high-acuity foveas are subdivided (Catania and Remple, 2004). The mole's tactile fovea consists of the paired 11th rays at the center of the star (Fig. 13.2*A*). Whenever moles touch something of interest with rays 1–10, they make a sudden movement of the star to position the 11th rays over the object for additional exploration [Fig. 13.2*E* and *F*; Catania (2012, Supporting Information, Movie S2, clips 1–3)]. These movements are similar to visual saccades in their form and time course (Carpenter, 1988; Catania and Remple, 2004).

STAR REPRESENTATION IN THE CNS

The segregated nature of the mole's sensory rays suggested that there could be a corresponding modular representation within cortical and subcortical areas, which was found for the whiskers of rodents (Woolsey and Van der Loos, 1970; Van Der Loos, 1976; Ma, 1991). This is indeed the case; flattened sections of cortex processed for cytochrome oxidase reveal a complex series of septa and stripes corresponding to the nose representations in several somatosensory areas (Fig. 13.3). Electrophysi-
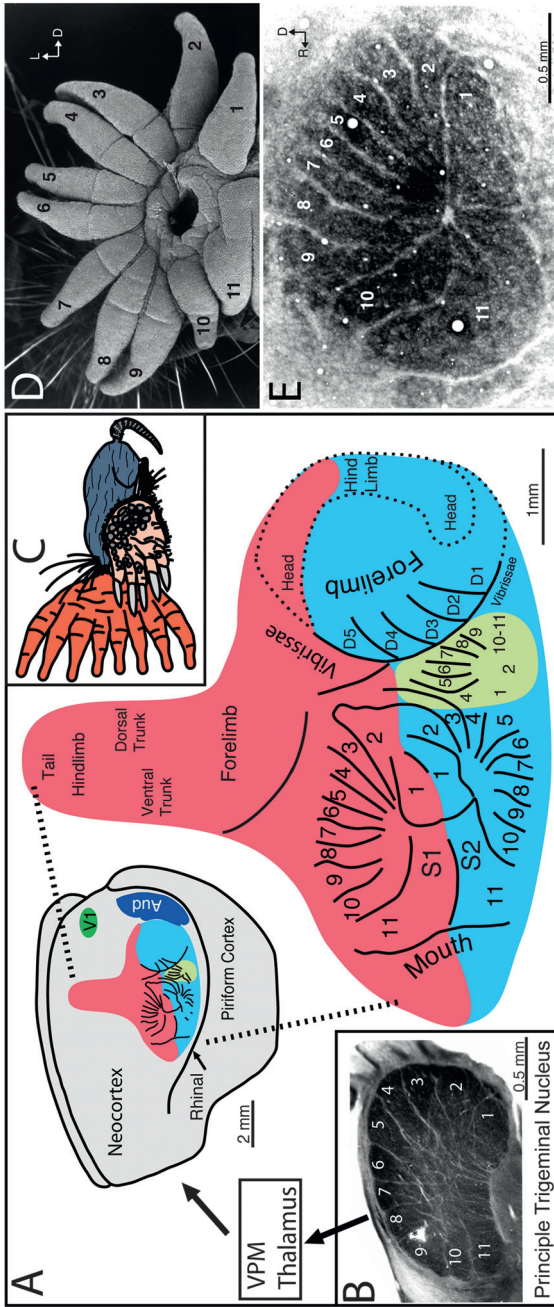
FIGURE 13.3 Mole somatosensory cortex. (*A*) The size and position of somatosensory areas with representations of different body parts labeled. (*B*) Anatomically visible star representation at the trigeminal level with each ray representation labeled. (*C*) A moleunculus showing the relative proportion of body parts represented in cortex. (*D* and *E*) The representation of the half star in primary somatosensory cortex with the 11 rays (rotated to match the cortex) under the scanning electron microscope (SEM) and the cortical representation in a cytochrome oxidase stain. Note the large representation of ray 11 (the tactile fovea) relative to its size on the star.

ological recordings reveal three maps of the contralateral star in lateral cortex (Catania and Kaas, 1995; Catania, 2000a). Each map can be seen as a separate series of stripes representing the nasal rays. The most distinctive area corresponds to the primary somatosensory representation (S1) of the star. The secondary somatosensory area (S2) also contains a large star representation. A third smaller star representation is located just caudal to S2. Injections of neuroanatomical tracers show that S1 is topographically interconnected with the corresponding ray representations in S2 and S3, forming a cortical processing network (Catania and Kaas, 2001). Finally, recent investigation of the principal trigeminal sensory nucleus (Catania et al., 2011) reveals a large, visible representation of the star consisting of 11 modules that bulge out of the brainstem (Fig. 13.3*B*). The mole's principal nucleus is proportionally much larger than the corresponding nucleus in rodents (Ashwell et al., 2006).

Four features of the neocortex highlight the specialized nature of star-nosed mole brains. First and most obviously, a large proportion of somatosensory cortex is devoted to the star. This example of extreme cortical magnification is schematically illustrated in Fig. 13.3*C*. Second, star-nosed moles are the only species with three anatomically visible cortical representations of a single sensory surface. Third, within S1, the 11th foveal appendage is greatly overrepresented relative to its size, the number of sensory organs on its surface, and the number of nerve fibers that supply the ray. This finding parallels the way that visual systems are organized (Azzopardi and Cowey, 1993) and suggests a general organizational framework for the evolution of high-resolution sensory systems [Suga et al. (1975) and Azzopardi and Cowey (1993) discuss bats]. Fourth, star-nosed moles have an extra cortical representation of the nose compared with other moles and shrews (Suga et al., 1987). This finding suggests that star-nosed moles have added a cortical area to their processing network.

OPTIMAL FORAGING AND THE FUNCTION OF THE STAR

Having outlined the unusual and specialized nature of the mole's somatosensory system, it seems natural to wonder why such a structure evolved. It is not enough to suggest that they simply have a very well-developed sense of touch, because many other moles are touch specialists. What can star-nosed moles do that other moles cannot? A likely answer comes from considering the star-nosed mole's behavior and environment in the context of optimal foraging theory.

Competition in the Swamp

Star-nosed moles are the only mole species that lives in the muddy soil of wetlands. Unlike typical mole habitats where soil is dense and stable,

tunnels in wetlands tend to be shallow, ephemeral, and interspersed with grassy runways and leaf litter. As a result, the tunnels are accessible to diverse mammals that also feed on the many invertebrates in the nutrient-rich soil. For example, when live-trapping star-nosed moles, we usually capture a greater number of other insectivores (Catania, 2012, Supporting Information, Fig. S1) that share the same tunnels (mostly shrews). Thus, star-nosed moles have substantial competition for prey.

A second feature of star-nosed mole habitats is the small size of the prey compared with more terrestrial settings. Wetlands are a rich source of small invertebrates (Anderson and Smith, 2000). Our preliminary comparisons of invertebrates around the wetland tunnels of star-nosed moles and more terrestrial tunnels of eastern moles (*Scalopus aquaticus*) found the wetland prey to be an average of 20 times smaller than the prey in the drier habitat (Catania, 2012, Supporting Information, Fig. S1). This finding is consistent with gut content studies of star-nosed moles, which show that they eat large numbers of these small invertebrates (Hamilton, 1931).

Prey Profitability and Star-Nosed Mole Behavior

The former considerations suggest that star-nosed moles live in a competitive environment with diverse prey. With these observations in mind, it is useful to turn briefly to mathematical models of predator behavior for additional clues to answer why the star might have evolved. Foraging theory (Stephens and Krebs, 1986) provides a framework for predicting how predators may behave, assuming that the goal is to maximize the rate of energy gained while foraging. In this paradigm, the rate of energy intake (R) is equivalent to $E/(T_s + T_h)$, where E is the energy gained from a prey item, T_s is the time spent searching for prey, and T_h is the time spent handling prey (handling time includes pursuit, capture, and consumption of prey). A key variable in considering which prey items should be included in the optimal diet is prey profitability (P). Prey profitability is simply the ratio of energy gained (E) to handling time (T_h). Prey profitability has the general form of the equation $y = 1/x$, with y (profitability) approaching infinity as x (handling time) approaches zero (Fig. 13.4A). In this formulation (often called the prey model) [Stephens and Krebs (1986) have a full treatment], the optimal diet is obtained by adding prey items to the diet if (based on their profitability) they increase the average rate of energy intake or alternatively, rejecting prey items if they decrease the average rate of energy intake while foraging.

The results of these measurements for star-nosed moles are astounding (Catania and Remple, 2005); star-nosed moles have the shortest handling time documented for any mammal when consuming small prey (Catania, 2012, Supporting Information, Movie S2, clips 4 and 5). In a
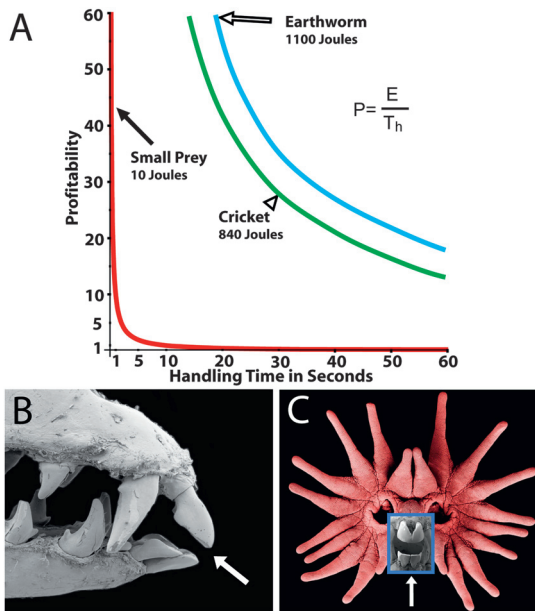
FIGURE 13.4 Profitability of prey. (*A*) A graph showing the profitability of prey relative to handling time for three different sizes corresponding to different amounts of energy. The red line represents profitability for small (10 J) prey vs. handling time, which was used in mole feeding experiments (Catania and Remple, 2005). For most handling times, small prey items are minimally profitable. However, star-nosed moles have very short (average of 227 ms) handling time, making small prey profitable (filled arrow). Larger prey items are much more profitable overall (green and blue lines) for similar handling times, but they take much longer to handle (open arrowhead and open arrow; 20–30 s), making them similar to small prey in profitability. (*B*) Unusual front teeth in star-nosed moles (arrow). (*C*) These teeth are located directly behind the tactile fovea and are used for rapidly picking up small prey (Catania, 2012, Supporting Information, Movie S2 shows tooth use). [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

laboratory setting, they were able to identify a prey item (small earthworm segments of 10 J energy content), make a saccadic movement to the tactile fovea, and then consume the prey in as little as 120 ms (Catania and Remple, 2005). The average handling time for small prey was 227 ms. When profitability for small prey is plotted relative to handling time, the value for star-nosed moles is surprisingly large (Fig. 13.4*A*, red line), corresponding to a position high on the vertical asymptote. It seems that star-

nosed moles have come as close as possible to zero handling time. This latter conclusion is supported by the frequent occurrence of double takes when food is first contacted (Catania and Remple, 2005). In these cases, moles contact the prey but briefly move in the wrong direction before foveating to the item (Catania, 2012, Supporting Information, Movie S2, clips 2 and 3), suggesting that nervous system processing of touch lags behind the rapid star movements.

To put short handling time in context, it is important to consider profitability for larger prey items. This profitability is illustrated for a cricket (840 J) and a large earthworm segment (1,100 J) by the green and blue lines, respectively, in Fig. 13.4A. These latter plots of prey profitability vs. handling time dwarf the plot for small 10 J prey. It is telling that star-nosed moles seldom eat chitinous crickets, whereas short-tailed shrews (*Blarina brevicauda*) eat these crickets and other insects. The handling time for a short-tailed shrew to consume a cricket is roughly 30 s (Catania and Remple, 2005). Remarkably, this time could make small prey items more profitable to star-nosed moles than much larger insects are to competing insectivores.

These considerations suggest that star-nosed moles should include small prey in their diet. However, there is additional evidence to support this interpretation. The front teeth of star-nosed moles are unique among mammals (Fig. 13.4B). They are tiny, with a refined shape that requires the union of two upper teeth and four lower teeth across the midline to form what appears to be a small beak. This tweezer-like structure is located directly behind the somatosensory fovea (Fig. 13.4C), and it is used to efficiently pluck small prey from the substrate (Catania, 2012, Supporting Information, Movie S2, clip 4). The behavioral sequence is closely integrated with star movements, such that the 11th foveal appendages spread apart to accommodate the small teeth. The tiny, specialized teeth are strong evidence of a long evolutionary history of star-nosed moles feeding on small prey. However, it is also important to note that star-nosed moles have larger back teeth for eating larger prey items, especially soft-bodied earthworms (Fig. 13.4B). This finding is consistent with optimal foraging theory, which predicts that large prey items are also profitable (Fig. 13.4A). The conclusion is that star-nosed moles can include small prey items in a broader diet of invertebrates.

Function of the Star

A number of facets of the mole's behavior and environment suggest that it is adapted to rapidly locate small prey, presumably providing a resource that is difficult for other competing species to exploit. For example, profitability would be many times lower for a competitor that handled

small prey for even 0.5 s longer than a star-nosed mole (Fig. 13.4*A*, red line). Observations of eastern moles (*S. aquaticus*) presented with arrays of small prey (Catania, 2012, Supporting Information, Movie S2, clip 6) clearly show that star-nosed moles are more efficient at exploiting this resource. Thus, numerous small Eimer's organs, modified teeth integrated with the star, and many CNS specializations seem to be adaptations that help to reduce handling time (T_h) such that small prey can be exploited.

There is an additional component of the sensory system that can be interpreted in light of optimal foraging theory—the large size of the star compared with the nose of other moles (which also have Eimer's organs). The expanded surface area means that star-nosed moles contact a large area with each touch, and this area, in turn, reduces time searching compared with the time searching by moles with a smaller array of sensors (Catania and Remple, 2005). Time searching (T_s) is the other part of the denominator used to calculate *R*, and therefore, minimizing both T_h and T_s maximizes the rate of energy intake. Put another way, profitable small prey items are only useful if they can be taken in large numbers, and for that to occur, prey must be located. Thus, optimal foraging theory suggests both the behavior and anatomy of the star-nosed are admirably adaptive.

HOW DID THE STAR EVOLVE?

Having described the function of the star and by extension, the likely selective pressures that led to its evolution, there remains the question of how it evolved. The star is a biological novelty consisting of many appendages, and it might be expected to have evolved by redeployment of conserved developmental mechanisms for appendage formation. Although we do not yet have evidence for genetic patterning mechanisms, the unusual morphology of the developing star seems to tell the story of its evolution (Catania et al., 1999). When the star first begins to emerge in embryos, it appears as if the rays are folded backward on the snout (Fig. 13.5*A*). However, sections through the developing nose (Fig. 13.5*B*) show that each nascent ray is simply a swelling of the epidermis with no underlying cell layer to form the bottom portion. Later in development (Catania et al., 1999), a second layer of epidermis extends below the nascent rays to form the bottom wall, and the rays become backward-facing cylinders embedded in epidermis of the face. Shortly after birth, these cylinders emerge, break free, and bend forward to form the adult star. To summarize, the rays develop in place as backward-facing cylinders that later detach and rotate forward (Fig. 13.5*C*). As a consequence, the tip of each ray is derived from tissue more caudal than the base of each ray, because each ray reverses its orientation during development—an unprecedented mechanism for appendage formation.
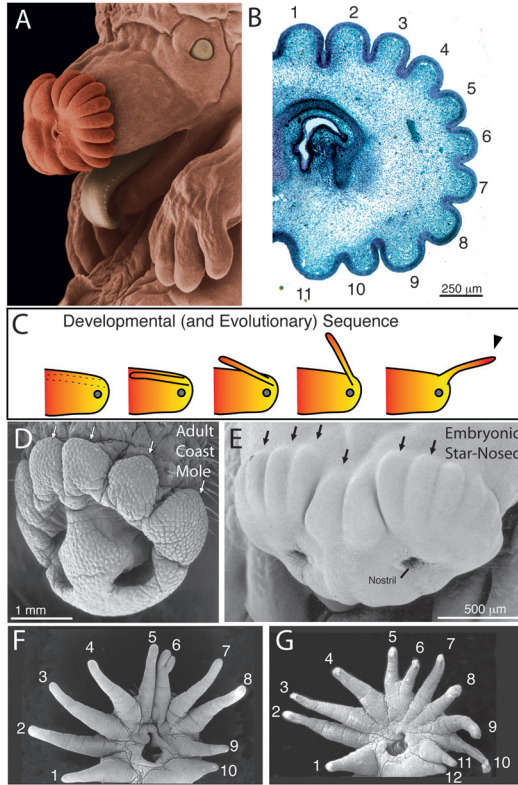
FIGURE 13.5 Development reveals evolution of the star. (*A*) An embryonic star-nosed mole showing the nascent rays. (*B*) A section of the snout (same stage as *A*) reveals the rays as swelling or waves in the epidermis with no underlying structure to form a complete cylinder. Later in development, a second layer of epidermis forms under these epidermal waves to form backward-facing cylinders. (*C*) The developmental sequence illustrated schematically for a single ray. The ray forms in place facing backward and then emerges from the side of the face to bend forward. The tip (arrowhead) is thus formed by caudal snout tissue (orange). (*D*) An adult coast mole showing extensions of Eimer's organs attached to the side of the face, which was hypothesized for ancestral star-nosed moles. (*E*) An early embryonic star-nosed mole nose looks strikingly similar to an adult coast mole nose. (*F* and *G*) Congenitally abnormal mole noses with (*F*) fewer or (*G*) greater numbers of rays. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

Why would such an apparently poorly engineered developmental sequence exist (Jacob, 1977)? Perhaps star-nosed moles evolved from an ancestor with strips of sensory organs on its snout that later raised up and

bent forward over many generations. In the absence of additional evidence, this hypothesis would have to remain very tentative. However, the discovery of a mole with just such an intermediate stage of sensory organs on its (adult) nose provides powerful support for this suggestion. The coast mole (*Scapanus orarius*) has a series of short strips of Eimer's organs that extend caudally on the snout. The adult coast mole nose has a striking resemblance to an early embryonic star-nosed mole nose (Fig. 13.5*D* and *E*). Of course, the coast mole is not the ancestor of the star-nosed mole, but the existence of this protostar in a living species strongly suggests that such an ancestor to the star-nosed mole existed. A similarity between the adult, ancestral anatomy and an extant embryonic form was predicted by Gould (1977) for developmental sequences that have been built upon with evolutionary changes occurring primarily at the terminal stages of development (Gould, 1977).The result is partial recapitulation of an evolutionary sequence during development (Gould, 1977; Northcutt, 1990).

The most obvious difference between the morphology of adult coast mole sensory swellings and embryonic star-nosed mole swellings is the greater number on the latter. This difference is not hard to account for, because sudden duplications of rays could readily occur. Such meristic changes are common in evolution (Raff, 1996). In fact, we commonly find star-nosed moles with congenitally abnormal noses (Catania et al., 1999). Approximately 5% of star-nosed moles have either greater or fewer than the usual 22 rays (Fig. 13.5*F* and *G*). This finding is a high rate of abnormality, much greater than for the tetrapod limb (Castilla et al., 1996; Zguricas et al., 1998). Darwin (1859) predicted this kind of variability in *On the Origin of Species by Means of Natural Selection*, stating that "in those cases in which the modification has been comparatively recent and extraordinarily great . . . we ought to find the generative variability, as it might be called, still present to a high degree" (Darwin, 1859). Thus, far from being inexplicable (Weston and Wieland, 2003), star-nosed moles provide strong support for basic evolutionary principles, including Darwin's predictions for rates of variation, Gould's (1977) theories of the relationship between ontogeny and phylogeny, and the "tinkering" nature of evolution (Jacob, 1977), which often produces new and unusual solutions to old developmental problems.

FISHING SNAKE

At first glance, the rays of the star-nosed mole and the tentacles of the tentacled snake seem superficially similar. Both are flexible extensions of the epidermis on the front of the face. However, the behavior of the two species and the function of their appendages are very different. Star-nosed moles are active explorers that move the rays in a flurry of

motion as they travel through their tunnels or forage in shallow water. In contrast, tentacled snakes are sit-and-wait predators (Fig. 13.6). They are fully aquatic and never leave the water, and they feed exclusively on fish. When hunting, the snake adopts a J-shaped posture (Fig. 13.6*A*) and waits for fish to enter the concave area formed by the bend of its neck and head. When fish are in this favorable position, the snake strikes explosively and typically reaches the position of the fish in about 25 ms (Catania, 2009). Given this hunting strategy, it seems reasonable to hypothesize that the tentacles function as fish detectors. This idea and others have been suggested for over a century, but only recently, experiments have been conducted to investigate various possibilities. The function of the tentacles was explored using a multifaceted approach that included anatomical investigation of their internal and external anatomy, electrophysiological recordings from the trigeminal afferents and the optic tectum, and behavioral observation based on slow motion analysis of high-speed video recordings under visible or infrared lighting (Catania et al., 2010).
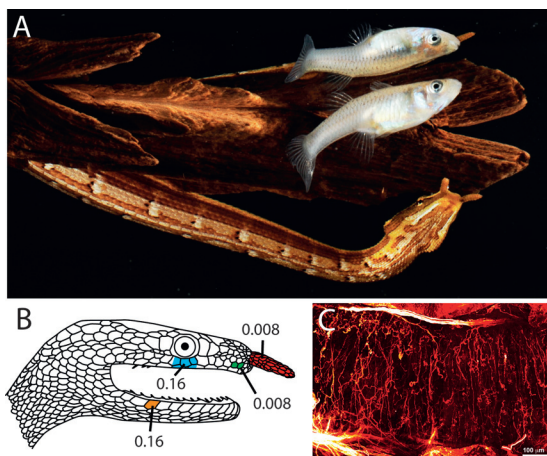
FIGURE 13.6 Tentacled snake hunting posture and sensory appendages. (*A*) The characteristic J-shaped hunting position for this sit-and-wait predator. (*B*) Examples of single-unit receptive fields for trigeminal afferents and the lowest forces (grams) that produced action potentials. (*C*) The dense network of fibers that traverse the center of the tentacle. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

APPENDAGES OF TENTACLED SNAKES ARE SENSITIVE MECHANOSENSORS

Two branches of the trigeminal nerve innervate each tentacle. Confocal microscopy of fluorescently (DiI) labeled fibers reveals a dense array of fine-nerve terminals that cross the middle of each tentacle orthogonal to the long axis (Fig. 13.6*C*). The fibers are poorly placed for detecting details of stimuli that compress the epidermis (in contrast to fibers in Eimer's organs) but are well positioned for detecting movement of the entire tentacle. Electrophysiological recordings from trigeminal afferents confirm this suggestion (Catania et al., 2010). The tentacles are sensitive to the slightest deflection caused by the finest calibrated von Frey hairs (Fig. 13.6*B*). When the snake's head is submerged in water, tentacle afferents also respond strongly to movement of a nearby vibrating sphere used to simulate moving fish. The tentacles are not responsive to electric fields, and there is no evidence of electroreceptors or chemoreceptors on their surface (Catania et al., 2010).

TACTILE AND VISUAL RESPONSES IN THE TECTUM

As would be expected, the snake's optic tectum is highly responsive to visual stimuli (Catania et al., 2010). Receptive fields for neurons in the superficial layers of the tectum form a visuotopic map of the contralateral eye, with superior fields represented dorsally, inferior fields represented laterally, nasal fields represented rostrally, and temporal fields represented caudally. Compared with vision, tactile responses in the tectum are less refined with larger receptive fields and weaker responses. Nevertheless, the overall topography of the somatosensory representation is in approximate register with the overlying visual representation, suggesting that mechanosensory and visual cues are integrated in this region (Catania et al., 2010).

TENTACLED SNAKE BEHAVIOR

To further explore the function of the tentacles, snakes were filmed under visible or infrared illumination (the latter is used to control for vision). The results under lighted conditions showed that fish seldom approach the tentacles, and therefore, a function as lures seems unlikely. This conclusion is also supported by the observation that snakes seldom strike at fish directly adjacent to their tentacles, probably because it is not possible to generate sufficient striking momentum over short distances. Under 950-nm wavelength illumination (Catania, 2012, Supporting Information, Movie S3), which they cannot see (Catania et al., 2010), tentacled

snakes are still able to strike and capture fish. However, they strike less often in the absence of eyesight and are less accurate. Overall, the results suggest that the main function of the tentacles is to aid in the localization of fish when eyesight is reduced at night or in murky water, thus allowing prey capture in a much wider range of conditions than for vision alone. However, a number of observations suggest that tentacled snakes rely most heavily on visual cues to guide their strikes when possible (Catania, 2009, 2010; Catania et al., 2010). This finding should not be too surprising, and it does not detract from the usefulness of the tentacles. For example, barn owls are renowned for their hearing but have acute vision that guides their attacks when available. Similarly, a pit viper can easily strike visible targets, but this ability does not detract from the use of the infrared-detecting pits for hunting warm-blooded prey at night or in underground burrows. Thus, many species have adaptations that importantly supplement more dominant visual systems.

TURNING THE TABLES ON FISH

Although tentacled snakes can make an explosive strike with remarkable speed (Smith et al., 2002; Catania, 2009), fish are expert escape artists with a well-studied neural circuitry that mediates high-speed evasion of predators (Zottoli, 1977; Eaton and Hackett, 1984; Faber et al., 1989; Korn and Faber, 2005). The fish C-start escape response has an onset latency of only about 7 ms from the detection of a water disturbance and begins with a C-shaped bend of the body followed by propulsion away from the predator. The C-start is mediated by two giant Mauthner cells (neurons), one cell on each side of the brainstem. Turning away from an approaching predator is important, and the decision about which direction to turn (which Mauthner cell fires first) occurs in the first few milliseconds after detecting a threatening stimulus. Within 25 ms, the fish is in mid C-start and primed to swim farther away. However, it takes a snake only about 25 ms to reach a fish when striking. Thus, the snake's strike and the fish's C-start have been consistently pitted against each other for the long evolutionary history of this predator–prey interaction. Fig. 13.7 outlines the senses and behaviors used by snakes and fish in this competition.

In adapting to this long-standing challenge, tentacled snakes have evolved a remarkable strategy to take advantage of the specialized escape circuitry of fish (Catania, 2009). Recall that tentacled snakes prefer to strike at fish that have entered the concave area formed by the J-shaped hunting posture. Just before the strike, the snake feints with its body, which is on the opposite side of the fish relative to the snake's jaws. As a result, fish usually (~80% of the time) turn away from the snake's body and thus toward the approaching jaws—sometimes swimming
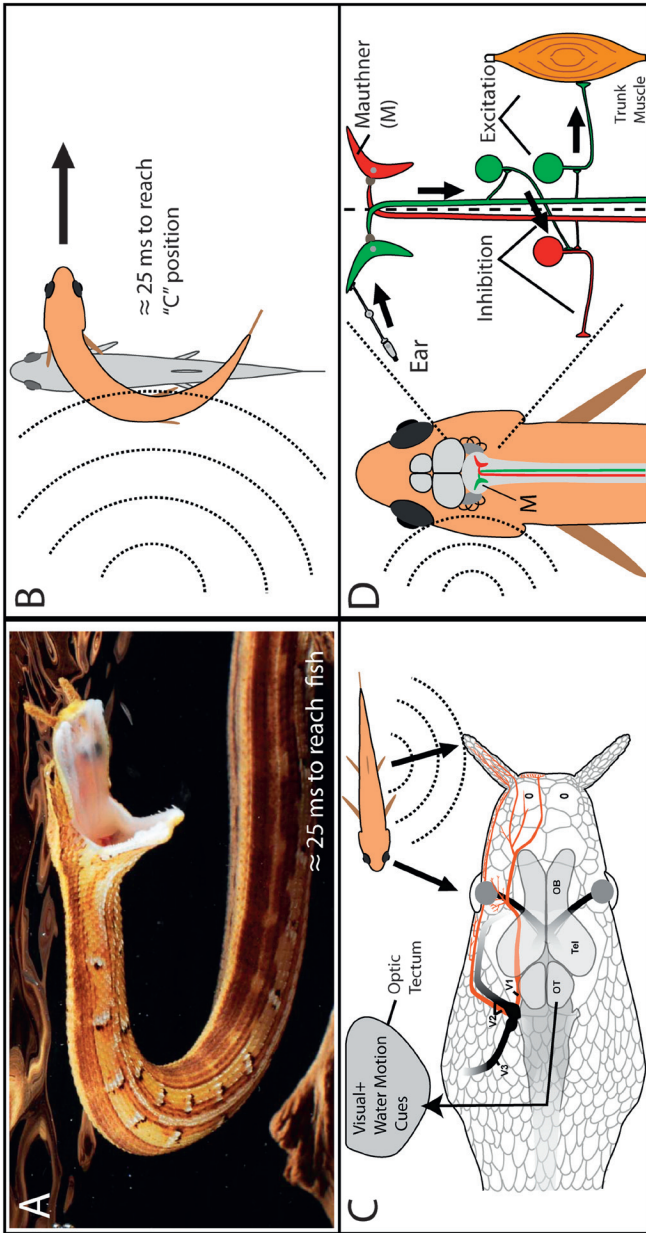
FIGURE 13.7 Summary of the sensory systems and behavior in the predator–prey interaction between snakes and fish. (*A*) A striking tentacled snake takes roughly 25 ms to reach a nearby fish (Catania, 2012, Supporting Information, Movie S4). (*B*) A directional C-start escape response occurs 7–8 ms after sound detection, and within 25 ms, the C-shaped posture is attained. (C) Visual and mechanosensory information converges in the snake's optic tectum. (*D*) The Mauthner circuitry mediating fish escape responses. When a threatening sound occurs on the left side, the left Mauthner neuron usually fires first (green), projecting to the right side of the body and stimulating right trunk muscles while inhibiting left muscles (red) to cause a right turn away from the stimulus. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

straight into the snake's mouth (Catania, 2012, Supporting Information, Movie S4). Hydrophone recordings correlated with video recordings confirm that a pressure wave is generated by the snake's initial body feint (Catania, 2009). These results explain an early description in the work by Cornellissen (1970) that fish are partially swallowed during strikes and the later observation in the work by Murphy (2007) that some fish disappeared completely in a single video frame when strikes were filmed at 30 frames/s, suggesting minimal or even nonexistent handling times (Murphy, 2007). Swimming into the mouth of your adversary certainly reduces handling time.

Startling fish toward the strike is an impressive adaptation, but this strategy is only feasible when fish are oriented roughly parallel to the snake's jaws (Fig. 13.8*A*). When fish are oriented at a right angle to the jaws, the C-start can translate fish to one side or the other but not directly toward the strike. In these cases, the snake uses an even more surprising strategy—it feints with its body and aims for the far side of the fish, which is the most likely future position of the head (Fig. 13.8*B*; Catania, 2012, Supporting Information, Movie S5). Most fish turn away from the body feint, often placing their head directly into the snake's oncoming jaws. Because the strike is ballistic and does not make use of visual feedback, these attacks require a prediction of future fish behavior. The latter is clear from the speed of the strike, which begins before the C-start, and the fact that snakes retract their eyes when they strike (Catania, 2009). It can also be shown by examining trials when no C-start (or opposite C-starts) occurred (Catania, 2009) and snakes usually struck to the most likely (but incorrect) future location of the moving head (Fig. 13.8*C*).

The snake's strategy of startling fish toward the strike has the obvious benefit of improving capture success. However, it also has the added advantage that most fish are caught head-first and often partly swallowed, greatly reducing handling time (Murphy, 2007). When this time was measured explicitly by manually presenting fish either head- or tail-first, the former allowed for much shorter swallowing (handling) times. Because tentacled snakes are cryptic sit-and-wait predators, the more quickly they can swallow prey, the more likely they are to remain camouflaged to other nearby fish, thus indirectly increasing their foraging efficiency. In addition, tentacled snakes often exhibit a tail-wiggling behavior when swallowing large fish (when handling time is long). This behavior may distract the snake's own predators, which would presumably attack the wiggling tail, allowing for escape. This subtle but important behavioral adaptation suggests that snakes are in danger of becoming prey themselves when movement (e.g., swallowing fish) breaks their camouflage. Thus, reduced handling time likely has benefits related to both long-term foraging efficiency and short-term survival.
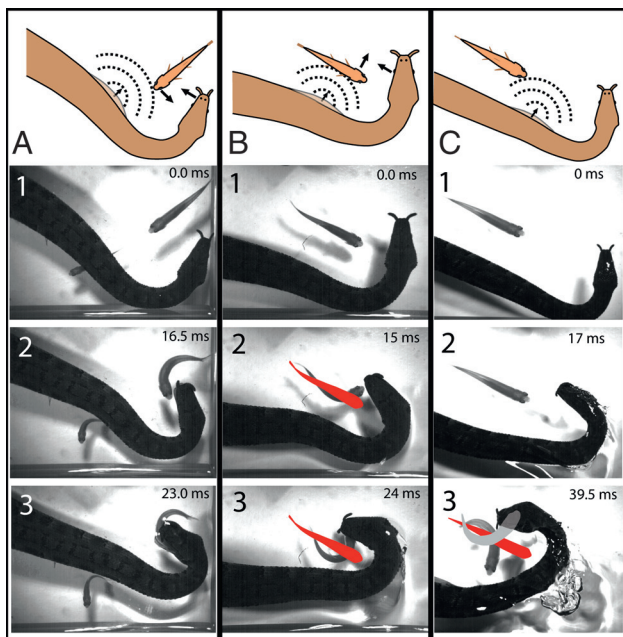
FIGURE 13.8 Frames captured from high-speed video illustrate tentacled snake strikes. (*A*) In this trial, a fish is oriented roughly parallel to the jaws. The snake startles the fish to its strike and into its mouth (Catania, 2012, Supporting Information, Movie S4). (*B*) In this trial, a fish is at an approximately right angle to the jaws. The snake startles the fish and strikes to the future location of the head (Catania, 2012, Supporting Information, Movie S5). The red outline shows the original position of the fish. (*C*) In this trial, the snake fails to elicit a C-start, and instead, the fish responds to the snake's moving head, turning to the body (Catania, 2009). Nevertheless, the snake aimed for the approximate future location of the fish's head had it responded to the body feint (gray; reflected C-start). Small numbers show the number of milliseconds from the first movement of the snake. [Note: Figure can be viewed in color in the PDF version of this volume on the National Academies Press website, www.nap.edu.]

BORN KNOWING

The observation that tentacled snakes can startle fish and predict their future movements raises the question of whether this strategy is an innate ability or learned through a lifetime of striking at escaping fish. This question was addressed in laboratory-born snakes that had never experienced live fish. To prevent the naïve snakes from learning during the trials, snakes were placed in a chamber above fish, separated by a

thin transparency sheet. The transparency sheet (instead of glass) was chosen to minimize the distance between the fish and snake. However, it also had the unexpected benefit of allowing the pressure wave generated by the snake's body feint to startle underlying fish. As a result, snake movements could be observed in relationship to escaping fish, although snakes could never contact fish (when the flexible barrier was replaced by glass, fish did not respond to the purely visual stimulus of striking snakes) (Catania, 2010).

The results clearly show that tentacled snakes are born with the ability to make predictive strikes (Catania, 2012, Supporting Information, Movie S6). The surprising ability of naïve snakes to predict the future behavior of their prey is a testament to the long evolutionary history of this predator–prey interaction. It is an example of selection acting on innate behavior over the course of evolution in contrast to learning, which selects behavior during an animal's lifetime. In this sense, tentacled snakes fall on the extreme nature side of the nature vs. nurture continuum, at least for striking behavior. Tentacled snakes also provide a compelling example of the rare enemy effect as outlined in the work by Dawkins (1982). In this scenario, a predator may take advantage of a trait that is usually adaptive. Because tentacled snakes are less common than a host of other predators, the best bet for a fish is to turn away from a water disturbance. It is an unlucky fish that encounters a tentacled snake and makes a wrong turn.

CONCLUSIONS

Specialized sensory systems are inherently interesting to biologists because they represent extremes in the process of evolution. However, they are more than curiosities—they represent a challenge and an opportunity. The challenge is to understand how they function and why they evolved in the context of their environment. The opportunity comes in the form of more general insights into biological processes that may be derived from this understanding. Star-nosed moles and tentacled snakes provide examples of these dual perspectives. Star-nosed moles conveniently show principles of mammalian brain organization owing to the multiple anatomically visible maps of the star in the neocortex. However, they also provide clues to more general biological principles such as theories of predator diet selection or the relationship between development and evolution. Tentacled snakes similarly show how information from different senses is integrated in the tectum and the importance of multiple cues for detecting environmental stimuli. However, the most surprising finding for this species is their ability to startle fish toward strikes, thus taking advantage of the neural circuitry that mediates obligatory fish escape responses. Tentacled snakes provide a concrete example of the

rare enemy effect, which suggests that uncommon predators may tap into prey behavior that is usually adaptive. These various discoveries in two divergent species illustrate the necessity of integrating neurobiological, behavioral, and ecological approaches to best understand adaptations.

ACKNOWLEDGMENTS

Part IV

PHYLOGENY OF HUMAN BRAINS AND HUMAN MINDS

The chapters in Part IV address the question of human uniqueness in brain organization and behavior. In Chapter 14, Todd Preuss focuses on molecular genetic differences between human brains and the brains of our closest relatives. Particular emphasis is given to the role of *foxP2*, which has, at times, been called the human language gene. Not surprisingly, the true story of *foxP2* is more complex, because as Preuss puts it, "we are trying to relate a multifunctional gene to a complex, high-level phenotype." To deal with this complexity, Preuss suggests that we need a better understanding not of single-gene variation, but of variation in many genes and, particularly, brain development. Preuss also notes that human brains mature more slowly than the brains of other species, which would explain why brain metabolic activity is surprisingly high and structural plasticity unusually protracted in humans. Particularly interesting is the observation that some patterns of gene expression in the prefrontal cortex of humans are seen only during development in other species. The mechanisms underlying this heterochrony as well as their functional sequelae remain unclear. However, childhood is well known to be more protracted in humans than in other apes.

Lizabeth Romanski reviews in Chapter 15 the anatomical and physiological organization of the ventrolateral prefrontal cortex (vlPFC) of macaque monkeys. This cortical region is of special interest because its homolog in humans includes several language-related areas (e.g., Broca's area). In a key experiment, Romanski and her colleagues took movies of vocalizing monkeys, separated them into audio and visual streams, and

showed them to other monkeys with recording electrodes in their vlPFC. This experiment revealed that the majority of vlPFC neurons integrate auditory and visual information in a nonlinear manner. This finding is important because human speech perception also involves a considerable amount of audiovisual integration, as demonstrated by the McGurk effect (McGurk and MacDonald, 1976). Of course, audiovisual integration of vocalization-related stimuli is not identical to speech perception, which requires the integration of sounds and visual information with meanings. The latter type of integration still eludes the understanding of neurobiologists and is extremely difficult to study in monkeys. Nonetheless, the audiovisual integration that Romanski describes in monkeys is likely to have played a major role in the evolution of human language.

In Chapter 16, Jessica Cantlon compares the mathematical abilities of nonhuman primates and humans, especially human children. Although we often think that mathematics requires symbols (e.g., numbers and operators), simple math can be performed without symbols. For example, one can compare two images and estimate, even without counting, which image contains more items of a particular sort. This kind of analog numerical estimation can also be performed by human infants and nonhuman primates. Cantlon further reports that the analog math task activates homologous brain areas in the parietal cortex of both humans and monkeys. Collectively, the data strongly suggest that analog math abilities evolved long before the origin of *Homo sapiens*. This finding is fascinating, but how did symbolic math evolve? Was it built on top of the more ancient analog skill, using the ancient circuitry with only minor modifications? Or did symbolic math evolve out of symbolic communication (i.e., language)? At this point, the answer is unknown.

In the final Chapter 17, Clark Barrett dispels the notion—promulgated by some evolutionary psychologists—that adaptive specializations in the brain must be hard-wired modules. To grasp the argument, consider face-selective neurons in primate brains. Given the importance of conspecific faces in the lives of most primates, the distinct patches of face-selective neurons in monkey and human brains were likely shaped by natural selection. Nonetheless, the development of face-selective neurons probably depends on extensive experience with faces. Indeed, Barrett hypothesizes that selection generated not an innate face-processing module but a set of mechanisms that, given experience with faces, will generate a large number of neurons that selectively encode faces. Given other types of experience, the same mechanisms would (and do) generate patches of neurons selective for other kinds of behaviorally important stimuli. Stated succinctly, Barrett argues that natural selection generates developmental norms of reaction rather than experience-independent specialized modules. This idea extends evo-devo neurobiology into the realm of evolutionary psychology.

14

Human Brain Evolution: From Gene Discovery to Phenotype Discovery

TODD M. PREUSS

The rise of comparative genomics and related technologies has added important new dimensions to the study of human evolution. Our knowledge of the genes that underwent expression changes or were targets of positive selection in human evolution is rapidly increasing, as is our knowledge of gene duplications, translocations, and deletions. It is now clear that the genetic differences between humans and chimpanzees are far more extensive than previously thought; their genomes are not 98% or 99% identical. Despite the rapid growth in our understanding of the evolution of the human genome, our understanding of the relationship between genetic changes and phenotypic changes is tenuous. This is true even for the most intensively studied gene, *FOXP2*, which underwent positive selection in the human terminal lineage and is thought to have played an important role in the evolution of human speech and language. In part, the difficulty of connecting genes to phenotypes reflects our generally poor knowledge of human phenotypic specializations, as well as the difficulty of interpreting the consequences of genetic changes in species that are not amenable to invasive research. On the positive side, investigations of *FOXP2*, along with genomewide surveys of gene-expression changes and selection-driven sequence changes, offer the opportunity for "phenotype discovery," providing clues to human phenotypic specializations that were previously unsuspected. What is more,

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at least some of the specializations that have been proposed are amenable to testing with noninvasive experimental techniques appropriate for the study of humans and apes.

The ability to sequence the whole genome of a species, along with other advances in molecular biology and in bioinformatics, has ushered in a remarkable new era of human evolutionary studies. We might reasonably expect that these developments have advanced our understanding of the evolution of the human brain and its functional capacities. Here, I will argue that this is the case, although the path connecting genes to phenotypes is not as straight as one might suppose.

COMPARATIVE GENETIC AND MOLECULAR BACKGROUND

To appreciate how far we have come in this field, and what we have yet to accomplish, it is useful to note where we were in the late 1990s, just before the comparative genomics revolution. What were scientists' expectations about the kinds of molecular changes that occurred in human evolution? What was the nature of the phenotypic changes that they expected to explain or illuminate with comparative molecular studies?

It has long been understood that the evolution of biological features that do not fossilize, including molecules, can be reconstructed by comparing appropriately chosen species. Human specializations are, by definition, features of the human species that evolved in our lineage after it separated from the lineage leading to chimpanzees and bonobos, our closest relatives. A claim about human specializations requires comparing the human species to its sister taxa (chimpanzee and bonobos), to demonstrate that there are differences between these species, and then comparing the human–chimpanzee–bonobo group vs. other apes and monkeys, to estimate whether the common ancestor of humans, chimpanzees, and bonobos resembled humans or chimpanzees and bonobos (Fig. 14.1). The more species that can be studied, the more reliable the evaluation of evolutionary change. Unfortunately, many of the ape species—including bonobos, gorillas, orangutans, and gibbons—are not readily accessible even for noninvasive studies, so that comparative analysis often involves comparing humans, chimpanzees, and macaque monkeys (Fig. 14.1, *Inset*).

The beginning of comparative molecular biology (at least as regards human evolution) is usually traced to Nuttall (1904), who found that rabbit antisera raised to human blood reacted strongly with human, chimpanzee, and gorilla blood, but less strongly with orangutan or gibbon blood, indicating that the molecular differences between species are consistent with their evolutionary relationships as inferred from differences in anat-
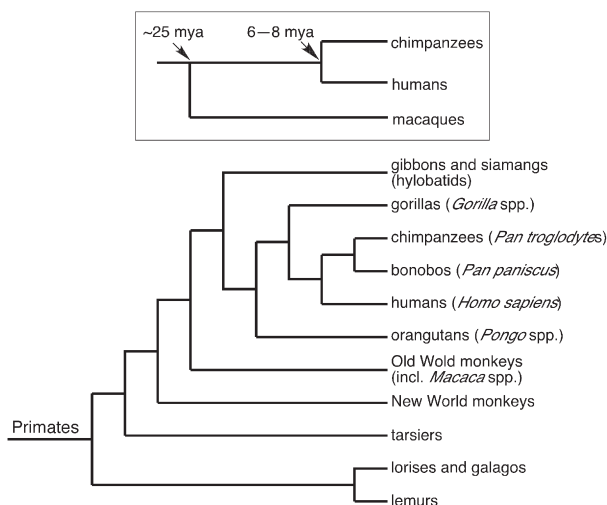
FIGURE 14.1 Identifying human specializations requires, first, determining that humans differ from the chimpanzee-bonobo group (the sister group of humans), and second, determining that the difference evolved in the human lineage. The latter judgement requires evidence about the character states of out-group taxa, such as other apes and monkeys. For practical reasons, evaluations of human specializations often use a minimal set of comparisons, involving humans, chimpanzees, and macaque monkeys (*Inset*). Humans and chimpanzees diverged 6 to 8 Mya, and the lineage leading to macaque monkeys diverged from the human-ape lineage ~25 Mya. Modified from Preuss et al. (2004).

omy (Goodman and Sterner, 2010). The beginning of modern ideas about human molecular evolution, however, can be traced to a landmark paper published in *Science* by King and Wilson (1975). King and Wilson were struck by the great similarity between humans and chimpanzees in the amino acid sequences of proteins that had been sequenced to that point: the differences amounted to only 1%, which is less than that between anatomically indistinguishable sibling species in some animal groups. This great molecular similarity between humans and chimpanzees seemed paradoxical, given the profound differences in behavior, brain size, and gross anatomy between the two species, most of which, they assumed, evolved in the human lineage rather than the chimpanzee lineage. To resolve this paradox, they proposed that humans underwent a "relatively small number of genetic changes in systems controlling the expression of genes," yielding a large phenotypic effect but only a small genetic signal (King and Wilson, 1975). Such changes could involve the sequences of

genes that code for regulatory proteins (including what we would today call "transcription factors") or by DNA rearrangements (translocations, inversions, duplications, and deletions) that change the relationships of genes to regulatory elements. These ideas were subsequently championed by Stephen Jay Gould, in his book *Ontogeny and Phylogeny* (Gould, 1977), who emphasized that the phenotypic consequences of gene-expression changes would be magnified if they occurred early in development. These writings established the expectation that we should be able to discover a few key genetic changes that account for many, if not most, of the phenotypic differences between humans and chimpanzees.

NEUROBIOLOGICAL AND BEHAVIORAL BACKGROUND

In the classical process of "gene discovery," geneticists start with a conspicuous phenotypic variant of a species, and then carry out mapping studies to identify the genetic locus that harbors the mutant allele. Thus, gene discovery begins with characterization of phenotypes. It seems reasonable to search for human genetic specializations related to characteristics of the brain, behavior, and cognition, as most people would regard humans as being highly specialized in these domains. Most people are not neurobiologists or behavioral scientists, however. Surprisingly, experts in these fields have often taken a very jaundiced view of supposed human evolutionary specializations, with the result that there is not at present a detailed, consensus view of human neurological or psychological specializations. In part, the reasons for this are pragmatic. Studying ape behavior and cognition is a daunting business, as the animals are large, powerful, and long-lived, and thus are difficult and expensive to maintain. Also, it has proven difficult to develop experimental procedures that convincingly tap the same psychological processes across multiple species. In neuroscience, the main difficulty has been the lack of suitable noninvasive methods for studying brain structure—and in particular, the axonal connections between brain regions that are such important components of the functional organization of the brain. Neuroscientists have powerful methods for studying connectivity, but until recently, these methods required invasive and terminal procedures, precluding their use in humans, as well as in apes. This situation led Crick and Jones (1993), to decry "the backwardness of human neuroanatomy."

There are also ideological obstacles to identifying human specializations. Claims of human neural or psychological specializations have been seen by some as contrary to Darwin's understanding of evolutionary continuity, and to represent special pleading on behalf of humans. In fact, Darwin (1871) insisted that there are no differences in kind between the minds of humans and related species; Huxley (1863a) made similar

claims with respect to the brain. However, Darwin and Huxley were not at all consistent about applying this principle to other animal groups or other biological systems, and modern evolutionary biology insists only that there be continuity across generations, not that every feature present in humans be present in some rudimentary form in other animals, a stance that would preclude the existence of any true evolutionary novelties (Preuss, 2009, 2012). Although some psychologists and neuroscientists now reject Darwin's narrow construction of continuity (Povinelli, 1993; Preuss, 1995, 2012; Premack, 2007; Penn et al., 2008), it retains considerable currency in those fields, and informs the current public understanding of the meaning of evolution (Penn et al., 2008; Preuss, 1995).

There is an additional problem in the neurosciences. As new, powerful (and invasive) methods for studying brains became available in the 1970s, a doctrine developed that held that, apart from size, which is highly variable across species, the organization of the cerebral cortex (the largest part of the brain in most mammals) does not vary in important respects across species [reviewed in Preuss (2001)]. This concept of the "basic uniformity" of cortical organization, and kindred ideas, did little to encourage comparative studies of brain organization, nor did it cause many neuroscientists to be alarmed by the "backwardness of human neuroanatomy" (Crick and Jones, 1993) because there was no reason to think the human brain exceptional except with respect to size. Human brains are, indeed, for mammals of our body size, bizarrely large, approximately three times the volume of those of chimpanzees and other great apes, the result of an enormous evolutionary expansion of the cerebral cortex during the past 2 million years (Preuss, 2011).

Much has changed in recent years, as investigations of human psychological and neurological specializations have begun to flourish [reviewed in Tomasello and Call (1997), Subiaul et al. (2007), Gazzaniga (2008), Passingham (2008), Sherwood et al. (2008), Preuss (2009, 2011), Premack (2010)]. In the neurosciences, the development of noninvasive brain imaging techniques, in particular, has made human neuroanatomy much less backward today than in 1993. One new technique, diffusion-tensor imaging, makes it possible to study the long-range connections of the cerebral cortex. This means we can now compare the cortical connectivity of humans, chimpanzees, and other primates in considerable detail by using the same technique (Rilling, 2008; Preuss, 2010). The first comparative human–chimpanzee–macaque studies that used diffusion-tensor imaging have recently been published, demonstrating human specializations in the connections between regions of higher-order association cortex (Rilling et al., 2008, 2011). Studies of brain tissue acquired postmortem are also revealing human specializations of the cellular and histological organization of the cortex (Sherwood et al., 2008; Preuss, 2010).

Comparative psychological investigations, too, have highlighted human specializations, usually involving aspects of higher-order cognitive abilities, such as the ability to represent abstract or unobservable properties, like force, weight, and mental states (Povinelli and Eddy, 1996; Povinelli, 2000, 2012; Premack, 2007, 2010), and the capacity for symbolic or analogical reasoning (Deacon, 1997a; Penn et al., 2008; Premack, 2010). Another line of research focuses on adaptations of the human mind for culture and social cognition (Henrich and McElreath, 2003; Richerson and Boyd, 2006; Herrmann et al., 2007). Claims for human psychological specializations remain controversial, however, as reflected in the peer commentary on Penn et al. (2008).

There is, however, one human specialization that is widely, if not universally, acknowledged by scientists today: language, that remarkable instrument for organizing and sharing the contents of minds. The upshot of the ape-language projects of the 1960s and 1970s is that apes are understood to be able to learn elements of human language (e.g., manual signs), but they do not organize these elements into expressions possessing the formal characteristics of human language (Terrace et al., 1979; Wallman, 1992; Pinker, 1994; Rivas, 2005; Premack, 2010).

Although progress is being made in understanding human neurobiological and psychological specializations, this is relatively new science, and at present there is no real consensus among specialists about the precise nature of the human specializations. From the point of view of biologists looking to understand the genetic bases of human phenotypic specializations, the most salient ones today are, simply, big brains, advanced cognition, and language.

FOXP2: CASE STUDY

The advent of comparative genomics has been accompanied by a remarkable proliferation of research on human evolutionary biology. The resulting studies have compared humans with chimpanzees and other primates on nearly every imaginable dimension of genetics and molecular biology. These include studies that used high-throughput techniques to identify human specializations of gene expression, primarily in the brain, including whole-genome screens to identify genes that underwent human-specific sequence changes as a result of selection; studies of chromosome segment duplications; identification of differences in alternative splicing between humans and chimps; and more [reviewed in Preuss et al. (2004), Khaitovich et al. (2006), Sikela (2006), Varki and Nelson (2007), Varki et al. (2008), Johnson et al. (2009), Preuss (2010)]. The field continues to grow, but even at this early stage, it is apparent that the genetic and molecular differences between humans and chimpanzees are much greater than had

been supposed. For instance, the number of genes that show expression differences in adult cortex is on the order of hundreds (Preuss et al., 2004) at least, and more likely thousands (Konopka et al., 2009b). Similarly, the number of genes that underwent positive selection in humans is on the order of hundreds at least (Clark et al., 2003; Bustamante et al., 2005). Humans possess species-specific genes, as a result of the numerous tandem duplications of chromosome segments that occurred in human evolution, and also recombination events (Wu et al., 2011; Zhang et al., 2011). One consequence of the numerous duplications, insertions, and deletions is that the total DNA sequence similarity between humans and chimpanzees is not 98% to 99%, but instead closer to 95% to 96% (Britten, 2002; Wetterbom et al., 2006; Varki and Nelson, 2007), although the rearrangements are so extensive as to render one-dimensional comparisons overly simplistic.

There is, then, no shortage of human genetic specializations to work with: the problem is connecting the genes to phenotypes. There is at least one example, however, of a gene that underwent selection-driven sequence change in human evolution and is related to a human-specific cognitive and behavioral phenotype: *FOXP2*. The history of the discovery of this gene, its connection to speech and language, and the research inspired by the discovery, illustrate the range of approaches now available to scientists pursuing the genetic underpinnings of human nature, as well as the elusive character of the relationship between single genes and complex phenotypes.

FOXP2: Gene Discovery

The first part of the *FOXP2* story is a straightforward narrative of gene discovery. Hurst et al. (1990), in England, identified an inherited defect of speech in three generations of a family, known as the KE family. The defect followed a pattern consistent with an autosomal dominant mutation, being present in approximately half the family members, both male and female. According to this initial report, the affected family members exhibited dysfluent, often simplified, speech, with difficultly constructing grammatical sentences. Hurst et al. attributed this to difficulties in making the rapid articulatory movements required in speech, although they did note problems with comprehension. They also reported that the affected members of the family had IQs in the normal range, although some were on the low side of normal. They characterized the disorder as "verbal apraxia" and "developmental verbal dyspraxia," the suffix "-praxia" implying that the defect was in motor control (i.e., praxis) and was not a true linguistic defect (i.e., aphasia). Other researchers, however, favored a linguistic interpretation, either of grammar, mainly (Gopnik, 1990), or of language more broadly (Vargha-Khadem and Passingham, 1990).

The discovery of the KE family led to a search for the specific gene responsible for the speech defect. Linkage analysis with microsatellite markers suggested a locus on the long arm of chromosome 7, specifically a 5.6-cM region of 7q31, and the locus was dubbed *SPCH1* ("speech and language disorder 1") (Fisher et al., 1998). Subsequently, Lai et al. (2001) identified an unrelated individual (case CS) with a speech deficit similar to that of the affected KE members and a chromosomal translocation in 7q31. The breakpoint mapped onto a bacterial artificial chromosome that contained a known gene, *FOXP2*, a transcription factor with a forkhead-box DNA-binding domain. By screening the coding regions of *FOXP2* with restriction-fragment mapping and direct sequencing, Lai et al. were able to identify the mutation shared by affected KE family members, which would result in an arginine-to-histidine substitution at position 553 (R553H). This falls within the DNA-binding forkhead domain of the FOXP2 protein, and the arginine residue in this part of the domain is highly conserved across members of the forkhead-box family of transcription factors. The implication is that R553H is a loss-of-function mutation.

At the same time geneticists were working to identify the mutation responsible for the deficit in affected KE family members, psychologists and neuroscientists were working to better characterize the phenotype. Vargha-Khadem and coworkers (Vargha-Khadem et al., 1995, 2005; Watkins et al., 2002a) ultimately argued for a primary deficit of orofacial apraxia, based in part on the fact that the mutation impairs the ability to repeat nonwords as well as words. While disputing the idea that *FOXP2* is a grammar gene, they did acknowledge that grammatical and syntactic language deficits result from R553H, but left open the possibility that the language problems are a secondary consequence of the dyspraxia. They also confirmed that affected KE family members have lower IQs on average than nonaffected members (Vargha-Khadem et al., 1995; Watkins et al., 2002a), although, again, it is unclear whether this is a core deficit or a secondary consequence of language impairment.

The brain phenotype of affected KE family members has been assessed with structural and functional neuroimaging. These studies have highlighted abnormalities of the motor system. For example, affected family members show a 25% reduction of the volume of the caudate nucleus of the basal ganglia bilaterally, compared with unaffected family members and controls (Vargha-Khadem et al., 1998; Watkins et al., 2002b). Notably, caudate volume is significantly correlated with performance on tests of oral apraxia (Watkins et al., 2002b). Other motor-related structures also show reduced gray matter bilaterally, including the cerebellum and the cortex of the precentral gyrus, as does the inferior frontal gyrus (Broca's area), whereas the superior temporal gyrus shows increased gray matter volume (Belton et al., 2003). Functional imaging studies using PET or functional

MRI reveal differences between affected KE family members compared with unaffected members and controls in tasks that required repeating words or nonwords, or in making orofacial movements (Vargha-Khadem et al., 1998; Watkins et al., 2002b; Liégeois et al., 2003, 2011). Although there are some inconsistencies in the functional imaging studies, they have also highlighted activation differences in motor structures—cortical motor areas, basal ganglia (striatum), and cerebellum—and Broca's area. Vargha-Khadem et al. (2005), in their review of imaging studies, emphasize abnormalities in systems involving frontal lobe connections with the basal ganglia and cerebellum (i.e., frontostriatal and frontocerebellar systems) likely to be related to orofacial movements. Notably, however, in the structural and functional imaging studies, the differences between affected KE family members and unaffected members were not limited to those structures, but involved additional and extensive regions of cortex not usually associated with motor (or language) function, such as the postcentral (somatosensory) cortex and occipital (visual) cortex.

Examination of fetal human brain tissue also makes it clear that *FOXP2* expression is not limited to brain regions usually associated with language (Ferland et al., 2003; Lai et al., 2003; Spiteri et al., 2007). For example, although it is expressed in the perisylvian cortical region (the cortex spanning the territory from Broca's to Wernicke's language areas), and is present in the striatum (caudate and putamen), as one might expect from neuroimaging studies of the KE family, it is expressed in the cortex of frontal pole and occipital pole, neither of which is critical for language. *FOXP2* is also expressed in the thalamus, cerebellum, and brainstem, and moreover, is expressed in a wide variety of tissues other than the brain (National Center for Biotechnology Information, 2012).

FOXP2 in Human Evolution

If language is a human specialization, and *FOXP2* plays an important role in the development of speech and language, it is natural to ask whether *FOXP2* underwent evolutionary changes in its sequence or expression patterns in human evolution. Enard et al. (2002) addressed this question, and found that, although the FOXP2 protein sequence is very strongly conserved in mammalian evolution generally, human FOXP2 differs by two amino acids from that of chimpanzees, gorillas, and macaques, all of which have identical sequences. Both substitutions are in exon 7: a threonine-to-asparagine substitution at position 303 (T303N) and an asparagine-to-serine substitution at position 325 (N325S); the latter substitution creates a potential phosphorylation site. The occurrence of two amino acid fixations is highly unlikely to have occurred by chance, so it is reasonable to conclude that these changes were the result of positive selec-

tion, a conclusion supported by analysis of variation in intronic regions of the gene. [Interestingly, at approximately the same time, two other groups independently reported that *FOXP2* is among the genes likely to have undergone positive selection in human evolution, based on the ratio of nonsynonymous to synonymous nucleotide changes (Ka/Ks) in genes for which sequence information was available for humans, chimpanzees, and other species (Clark et al., 2003; Zhang et al., 2002a).] Enard et al. (2002) argued that the data are consistent with a selective sweep resulting in fixation of the two human-specific amino acid changes within the past 200,000 years, and speculated that this event occurred coincident with or subsequent to the appearance of modern *Homo sapiens* and is related to the ability to produce the orofacial movements required for speech.

With the publication of the *FOXP2* evolutionary story, we now had a human-specific modification of a gene that seems to influence a human-specific phenotype. However, important questions remain. For one, were the sequence changes in human *FOXP2* driven by selection for speech or language ability? This is not necessarily the case. The mutations in the KE family and in CS (Lai et al., 2001) do not recapitulate the evolutionary changes in the sequence of *FOXP2*: they are essentially knockout mutations, and the orthologs of *FOXP2* present in other species are presumably functional. What does *FOXP2* do in nonhuman species? If it is involved in speech or language in humans, are its actions narrowly tied to the development of vocalization systems in other animals, or does it also support the development of other systems?

FOXP2: Comparative Expression and Sequencing Studies

The expression of *FOXP2* orthologs in the brains of nonhuman species has received considerable attention, and the anatomical patterns of expression have much in common with those documented in humans [reviewed in Scharff and Petri (2011)]. Fetal macaques express *FOXP2* in the basal ganglia (specifically the striatum), thalamus, and extensive regions of cerebral cortex, especially in cortical layer 6 (Takahashi et al., 2008). In mice, the *FOXP2* ortholog is expressed from embryonic day 12.5 through adulthood, and its expression is widespread, including the striatum (where it is very strongly expressed), layer 6 of the cerebral cortex, the thalamus, hypothalamus, the Purkinje cells of the cerebellum, the substantia nigra, superior and inferior colliculi, and the inferior olive (Ferland et al., 2003; Lai et al., 2003). Rats show similar patterns of expression, with especially strong expression in the striatum (Takahashi et al., 2003). The similarities in anatomical localization extend to other vertebrates as well, including birds and reptiles (Haesler et al., 2004; Teramitsu et al., 2004). *FOXP2* orthologs are also expressed in the brains of amphibians and fish

(Bonkowsky and Chien, 2005; Shah et al., 2006; Schön et al., 2006; Itakura et al., 2008).

The presence of *FOXP2* orthologs in birds has prompted investigations to determine whether song-learning birds underwent sequence changes in *FOXP2* analogous to those documented in humans [reviewed in Scharff and Haesler (2005), Vargha-Khadem et al. (2005), Scharff and Petri (2011)]. Particular attention has been paid to zebra finches, a favorite species for students of bird song because the male develops its song at sexual maturity, the animal gradually shaping its song to match the memory of a tutor's song (usually that of its father). In zebra finches, *FOXP2* expression is especially strong in area X [Haesler et al. (2004), Teramitsu et al. (2004)], a critical element of the avian song-learning system that is composed of tissue homologous to the striatum of mammals. What is more, in zebra finches and in canaries, area X expresses more *FOXP2* than the surrounding region of the striatum during the period of vocal learning (Haesler et al., 2004; Teramitsu et al., 2004). Zebra finches recruit new neurons during the period of song learning, and those neurons express *FOXP2* (Rochefort et al., 2007). In addition, knockdown of *FOXP2* in area X of zebra finches impairs song learning (Haesler et al., 2007). Thus, *FOXP2* is probably involved in the development of the song system in zebra finches, and probably in other bird species as well. However, zebra finch *FOXP2* lacks the specific nucleotide substitutions that evolved in the human terminal lineage (Haesler et al., 2004; Teramitsu et al., 2004).

A recent comparative sequencing study suggests that there is somewhat more evolutionary diversity in *FOXP2* sequences than initially believed. Li et al. (2007) partly or completely sequenced *FoxP2* in a large number of bats (42 species), including species that are echolocating and vocal learners, as well as other bats; in cetaceans (18 species of whales and dolphins, 15 of which are echolocating); in the African elephant (reputedly a vocal learner); and in other species from diverse mammalian orders. Although the *FoxP2* sequence was confirmed to be highly conservative in most mammalian orders, bats proved to be markedly divergent, with nonsynonymous nucleotide changes concentrated in exons 7 and 17. Within exon 7, none of the bats or other mammalian species have a human-like asparagine substitution at position 303, but many echolocating bats, and also two carnivore species sequenced, do have human-like serine substitutions at position 325. In exon 17, no nonsynonymous substitutions were found in any of the mammals sequenced except bats, in which variation is considerable, with as many as eight substitutions in one species. Although they argued that *FoxP2* was likely the target of positive selection in bats, however, Li et al. are skeptical that there is a relationship between vocal learning ability and specific *FoxP2* substitutions, because they could identify no nonsynonymous substitution that is shared by all

the vocal learning species they examined or in other published studies of mammals or birds. Comparative studies of FOXP2, therefore, draw no clear connection between specific sequence changes and vocal learning capacities, although the increased levels of FOXP2 expression reported in birds during periods of song acquisition suggests that this transcription factor is involved in learning-related neural changes.

FOXP2: Mouse Model of R552H Substitution

One approach that has been adopted to understand the function of *FOXP2* in humans is to make mouse models that express the mutant form of *FOXP2* present in affected members of the KE family (R552H), as Groszer et al. (2008) have done. The homozygotes were severely developmentally delayed, and died by 3 to 4 wk after birth. The cerebellum was abnormally small, with decreased foliation, and behaviorally, the pups emitted fewer ultrasonic distress calls than heterozygotes or WT mice. The heterozygous pups, however, were fully viable and healthy, and were not developmentally delayed. These animals were screened for abnormalities of the motor system, particularly the striatum and cerebellum, brain regions that show structural and functional changes in affected KE family individuals. The brains of the R553H heterozygotes appeared to be grossly normal and the cerebellum was of normal size. Nevertheless, the animals showed impaired motor learning on a running wheel. Moreover, the physiology of the striatum and the cerebellum differed from WT mice. In the striatum, high-frequency electrical stimulation of glutamatergic synapses, which in WT animals produces long-term depression (LTD; a form of synaptic plasticity), failed to produce any significant LTD in R552H heterozygotes. Stimulation of the parallel fiber/Purkinje cell system in cerebellar tissue-slice preparations produced weaker effects on LTD, and in the opposite direction, with slightly stronger LTD in R552H heterozygous animals than in WT mice.

FOXP2: Mouse Model of Human Evolution?

A different approach to understanding the functions of *FOXP2* in humans was adopted by Enard et al. (2009), who produced a transgenic mouse that expressed a humanized version of mouse *FOXP2*, with the characteristically human threonine-to-asparagine substitution at position 303 and asparagine-to-serine substitution at position 325. They also made Herculean efforts to screen the phenotype that involved behavioral, neurohistological, neurophysiological, and neurogenetic comparisons of the transgenic and normal mice. Unlike R552H mice, humanized *FOXP2* homozygous mice are fully viable and fertile and show no gross behav-

ioral or anatomical abnormalities. Moreover, they expressed *FOXP2* in the brain structures one would expect from previous studies: cerebral cortex (cells in layer 6), the striatum, thalamus, and cerebellar Purkinje cells. Behaviorally, the humanized mouse pups showed no difference with WT mice in the number of isolation calls made per unit time, although the spectral characteristics of their calls differed, with lower peak frequencies at the start of calls, and lower mean and maximum peak frequencies during calls. Interestingly, the humanized *FOXP2* mice showed no evidence of motor impairment compared with WT mice, although they did have lower scores on several measures of exploratory behavior. Neurochemically, humanized *FOXP2* mice showed lower dopamine concentrations in cortex, striatum, pallidum, and cerebellum compared with WT, but concentrations of other neurotransmitters (serotonin, glutamate, and GABA) did not differ. Humanized *FOXP2* mice also showed differences in gene expression in the striatum compared with WT mice, with a number of genes downregulated in the humanized mice that are known to be expressed by medium spiny cells that express the dopamine D1 receptor, a class of cells that also express *FOXP2* (Heiman et al., 2008). The electrophysiological characteristics of striatal medium spiny cells were examined in cells cultured from humanized *FOXP2* mice, and found to support stronger LTD than WT cells, the opposite of the finding with R552H mice. Perhaps the most notable finding of this study (Enard et al., 2009) is that cultured striatal neuronal precursors from humanized *FOXP2* mice had longer neurites (i.e., dendrites and axons) than those from the cells of WT mice. Subsequent examination of medium spiny neurons in brain sections from the striatum showed that neurons from humanized *FOXP2* mice had longer dendrites that those from WT mice.

Regulation of Gene Expression by FOXP2

The study by Enard et al. (2009) suggests yet another, more mechanistic, approach to understanding the functions of *FOXP2*: identifying the genes regulated by *FOXP2*. Several studies have been carried out with this goal, using chromatin immunoprecipitation (CHiP) to identify DNA sequences bound by FOXP2 protein. DNA sequences bound with FOXP2 antibodies were then identified by hybridization to gene chips representing human promoters (i.e., ChIP-chip) or, more recently, by direct sequencing of the targeted sequences (i.e., ChIP-seq).

Spiteri et al. (2007) identified 285 gene targets in fetal human tissue from the inferior frontal gyrus and basal ganglia, and determined their representation in gene ontology (GO) and pathway categories. GO categories represented at greater than chance levels included morphogenesis, intracellular signaling, cation homeostasis, neuron outgrowth, and axo-

nal morphology. Overrepresented pathway categories included dendritic branching, calcium mobilization, calcium concentration, and learning. Notably, 14 genes that were targeted by FOXP2 (which underwent selective change in human evolution) also showed evidence of positive selection in human evolution. A comparison of published datasets identified 47 gene targets of FOXP2 that were differentially expressed in the cortex of humans and chimpanzees, including a number of genes involved in neural development, central nervous system patterning, and neural transmission. Vernes et al. obtained qualitatively similar results in GO and pathway analyses after examining expression in human neuron-like cells (SH-SY5Y cells) transfected with human *FOXP2* (Vernes et al., 2007), and also in a ChIP study of FOXP2 promoter occupancy in embryonic mice (Vernes et al., 2011). The latter study also directly examined the effect of mouse *Foxp2* expression on neurite outgrowth by transfecting mouse neuron-derived Neuro2a cells with *Foxp2* or an empty vector (used as a control). They then treated the cells with retinoic acid, which causes Neuro2a cells to extend neurites as they adopt a more differentiated, neuron-like phenotype. Cells transfected with *Foxp2* were found to have longer neurites than control cells.

To investigate the transcriptional consequences of the human-specific substitutions in *FOXP2* (T303N and N325S) more directly, Konopka et al. (2009a) transfected SH-SY5Y cells with human *FOXP2* or with a construct that restored the ancestral sequence found in chimpanzees, and used microarrays to assess gene expression. Although the two versions of *FOXP2* regulated many of the same genes, there were also differences in transcriptional activity, with 61 genes being more strongly expressed with human *FOXP2* than chimpanzee *FOXP2* and 55 less strongly expressed with human *FOXP2*. They then examined expression of *FOXP2*-regulated genes in adult brain tissue from humans and chimpanzees and found that many of the same genes were expressed in tissue as in the SH-SY5Y cells. Moreover, the differences in expression between human and chimpanzee brain tissue mirrored the differences found in cells transfected with human *FOXP2* or chimpanzee *FOXP2*. GO analysis of differentially expressed genes in the brain showed an enrichment of categories related to tissue and organ development and cell–cell signaling.

CONNECTING GENES AND PHENOTYPES

As a gene associated with a human-specific trait, *FOXP2* would at first glance seem to be a dream come true for evolutionary geneticists. Moreover, it is hard not to be impressed by the depth and breadth of the research related to *FOXP2*. Nevertheless, there is still no clear or direct connection between the human-specific amino acid substitutions in FOXP2 and speech

or language—not from the comparative studies, or from the mouse-model studies, or from the gene expression studies. The fact that mutations of FOXP2 in humans result in speech impairments shows that it plays a role in speech development, but the nature of its role remains unclear. It might play a very specific role, for example, by orchestrating a whole set of genes that switch brain development from an ancestral program to a human program that causes cells and connections to differentiate into systems that sustain speech or language. It might even regulate the development of other parts of the anatomy, such as the lungs and larynx, involved in speech production. Alternatively, FOXP2 might have a permissive role, for example, by regulating some aspects of cell behavior required for the normal development of language systems, but also for the normal development of other structures and systems. Both options would be consistent with the action of a loss-of-function mutation in *FOXP2*, such as the R553H mutation in the KE family. In neither case, however, do we have a direct connection between language and the specific FOXP2 substitutions that took place in human evolution (T303N and N325S). There is not much question that these changes were the result of selection, and that they affect gene expression in the brain. However, given the widespread pattern of *FOXP2* gene expression in the body, those substitutions are likely to affect gene expression in other organs, so it remains possible that the substitutions were driven by selection acting on non–speech-related parts of the brain or nonbrain tissues and organs. Humans are, after all, not just apes with unusually large, complex brains: other aspects of anatomy and physiology were extensively modified in human evolution as well. It could also be the case that FOXP2 has a speech- or language-specific function in the human brain, by virtue of the action of other transcription factors that bind to the same promoters in brain cells targeted by human FOXP2. However, then we would be talking about the interactions of genes involved in building a human organism, rather than a single gene, and it still would not be clear, without additional evidence, that the amino acid substitutions in FOXP2 were selected for their effects on developmental pathways specific to language.

It would seem that the crux of the problem with tying *FOXP2* to language is that we are trying to relate a multifunctional gene to a complex, high-level phenotype, by which I mean a phenotype that encompasses a diverse collection of tissues and cell types. It is probably not realistic to think that the development of such systems has simple genetic triggers and they are the products of epigenetic programs acting in isolation from other epigenetic programs. This conclusion merely restates two of the important lessons of experimental population genetics: first, that most phenotypes arise through the interactions of multiple genes (the principle of epistasis), and, second, that most genes influence multiple phenotypes (the principle of pleiotropy) (Mayr, 1970; Dobzhansky et al., 1977).

These considerations, along with the great number and variety of human genetic specializations, make it unlikely that evolutionary changes in just a few regulatory genes, acting early in development, can explain most of the phenotypic differences between humans and chimpanzees, as King and Wilson (1975) and Gould (1977) suggested. There might, in fact, be a relatively small number of genes that do act early in development and do have profound effects, but as we have seen with *FOXP2*, the genes downstream of a regulatory gene themselves can undergo evolutionary changes (Spiteri et al., 2007). Without a deeper understanding of development and, especially, of the human-specific aspects of human development, we are not likely to be able to make many definite connections between high-level phenotypes and the role of early-acting regulatory genes.

This raises a matter of serious concern: our lack of direct information about the human organism. I have noted the lack of detailed accounts of the human neurobiological and psychological phenotypes, and I think it would be fair to say we are quite ignorant about many other human-specific features of human biology, including human-specific modifications of the developmental programs that generate human-specific phenotypes. In part, this lack of knowledge reflects technical limitations to our ability to study humans: just as we cannot do invasive neurobiological investigations, we cannot do invasive or terminal developmental investigations or transgenic experiments either. Given this, it is not surprising that we rely so much on studies of model animals to understand normal human biology and its development, with the hope that there is enough commonality in epigenetic systems across species that results in models will translate to humans. This approach seems particularly problematic when human-specific phenotypes are at issue. Presumably, no one expected that expressing human *FOXP2* in a mouse would yield a mouse that talks. It is, after all, a human gene on a mouse background, and without understanding the similarities and differences between human and mouse developmental programs, predictions about the specific effects of a mutant human gene on the high-level phenotypes of mice are hazardous.

In offering this critique, I do not mean to say that mouse models are not useful or informative, but rather that they are not enough by themselves. Certainly, every aspect of our science would benefit from the better characterization of human-specific phenotypes, and the most direct way to obtain that information, especially for high-level phenotypes, is by studying humans directly. However, we do need other experimental paradigms to help us explore the phenotypic consequences of human-specific genetic changes. Investigations of gene functions in cell culture, as exemplified by a number of the studies cited earlier, provide another avenue for empirical research. This approach could be further empowered

by the emerging technology for generating differentiated cells of various types (including neurons) from induced pluripotent stem cells (Hansen et al., 2011; Shi et al., 2012).

There is another approach we can adopt, one that takes as its starting points not some known human phenotypic specializations (of which there are rather few), but instead starts with the genetic and molecular differences themselves and uses them as clues to previously unsuspected phenotypes: that is, "phenotype discovery" (Preuss et al., 2004; Varki et al., 2008; Preuss, 2010), in contrast to "gene discovery" (Fig. 14.2). How this works is illustrated by the studies reviewed in the previous section showing that *FOXP2* expression affects neurite outgrowth and synaptic plasticity (Vernes et al., 2007, 2011), and that mice expressing human *FOXP2* show more neurite outgrowth and synaptic plasticity than mice expressing mouse *Foxp2* (Enard et al., 2009). There are additional lines of evidence suggesting that evolution targeted these features of human neuronal biology. Some of the first microarray studies comparing adult humans to other primates (Cáceres et al., 2003; Preuss et al., 2004; Uddin et al., 2004), and follow-up validation studies (Cáceres et al., 2007), noted expression changes in genes related to synaptic plasticity, as has a recent comparative study of metabolite concentrations (Fu et al., 2011). It is noteworthy, too, that there is genomic evidence for positive selection on genes
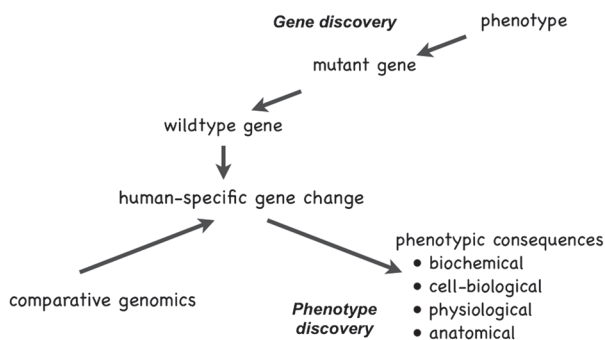
FIGURE 14.2 Gene discovery starts with an unusual, heritable phenotype, and then proceeds to determine the chromosomal locus of the mutation, and finally to identify the mutated gene itself. Phenotype discovery starts with species differences in genes or gene expression, identified through gene discovery, comparative genomics, or other comparative molecular methods, and proceeds to identify the biochemical, cell-biological, and other phenotypic consequences of the genetic differences.

involved in aerobic energy metabolism (Grossman et al., 2001; Goodman and Sterner, 2010), and evidence for upregulation of energy-metabolism genes in human evolution (Cáceres et al., 2003; Uddin et al., 2004). One study that examined patterns of gene coexpression in the adult cortex of humans and chimpanzees found evidence for differences both in genes related to synaptic plasticity and energy metabolism (Oldham et al., 2006). What is emerging is a picture of humans as having adult brains that are unusually active physiologically and unusually dynamic anatomically. This may reflect the extension in humans of patterns of gene expression normally restricted to early life stages well into adulthood (Somel et al., 2009, 2010; Fu et al., 2011), a phenomenon that has been termed "transcriptional neoteny" (Somel et al., 2009).

Although the idea that human brains are unusually active and malleable might make intuitive sense to many, there is, in fact, little in the primary scientific literature hinting at this. In fact, one of the most robust generalizations in physiology is that as biological entities—be they organisms, organs, or organelles—become bigger, they use less energy per unit mass of tissue (West and Brown, 2005). However, PET studies in awake individuals suggest that the human brain uses approximately the same amount of glucose per unit of tissue as do rhesus macaques, animals with brains less than one-tenth the volume of human brains (Bohnen et al., 1999; Bentourkia et al., 2000; Cross et al., 2000; Noda et al., 2002). This is consistent with the idea that human brains are "running hot" (Preuss, 2011).

What is notable about this example is not simply that the genomic evidence suggests phenotypic specializations of humans that were not previously suspected, but also that the claims can be tested empirically: the energetics claim by in vivo physiological techniques such as PET, and the claim of neural dynamism by studies in cell and tissue culture, and possibly even in postmortem tissue (Enard et al., 2009). Perhaps when we know enough about the cell-physiological consequences of enhanced neural dynamism, we can generate additional testable predictions at levels that are amenable to investigation by PET and other in vivo techniques.

I suggest that, in general, comparative molecular data are particularly well suited to phenotype discovery at the level of cells, and especially their biochemical and physiological characteristics, because the route from gene changes to cellular changes is more direct than that from genetic changes to high-level phenotypes [see also Varki et al. (2008)]. That does not mean we should abandon phenotype-driven gene discovery studies, but gene-driven phenotype discovery may represent an approach with a greater payoff, at least in the short term, and has the additional advantage of being able to reveal previously unsuspected aspects of human nature.

ACKNOWLEDGMENTS

15

Integration of Faces and Vocalizations in Ventral Prefrontal Cortex: Implications for the Evolution of Audiovisual Speech

LIZABETH M. ROMANSKI

The integration of facial gestures and vocal signals is an essential process in human communication and relies on an interconnected circuit of brain regions, including language regions in the inferior frontal gyrus (IFG). Studies have determined that ventral prefrontal cortical regions in macaques [e.g., the ventrolateral prefrontal cortex (VLPFC)] share similar cytoarchitectonic features as cortical areas in the human IFG, suggesting structural homology. Anterograde and retrograde tracing studies show that macaque VLPFC receives afferents from the superior and inferior temporal gyrus, which provide complex auditory and visual information, respectively. Moreover, physiological studies have shown that single neurons in VLPFC integrate species-specific face and vocal stimuli. Although bimodal responses may be found across a wide region of prefrontal cortex, vocalization responsive cells, which also respond to faces, are mainly found in anterior VLPFC. This suggests that VLPFC may be specialized to process and integrate social communication information, just as the IFG is specialized to process and integrate speech and gestures in the human brain.

The area dedicated to language processing in the frontal lobe is located within the inferior frontal gyrus (IFG), which can be further subdivided into the pars opercularis (most posterior portion of

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the IFG), the pars triangularis, and the pars orbitalis (cortex inferior and anterior to the horizontal ramus of the lateral fissure). These subdivisions include Brodmann areas 44, 45, and 47. Our understanding of the functions within this specialized area of cortex is hampered by the fact that no other mammal has a frontal lobe of similar organization or complexity, leaving few animal models to investigate. Within nonhuman primates, only catarrhines have a well-developed frontal lobe with cytoarchitectonic evidence of Brodmann areas 44, 45, and 47 (Petrides and Pandya, 2002). In contrast, New World monkeys including marmosets and squirrel monkeys have a lissencephalic frontal lobe with previously identified motor and premotor cortices but less clearly defined prefrontal regions (Preuss, 2007). Recent work has identified an area in the ventrolateral prefrontal cortex (VLPFC) of rhesus macaques (*Macaca mulatta*) that is involved in the processing and integration of vocalizations and faces. We have hypothesized that the ventral prefrontal cortex (VLPFC) became specialized for the processing and integration of auditory and visual communication signals, in at least early anthropoid primates, and ultimately this region was modified and lateralized to the left cerebral hemisphere to subserve language in modern humans.

VENTRAL PFC: ANATOMICAL CONSIDERATIONS

Organization of VLPFC

The frontal lobe of the macaque monkey has been studied extensively with anatomical, electrophysiological, and functional methodologies compared with other primate species. The area of the VLPFC, also referred to as the inferior convexity of the PFC, in the macaque monkey, includes the cortical region ventral to the principal sulcus and anterior to the inferior limb of the arcuate sulcus (Fig. 15.1). The cytoarchitectonic areas of VLPFC in the macaque are arranged in a similar fashion to that of the human frontal lobe and include regions on the lateral frontal surface: area 45, which lies just anterior to the inferior arcuate sulcus; area 12 (or 12/47), which lies anterior to area 45 and ventral to area 46; and the most ventrolateral extent of the inferior convexity, which wraps around the inferior gyral surface and extends to the lateral orbital sulcus: area 12 orbital (Preuss and Goldman-Rakic, 1991). Additional architectonic studies have described areas within the arcuate sulcus and premotor cortex as well as the subdivisions of the orbital cortex (Carmichael and Price, 1995; Petrides and Pandya, 2002; Saleem et al., 2008; Gerbella et al., 2010), but we will confine our discussion to VLPFC, areas 45 and 12/47. We will refer to area 12 in general as area 12/47 to convey the homology of area 12 in the macaque with human area 47 as introduced by Petrides and Pandya
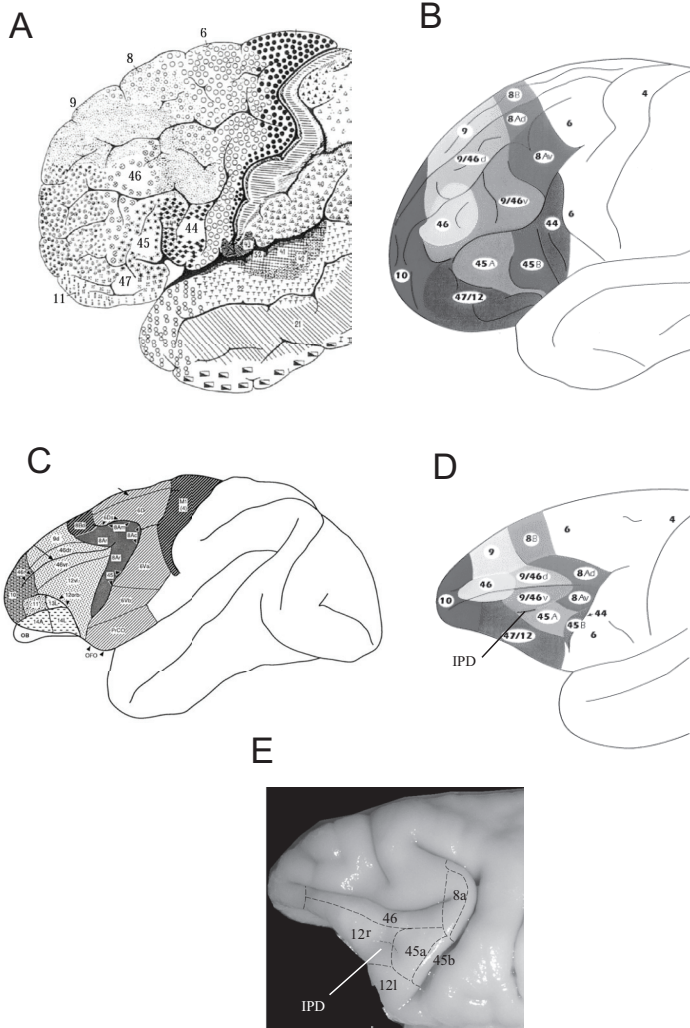
FIGURE 15.1 Organization of ventral PFC. Maps of the cytoarchitectonic organization of ventral PFC are shown for the human (*A* and *B*) and macaque brain (*C–E*). (*A*) Brodmann map (1909) of the human brain with areas 44, 45, and 47 marked on the IFG. Reproduced with permission from Brodmann (1909). (*B*) Map of the human brain color-coded to match the corresponding homologous regions in the macaque brain shown in *D*. Reproduced with permission from Petrides and Pandya (2002). (*C*) Map of *M. mulatta*. Reproduced with permission from Preuss and Goldman-Rakic (1991). (*D*) Map of macaque brain. Reproduced with permission from Petrides and Pandya (2002). (*E*) Photomicrograph of VLPFC in macaque monkey with cytoarchitectonic areas labeled. The IPD is marked in *D* and in *E*. Image courtesy of G. Luppino (Gerbella et al., 2010).

(2002). However, to differentiate between the ventrolateral region below the principal sulcus and the lateral orbital cortex we will use 12 ventrolateral (12vl) and 12 orbital (12o), respectively, as defined by Preuss and Goldman-Rakic (1991).

The VLPFC also commonly includes a small sulcus—termed the inferior frontal sulcus by Winters et al. (1969) and the infraprincipal dimple or the inferior prefrontal dimple (IPD) by others (Paxinos et al., 2000; Petrides and Pandya, 2002; Petrides et al., 2005)—which varies in its position and depth in *M. mulatta*. Some schematics of VLPFC depict the IPD as running in a rostral-to-caudal direction and separating area 45A from area 46 (Petrides and Pandya, 2002). However, in our neurophysiological recordings (Romanski and Goldman-Rakic, 2002; Romanski et al., 2005) and in other studies (Petrides et al., 2005), it is depicted as running dorsal to ventral and separating area 45 from area 12/47. It is not always described or visible in studies of other subspecies of macaque monkeys. Thus, there is variability in the position of area 12/47 and area 45 not only between the subspecies of macaques but also within *M. mulatta* individuals. As explained later, the IPD is the primary location in which auditory responsive neurons and audiovisual responsive cells have been reliably located in several studies, and may be a critical landmark for delineating the functional auditory responsive prefrontal region in macaques. Whether it defines the border of areas 12/47 and 45 is unclear.

In the human brain, areas 44 and 45 have been associated with language processing confirmed by electrical stimulation, PET and functional MRI (fMRI). However, the areas that control vocalization production in Old World monkeys are not as well understood and could include VLPFC, whereas other studies have implicated ventral premotor and the cingulate vocalization area (Petrides et al., 2005; Jürgens, 2009; Coudé et al., 2011).

Cytoarchitectonic Organization of VLPFC

The cytoarchitectonic descriptions here are taken from Preuss and Goldman-Rakic (1991), who described the frontal lobe of the rhesus macaque, *M. mulatta*, which is the same species that has been examined in most neurophysiology studies of VLPFC. These descriptions are in general agreement with Petrides and Pandya (2002). Area 45 is located ventral to the caudal principal sulcus within the ventral limb of the arcuate and extends onto the cortical surface (Fig. 15.1C–E). It is composed of large pyramidal cells in layer V and deep layer III. Layer IV is thick with densely packed small cells, with some of the larger pyramidal cells from deep layer III and superficial layer V intruding on layer IV. It is densely myelinated. Area 45 is bordered dorsally by area 8a (Preuss and Goldman-Rakic, 1991), which can be distinguished from area 45 by the presence of

extremely large pyramidal cells in layer Va of area 8. Area 12 (areas 12vl and 12o), which covers the surface of the ventrolateral convexity and extends onto the lateral orbital surface as far as the lateral orbital sulcus, can be distinguished from area 46 by its more heavily myelinated appearance. The disappearance of the large layer III pyramidal cells marks the transition from area 45 to area 12vl. Area 12vl on the ventrolateral surface has been distinguished from area 12o by the more diffuse myelinated appearance of 12o and the more granular layer IV of 12vl. A series of comparative cytoarchitectonic studies have examined the similarities of area 12 in macaque and area 47 in the human brain (Petrides and Pandya, 2002). As a result, area 12 has been referred to as area 12/47 even though the assignation of 12 was renewed in a recent analysis of VLPFC connections (Gerbella et al., 2010). In addition, studies by Petrides and Pandya (2002) and Gerbella et al. (2010) have suggested that area 45 be divided into subdivisions 45B, closest to and within the anterior limb of the anterior bank of the arcuate sulcus, and area 45A, located rostral to 45B and extending across the surface of the inferior convexity to the IPD, with area 12vl (i.e., 47) bordering 45A rostrally and ventrally (Fig. 15.1). The precise location of area 12vl relative to area 45A may vary somewhat in individuals and may be better determined from a combination of connectivity studies and physiological recordings.

Cortical Connectivity of VLPFC

Connectivity of VLPFC with Cortical Visual Processing Regions

Much of what we know about the cellular functions of the primate PFC is based on the processing of visual information. Thus, it is not surprising that many studies have examined projections from visual association cortex to the primate PFC. Results indicate that VLPFC receives afferents from extrastriate visual cortical areas in the inferotemporal cortex, including area TE. Early anatomical studies by Barbas, Pandya, and others examined the innervation of the entire prefrontal mantle by visual association areas (Chavis and Pandya, 1976; Barbas, 1988; Barbas and Pandya, 1989). Barbas was among the first to note that basoventral prefrontal cortices were more strongly connected with extrastriate, ventral visual areas, which have been implicated in object recognition and feature discrimination. In contrast, medial and dorsal prefrontal cortices are more densely connected with medial and dorsolateral occipital and parietal areas, which are associated with visuospatial functions (Barbas, 1988). This dissociation was confirmed by Bullier et al. (1996), who found a segregation of inputs to caudal PFC when paired injections of tracers were placed into temporal and parietal visual processing regions. In their study, visual temporal

cortex projected mainly to ventrolateral PFC, area 45, whereas parietal cortex sent projections to ventrolateral PFC and dorsolateral PFC (areas 8a and 46) (Bullier et al., 1996). Tracing and lesion studies by Ungerleider et al. (1989) showed that area TE projected specifically to three ventral prefrontal targets, including the ventral limb of the arcuate sulcus (area 45), the inferior convexity just ventral to the principal sulcus (area 12vl), and within the lateral orbital cortex (areas 11 and 12o). These projections are via the uncinate fasciculus (Ungerleider et al., 1989). Furthermore, ventrolateral PFC areas 12vl and 45, which contain object- and face-selective neurons (Wilson et al., 1993; Ó Scalaidhe et al., 1997, 1999), were shown to be connected with inferotemporal areas TE and TEO (Webster et al., 1994), with the strongest innervation of ventrolateral PFC and orbitofrontal areas 11 and 12o originating in TE.

Auditory Projections to PFC

In contrast to the visual pathways, the prefrontal targets of central auditory pathways have not been studied as extensively despite the accepted role of the frontal lobe in language. In early anatomical studies, lesion/degeneration techniques were used to reveal projections from the caudal superior temporal gyrus (STG) to the principal sulcus region, the arcuate cortex, and the inferior convexity of the frontal lobe, and from the middle and rostral STG to the rostral principal sulcus and orbital regions (Pandya et al., 1969; Jones and Powell, 1970; Chavis and Pandya, 1976). Additional studies revealed connections between the lateral PFC and cortical areas within the STG (Galaburda and Pandya, 1983; Barbas and Mesulam, 1985; Barbas, 1988; Barbas and Pandya, 1989). There was a suggestion of rostrocaudal topography in these studies whereby anterior and middle aspects of the principal sulcus, including areas 9, 10, and rostral 46, were connected with the middle STG, whereas area 8 received projections from mostly caudal STG (Barbas and Mesulam, 1985; Barbas, 1988; Petrides and Pandya, 1988). It became clear that VLPFC received afferents from the STG, inferotemporal cortex, and multisensory regions within the superior temporal sulcus (STS).

Importantly, detailed anatomical studies by Morel et al. (1993), Jones et al. (1995), and Hackett et al. (1998), together with parallel neurophysiological studies by Rauschecker et al. (1995), provided evidence that primate auditory cortices were organized as a core-belt system with a third zone, the parabelt just lateral to the belt (Morel et al., 1993; Jones et al., 1995; Hackett et al., 1998). A series of landmark neurophysiology studies provided the first electrophysiological evidence for three separate tonotopic regions (AL, ML, and CL) in the belt cortex that could be distinguished from the core A1 (Rauschecker et al., 1995). Additional studies have described functional dissociations of anterior and posterior belt and

parabelt regions (Tian et al., 2001). Rauschecker et al. (1995), Morel et al. (1993), and Hackett et al. (1998) used a common terminology to delineate auditory cortex in anatomical and physiological studies, which enabled cross-talk and comparisons that fostered progress in the study of auditory cortical processing and organization.

Combining physiological recording with anatomical tract tracing, Romanski et al. (1999b) analyzed the connections of physiologically defined areas of the belt and parabelt auditory cortex. They determined that rostral and ventral PFC receives projections from the anterior auditory association cortex (areas AL and anterior parabelt) and caudal prefrontal regions are innervated by posterior auditory cortex (areas CL and caudal parabelt; Fig. 15.2). Together with auditory physiological recordings from the lateral belt (Tian et al., 2001) and from the PFC (Romanski and Goldman-Rakic, 2002; Romanski et al., 2005), these studies suggest that separate auditory streams originate in the caudal and rostral auditory cortex and target dorsolateral spatial and anterior-ventrolateral object domains in the frontal lobe, respectively (Romanski, 2007). This is similar to the dorsal and ventral streams described for the visual system (Ungerleider and Mishkin, 1982). Ultimately, this also implies that auditory and visual afferents target similar functional domains of dorsal and ventral PFC (Romanski et al., 2005). The convergence of auditory and visual ventral stream inputs to the same VLPFC domain suggests that they may be integrated and combined to serve a similar function, for example, that of object recognition, which is aided by the integration of multiple sensory inputs.

Examination of the connections of VLPFC without accompanying physiology has suggested that area 45A receives greater inputs from the STG than from inferotemporal cortex (Petrides and Pandya, 2002; Gerbella et al., 2010). This is in contrast to previous analysis of anterograde projections of the STG and of inferotemporal cortex. These anterograde studies suggest that STG and STS innervate area 12/47 whereas inferotemporal and STS cortex project to area 45 and area 12/47. Much of the debate appears centered on where the boundary between area 45 and area 12/47 occurs, and may be clarified with additional neurophysiological recordings and combined anatomical connectivity studies.

FUNCTIONAL STUDIES OF VLPFC

Visual Processing in VLPFC

Decades of research have demonstrated the frontal lobe's involvement in cognitive functions including working memory, decision making, and social communication processes such as language and face–voice processing. Single unit recording studies in animal models have characterized dorsolateral prefrontal cortex (DLPFC) neuronal involvement in visuospa-
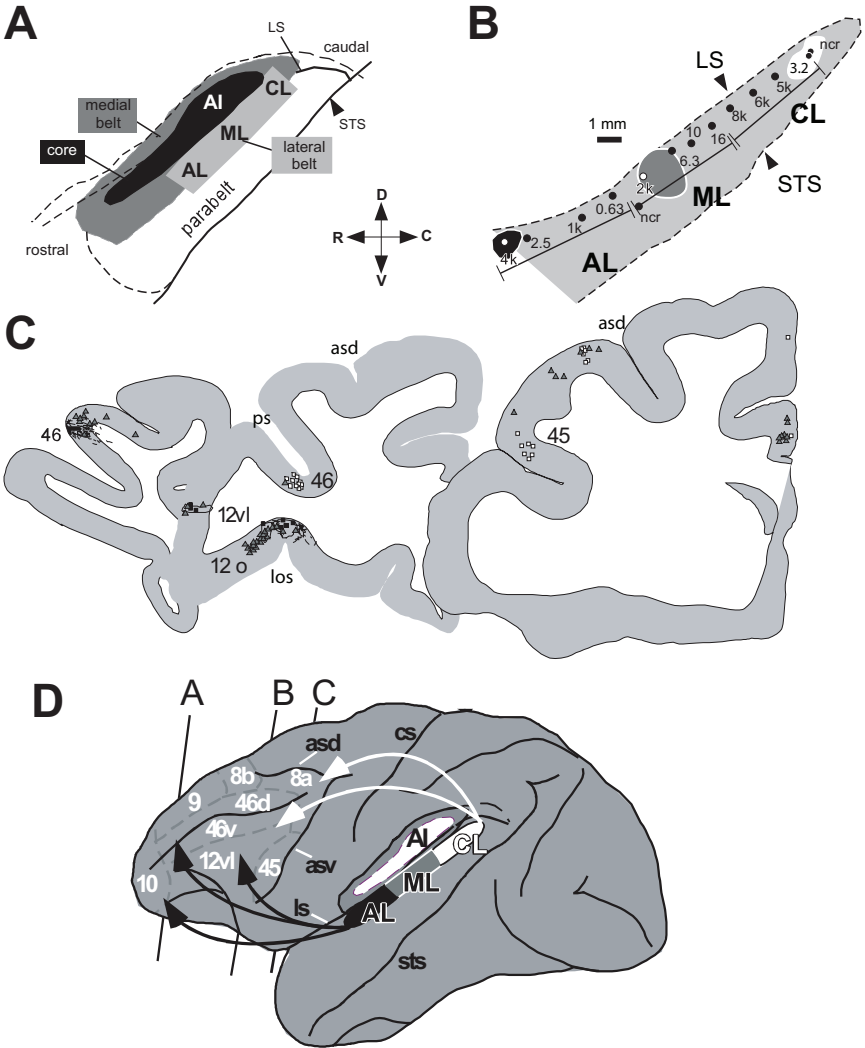
FIGURE 15.2 Dual streams of auditory afferents target the PFC. (*A*) The auditory cortex core (A1) surrounded by the belt (box delineates the lateral belt cortex shown in *B*). Injections placed into the auditory belt cortex at similar frequency-mapped locations in AL (black), ML (gray), and CL (white) are shown as colored polygons. (*C*) The resulting labeled retrograde cells and anterograde fibers are shown in three coronal sections through the PFC from rostral to caudal, with the same color coding as in *B* to indicate the source of the injections. (*D*) Summary of projections. Rostral and VLPFC receives stronger innervation from the anterior belt and adjacent parabelt regions whereas dorsolateral PFC receives the greatest innervation from caudal auditory belt and parabelt regions. Adapted from Romanski et al. (1999b).

tial processing, saccadic eye movements, and working memory (Bruce and Goldberg, 1985; Funahashi et al., 1989, 1993; Quintana and Fuster, 1992; Chafee and Goldman-Rakic, 1998). Further investigations have emphasized a process-oriented role for DLPFC and have described single-unit activity of prefrontal neurons during decision making, categorization, numerosity, and the coding of abstract rules (Kim and Shadlen, 1999; Miller and Cohen, 2001; Nieder et al., 2002; Freedman and Miller, 2008).

In contrast, investigation of the cellular activity in the VLPFC has focused on object processing and social communication. Early studies of VLPFC showed that neurons in this region were responsive to simple and complex visual stimuli presented at the fovea (Rosenkilde et al., 1981; Suzuki and Azuma, 1983). Face-responsive neurons were documented by Thorpe et al. (1983) and Rolls et al. (2006) and later described in detail by Goldman-Rakic and coworkers (Ó Scalaidhe et al., 1997, 1999; Wilson et al., 1993). In these studies, Wilson et al. (1993) showed that DLPFC and VLPFC neurons responded differentially to spatial and object features of visual stimuli. These studies were the first to demonstrate a functional dissociation between DLPFC and VLPFC by using single-unit electrophysiology. Wilson et al. (1993) showed that DLPFC neurons were selectively engaged by visuospatial memory tasks and VLPFC neurons were selective for color, shape, or type of visual objects. An earlier study by Mishkin and Manning (1978) showed that lesions of VLPFC in nonhuman primates interfere with the processing of nonspatial information, including color and form. Electrophysiological recordings demonstrated that VLPFC face cells had a twofold increase in firing rate to face stimuli compared with nonface stimuli during passive presentations or during working memory tasks (Ó Scalaidhe et al., 1997, 1999). Face cells were found only in the VLPFC and not in DLPFC, and were localized to three small parts of VLPFC, including a patch on the lateral convexity close to the lower limb of the arcuate sulcus (area 45), within and around the IPD (area 12vl), and a small number of cells in the lateral orbital cortex (Ó Scalaidhe et al., 1997). VLPFC face cells were sensitive to changes in facial features, expressions, or the angle of gaze, much like the inferotemporal cortical regions, which project to these VLPFC cells. These studies have suggested that VLPFC cells may encode identity, expression, and face view (Ó Scalaidhe et al., 1997, 1999; Rolls et al., 2006; Romanski and Diehl, 2011). Data from the single-unit recordings have been confirmed with fMRI studies in macaque monkeys (Tsao et al., 2008b), which have demonstrated activation of face-responsive "patches" in the same arcuate, ventrolateral, and orbitofrontal locations shown by Ó Scalaidhe et al. (1997, 1999). Demonstration by both methods of visual responsiveness and face selectivity substantiates the notion that VLPFC in the macaque monkey is involved in object and face processing (Fig. 15.3).
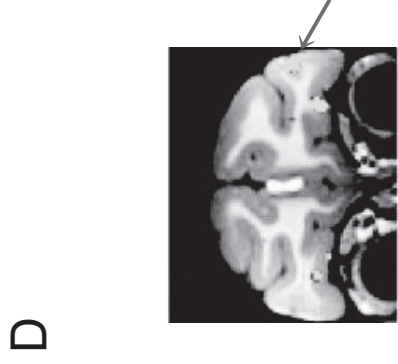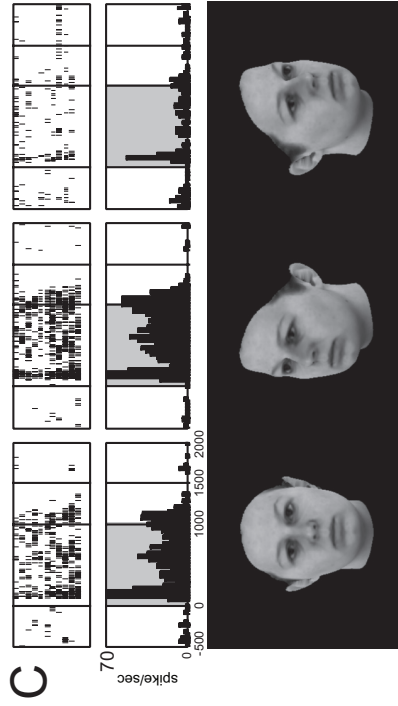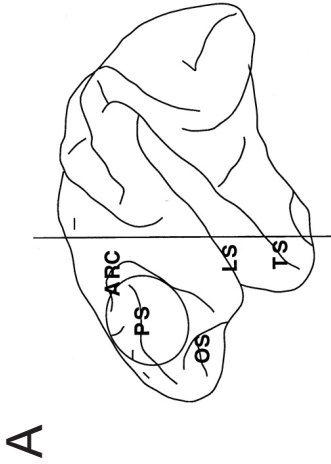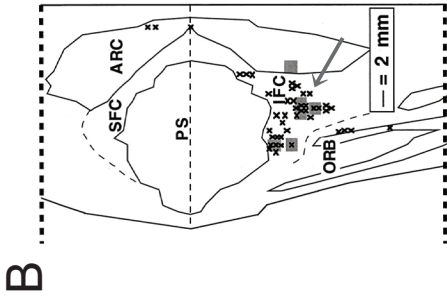
Auditory Responses and Function in Ventral PFC

The ventral frontal lobe has long been linked with complex auditory function through its association with language functions in the IFG. The results of some studies have suggested parcellation of function in the human IFG. The anterior region, the pars triangularis (area 45), along with the pars orbitalis (area 47), has been suggested to be more involved in semantic processing, comprehension, and auditory working memory (Démonet et al., 1992; Paulesu et al., 1993; Buckner et al., 1995; Demb et al., 1995; Stromswold et al., 1996; Price, 1998; Poldrack et al., 1999; Gelfand and Bookheimer, 2003). In contrast, the pars opercularis (area 44) and ventral premotor cortex are more active during phonological processing and speech production. The precise neuronal mechanisms that occur in the frontal lobe during the processing of complex auditory information are unknown but might be indirectly assessed with neurophysiological recordings in animals with similar ventral frontal lobe regions, such as macaque monkeys.

Neuronal responses to acoustic stimuli have been sporadically noted in the frontal lobes of Old and New World monkeys (Newman and Lindsley, 1976; Wollberg and Sela, 1980; Tanila et al., 1993). However, when recordings targeted cortical areas that had been shown to receive projections from acoustically characterized regions of the auditory belt and parabelt cortex (Romanski et al., 1999b), a discrete auditory responsive region was localized in VLPFC (Romanski and Goldman-Rakic, 2002). This VLPFC cortical region is thought to be the termination of a ventral

FIGURE 15.3 Face-responsive neurons in the VLPFC. (*A* and *B*) Face-responsive neurons recorded by Ó Scalaidhe et al. (1997) are depicted. Adapted from Ó Scalaidhe et al. (1997). (*A*) Region recorded in the PFC is indicated with a circle on the lateral brain schematic of the rhesus macaque brain. (*B*) Flat map of the recorded region in the PFC is shown with face cells outlined in black and gray. (*C*) Face-responsive cells that were selective for forward and 30° rotated face view (Romanski and Diehl, 2011). Stimulus images courtesy of Michael J. Tarr, Center for the Neural Basis of Cognition and Department of Psychology, Carnegie Mellon University, http://www.tarrlab.org/. (*D*) Location of face-selective patches in the VLPFC and in the orbitofrontal cortex is shown in white (Tsao et al., 2008b). A gray arrow indicates a similar portion of the VLPFC in the single-unit data portrayed in *B* and in the fMRI data in *D*. Reprinted by permission from Macmillan Publishers Ltd: *Nature Neuroscience* (Tsao et al., 2008b), copyright (2008).
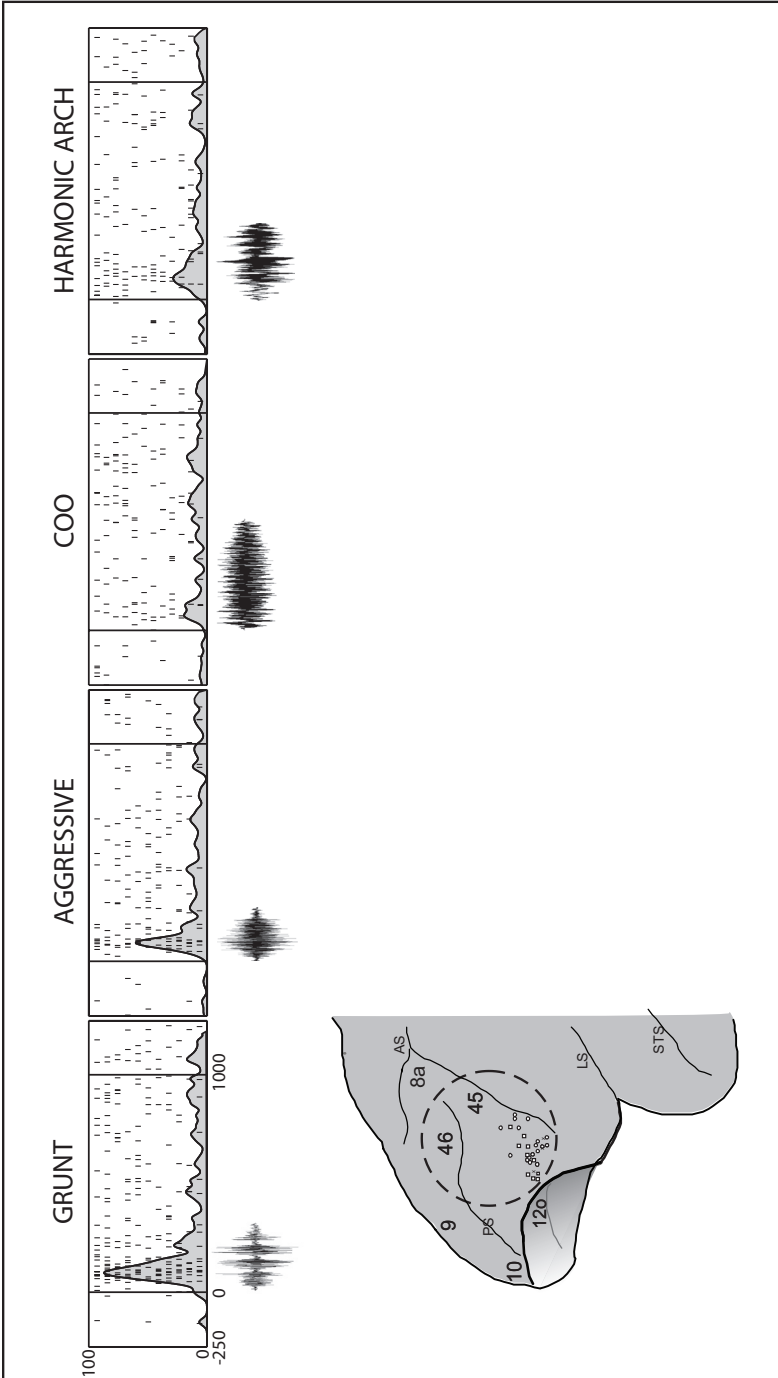
auditory processing stream, specialized for the processing of nonspatial (i.e., object) auditory information (Romanski et al., 1999b; Romanski, 2007; Cohen et al., 2009; Romanski and Averbeck, 2009). The auditory responsive region of VLPFC is located rostral to the ventral limb of the arcuate sulcus below the principal sulcus, in the area of the IPD. This region receives projections from ventral stream auditory cortical regions and polymodal cortex of the STS, as discussed earlier (Carmichael and Price, 1995; Hackett et al., 1999; Romanski et al., 1999a,b; Petrides and Pandya, 2002). VLPFC auditory neurons are responsive to complex auditory stimuli, including vocalization and complex nonvocal stimuli (Romanski and Goldman-Rakic, 2002). This small ventrolateral prefrontal auditory region has also been shown to be active in neuroimaging studies in rhesus monkeys during presentation of complex acoustic stimuli (Poremba and Mishkin, 2007).

The VLPFC auditory area was analyzed with a large library of rhesus macaque vocalizations to test selectivity to specific call categories, as previous analysis had implied some selectivity for calls with common functions (Gifford et al., 2005). Analysis of these vocalization responses with exemplars from 10 different types of calls demonstrated that neurons tended to respond to two or three vocalization types that had similar acoustic morphology rather than similar behavioral referents (Fig. 15.4; Romanski et al., 2005). Additional electrophysiological recording studies by Gifford et al. (2005) and Russ et al. (2007) have suggested that VLPFC neuronal activity is modulated during categorization of acoustic stimuli and in auditory decision making (Lee et al., 2009). These combined data are consistent with a role for VLPFC in a ventral auditory processing stream for auditory objects, including vocalizations. The localization of this auditory processing area to the ventral prefrontal region of Old World monkeys suggests a functional similarity between it and human language-

FIGURE 15.4 Auditory responsive neurons in VLPFC. A single-cell example of responses to four different vocalization stimuli is shown in the top part of the figure. The response is shown as raster and shaded spike density function in response to a "grunt" vocalization (low-value food call), an aggressive "pant threat" vocalization, a "coo" vocalization (low-value food and affiliative), and a harmonic arch (high-value food). The waveforms of the calls are shown below the rasters. A schematic of the PFC is shown indicating the locations of auditory responsive neurons. Adapted from Romanski et al. (2005).

processing regions in the ventral or inferior frontal lobe of the human brain (Deacon, 1992; Romanski and Goldman-Rakic, 2002; Aboitiz, 2012).

Multisensory Responses in VLPFC

The initial physiological studies of VLPFC suggested that auditory and visual object processing regions were located adjacent to one another in VLFPC. Any overlap in these auditory and visual responsive zones could thus be sites for multisensory integration of complex auditory and visual information. As neurons in this region are face- and vocalization-responsive, multisensory neurons in the macaque VLPFC might integrate face and vocal information. Given that the percentage of neurons responsive to visual stimuli was much greater than the number of auditory responsive cells (55% vs. 18%), we reasoned that multisensory cells are more likely to be located in regions where auditory cells had been recorded and predicted that multisensory neurons might be found only in this region. In our neurophysiological investigation, we presented movies of familiar monkeys vocalizing to macaque monkeys while single neurons were recorded from the VLPFC (Sugihara et al., 2006). These movies were separated into audio and video streams, and neural responses to the unimodal stimuli were compared with the responses to the combined audiovisual stimuli. Interestingly, approximately half the neurons recorded in the VLPFC were multisensory in that they responded to unimodal auditory and visual stimuli, that is, bimodal responses; or were multisensory because of an enhanced or decreased response to the combined audiovisual stimulus (face and vocalization) compared with the response to the unimodal stimuli (Sugihara et al., 2006). This is likely to be an underestimate of the percentage of multisensory responses because we used a limited set of audiovisual stimuli and neurons were found to be selective for particular face–vocalization pairs.

VLPFC neurons exhibited multisensory enhancement or suppression (Fig. 15.5) just as neurons do in the superior colliculus, the STS, and auditory cortex during multisensory integration (Stein and Meredith, 1993; Barraclough et al., 2005; Ghazanfar et al., 2005; Lakatos et al., 2009). It was also interesting that face/voice stimuli evoked multisensory responses more frequently than nonface/nonvoice audiovisual stimuli. This adds support to the notion that VLPFC is part of a circuit that is specialized for the integration of social communication information rather than sensory stimuli in a general sense. In localizing these multisensory responses to the PFC, there appeared to be two somewhat separate VLPFC regions for multisensory processing. Interestingly, these two separate clusters of multisensory neurons overlap with two prefrontal face patches described by Ó Scalaidhe et al. (1997) and Tsao et al. (2008b) in the arcuate and
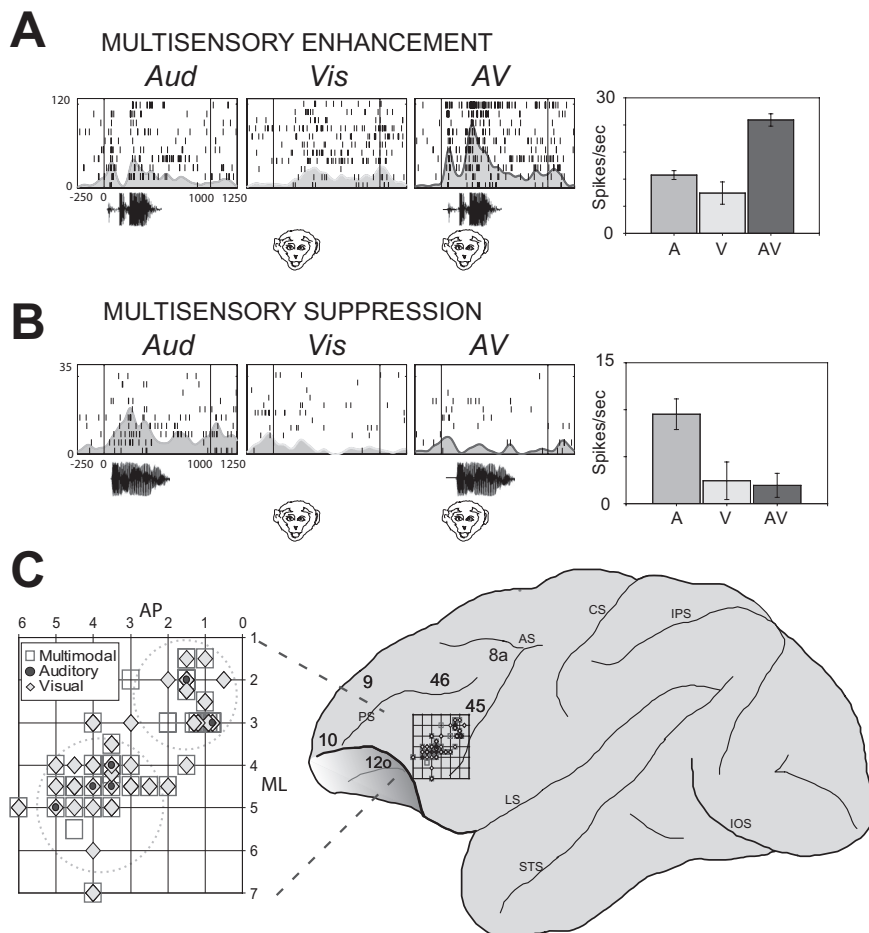
FIGURE 15.5 Multisensory neurons in the VLPFC. The responses of two single units are shown in *A* and *B* as raster/spike density plots to an auditory stimulus alone (a vocalization, AUD) and face (Vis) and both presented simultaneously (AV). *Right*: Bar graph of mean response to these stimuli. Cell in *A* exhibited multisensory enhancement and cell in *B* exhibited multisensory suppression. Locations where multisensory stimuli (open squares), visual responses (gray diamonds), and auditory responses (dark circles) are depicted on lateral view of frontal lobe in *D*, with the inset shown larger in *C*. Adapted from Romanski and Averbeck (2009).

ventrolateral PFC areas. In our study, there was a large pool of unimodal visual neurons with a small number of multisensory cells located in posterior VLPFC (area 45). Unimodal neurons in this area are mostly visual and respond to faces and nonface stimuli such as objects, shapes, and

patterns. The multisensory neurons in this arcuate region (Fig. 15.5) have strong visual responses modulated by the simultaneous presentation of auditory stimuli. There are strong projections to this area from the infero-temporal cortex and the polymodal STS, which have been associated with the processing of facial identity and facial expression. Previous studies in nonhuman primates of visual working memory, decision making, and visual search (Wilson et al., 1993; Kim and Shadlen, 1999; Freedman and Miller, 2008) have noted responsive neurons within this arcuate region as well as in the more commonly recorded principal sulcus region.

A smaller, potentially more specialized pool of multisensory neurons is located in VLPFC, anterior and lateral to the first pool (Fig. 15.5). These neurons are found near the IPD, and within its banks in area 12vl of Preuss and Goldman-Rakic (1991). This is the region where unimodal auditory responsive neurons were predominantly localized in previous studies (Romanski and Goldman-Rakic, 2002; Romanski et al., 2005). These anterolateral VLPFC neurons respond to vocalizations and to faces, but only weakly to other visual stimuli (Romanski and Goldman-Rakic, 2002; Sugihara et al., 2006). This area receives afferents from mainly polymodal STS cortical regions and also from auditory association cortex, including a small amount of afferents from the belt, more from the parabelt, and the largest contribution from the rostral temporal lobe (Romanski et al., 1999a,b; Hackett et al., 1999). Multisensory responses here favor vocalizations and their corresponding faces, suggesting a more specialized role in the integration of social communication information. Face-responsive cells recorded in this area, which were selective for forward gaze, such as that which occurs in face-to-face communication, were also more likely to be auditory responsive (Romanski and Diehl, 2011). Most of the multisensory neurons exhibited suppression rather than enhancement. This nonlinear interaction has also been noted in auditory cortex (Ghazanfar et al., 2005; Lakatos et al., 2009). This anterolateral pool of multisensory neurons may be specialized for the integration of social communication sounds with facial gestures and other communication-oriented information. In contrast, the more posterior multisensory neurons may serve a more general integrative purpose.

AUDIOVISUAL INTEGRATION IN HUMAN BRAIN AND IMPLICATIONS FOR EVOLUTION OF AUDIOVISUAL SPEECH

How does this compare with the ventral frontal lobe in the human brain? Whereas many associate the human IFG with only spoken language and verbal processing, communication is, in fact, a multisensory process. Several well-known illusions owe their effects to specific aspects of multisensory integration, including the McGurk and ventriloquist

effects (McGurk and MacDonald, 1976; Bertelson and Aschersleben, 2003). Although cross-modal integration takes place over a network of areas in the brain (Driver and Noesselt, 2008; Stein and Stanford, 2008), the same areas that underlie speech and language processing in the temporal and frontal cortex play an essential role in the integration of audiovisual communication information. The STS and the ventral frontal lobe are both sites of activation during the processing and integration of speech and gestures (Homae et al., 2002; Jones and Callan, 2003; Beauchamp et al., 2010; Noppeney et al., 2010). In an fMRI study of speech and gesture, Xu et al. (2009) found overlap of activation in two regions of the IFG when subjects viewed gestures or listened to a voicing of the phrase that fit the gesture. The activated regions included a large cluster in the pars triangularis and pars opercularis (areas 44 and 45) and a smaller focal cluster in pars orbitalis, area 47 (Fig. 15.6). Xu et al. (2009) argued that the IFG most likely plays a larger role in communication than classical auditory-speech processing and theorized that linking meaning with acoustic or visual symbols may be the essential function of these inferior frontal regions.

Thus, the process of linking, or integrating phonological constructs with auditory objects, results in the perception of spoken words, whereas integration of a visual image of letters with their learned meanings conveys the concept of a word. Integration might then be one of many basic processes the human frontal lobe performs during speech, language, and communication. The linking, or integrating of face and vocal information, in the macaque monkey frontal lobe could be seen as a precursor to the more complex functions that the IFG performs in the human brain whereby abstract concepts are united with images and sounds. In the human brain, words, sounds, gestures, and visual images are each integrated with meaning and with each other (Xu et al., 2009). In more primitive primates, such as macaques, in which abstraction is not likely to occur, faces are integrated with vocalizations. It has been previously suggested that the ventral PFC is essential in associating a visual cue with an action (Passingham et al., 2000). Our data suggest that VLPFC may associate auditory cues with gestural actions, which is necessary during communication.

Depending on the aspects of the stimuli that are integrated, several communication-relevant functions may be accomplished through integration. In humans, adding mouth movements or facial expressions to spoken words can clarify or even alter the meaning of an utterance (McGurk and MacDonald, 1976). Incongruency between face identity and a voice or between a facial expression and a vocal sound is detected by humans and activates prefrontal and temporal cortical regions. Some human neuroimaging studies have demonstrated a decrease in ventral prefrontal activity for incongruent faces and voices (Calvert et al., 2001; Homae et al., 2002;
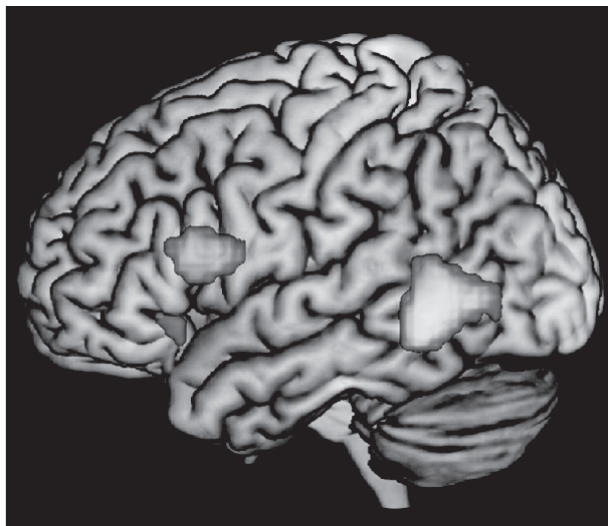
FIGURE 15.6 Convergence of speech- and gesture-responsive regions in the human frontal lobe. Shown are three activation clusters that were active for speech and gesture conditions. There is a large activation cluster in the posterior temporal lobe and in the IFG areas 44 and 45 and a small focal activation rostrally in area 47. Reproduced from Xu et al. (2009).

Jones and Callan, 2003). Others report increased activations during incongruent stimuli (Miller and D'Esposito, 2005; Ojanen et al., 2005; Hein et al., 2007). Recordings of macaque VLPFC neurons show that incongruent stimuli also evoke an increase or a decrease in neuronal activity depending on the original response to bimodal stimuli (Romanski and Diehl, 2011). The sign of the neuronal response may also be affected by facial features and emotional valence of the audiovisual stimuli.

Identity, or recognition, is another process that greatly benefits from the integration of face and vocal information [reviewed in Campanella and Belin (2007)] and studies have shown that animals match faces and corresponding voices as we do (Jordan et al., 2005; Sliwa et al., 2011). The circuit for the processing of face identity includes the cortex within the STS and inferotemporal cortex, and single cells in these areas respond to facial identity and facial expression (Sugase et al., 1999; Eifuku et al., 2004). How multisensory neurons in the STS integrate face and vocalization information to enhance recognition is not known at the single-cell level even though pairing of incongruent faces and vocalizations alters activity

in this region. The STS has a robust connection with VLPFC and is likely to send unimodal and multisensory identity information to VLPFC neurons. Selectivity of face-responsive cells in VLPFC has been shown for particular individuals, expressions, or categories of face stimuli (Ó Scalaidhe et al., 1997, 1999; Rolls et al., 2006; Romanski and Diehl, 2011).

The accumulation of evidence to date shows that cells in the ventral PFC of the macaque monkey respond to and integrate audiovisual information. VLPFC cells respond optimally to face and vocalization stimuli and exhibit multisensory enhancement or suppression when face-vocalization stimuli are combined. Thus, the ventral frontal lobe of nonhuman primates may have some basic functional homologies to the human frontal lobe, although more evidence from additional primate species is needed. The basic process of associating a face, or facial gesture, with a vocal stimulus, which occurs in the macaque PFC, may be a precursor to the more complex functions of the human frontal lobe, where semantic meaning is linked with acoustic or visual symbols.

16

Math, Monkeys, and
the Developing Brain

JESSICA F. CANTLON

Thirty thousand years ago, humans kept track of numerical quantities by carving slashes on fragments of bone. It took approximately 25,000 years for the first iconic written numerals to emerge among human cultures (e.g., Sumerian cuneiform). Now, children acquire the meanings of verbal counting words, Arabic numerals, written number words, and the procedures of basic arithmetic operations, such as addition and subtraction, in just 6 years (between ages 2 and 8). What cognitive abilities enabled our ancestors to record tallies in the first place? Additionally, what cognitive abilities allow children to rapidly acquire the formal mathematics knowledge that took our ancestors many millennia to invent? Current research aims to discover the origins and organization of numerical information in humans using clues from child development, the organization of the human brain, and animal cognition.

This review traces the origins of numerical processing from "primitive" quantitative abilities to math intelligence quotient (IQ). "Primitive" quantitative abilities are those that many animals use to estimate the value of an object or event, for instance its distance, length, duration, number, amplitude, saturation, or luminance (among others).

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The constraints on how human and animal minds process these different quantities are similar (Gallistel and Gelman, 1992). For example, all of these quantities show cognitive processing limitations that can be predicted by Weber's law. Weber's law states that quantity discrimination is determined by the objective ratio between their values. This ratio-based psychological and neural signature of quantity processing indicates that many quantities are represented in an analog format, akin to the way in which a machine represents intensities in currents or voltages (Gallistel and Gelman, 1992). I discuss the types of constraints that influence quantity discrimination, using "number" as the initial example, and then consider the psychological and neural relationship between "number" and other quantitative dimensions. Similar constraints on processing across different quantities have been interpreted as evidence that they have a common evolutionary and/ or developmental origin and a common foundation in the mind and brain (Zorzi et al., 2002; Walsh, 2003; Pinel et al., 2004; Feigenson, 2007; Ansari, 2008; Cohen Kadosh et al., 2008; Cantlon et al., 2009c; de Hevia and Spelke, 2009; Lourenco and Longo, 2011; Bonn and Cantlon, 2012). The resolution of these issues is important for understanding the inherent organization of our most basic conceptual faculties. The issue is also important for understanding how our formal mathematical abilities originated.

Primitive quantitative abilities play a role in how modern humans learn culture-specific, formal mathematical concepts (Gallistel and Gelman, 1992). Preverbal children and nonhuman animals possess a primitive ability to appreciate quantities, such as the approximate number of objects in a set, without counting them verbally. Instead of counting, children and animals can mentally represent quantities approximately, in an analog format. Studies from our group and others have shown that human adults, children, and nonhuman primates share cognitive algorithms for encoding numerical values as analogs, comparing numerical values, and arithmetic (Meck and Church, 1983; Gallistel, 1989; Feigenson et al., 2004; Cantlon et al., 2009c). Developmental studies indicate that these analog numerical representations interact with children's developing symbolic knowledge of numbers and mathematics (Gelman and Gallistel, 1978; Feigenson et al., 2004). Furthermore, the brain regions recruited during approximate number representations are shared by adult humans, nonhuman primates, and young children who cannot yet count to 30 (Dehaene et al., 2003; Nieder, 2005; Ansari, 2008). Finally, it has recently been demonstrated that neural regions involved in analog numerical processing are related to the development of math IQ (Halberda et al., 2008). Taken together, current findings implicate continuity in the primitive numerical abilities that are shared by humans and nonhumans, as well as a degree of continuity in human numerical abilities ranging from primitive approximation to complex and sophisticated math.

OLDEST NUMBERS IN THE WORLD

The fact that humans have been recording tallies with sticks and bones for 30,000 years is impressive, but the critical issue is this: what cognitive abilities enabled them to encode quantities in the first place? To identify the inherent constraints on humans' ability to process numerical information, it is helpful to consider the evolutionary history of numerical thought. We can look for clues to the evolutionary precursors of numerical cognition by comparing human cognition with nonhuman primate cognition. The degree to which humans and nonhuman primates share numerical abilities is evidence that those abilities might derive from a common ancestor, in the same way that common morphology like the presence of 10 fingers and toes in two different primate species points to a common morphological heritage.

So far, there is evidence that nonhuman primates share three essential numerical processing mechanisms with modern humans: an ability to *represent* numerical values (Brannon and Terrace, 1998; Nieder, 2005; Cantlon and Brannon, 2006, 2007b), a general mechanism for mental *comparison* (Cantlon and Brannon, 2005), and *arithmetic* algorithms for performing addition and subtraction (Beran and Beran, 2004; Cantlon and Brannon, 2007a). These findings compliment and extend a long history of research on the numerical abilities of nonhuman animals [see Emmerton (2001) for review].

Representation

When adult humans and monkeys are given a task in which they have to rapidly compare two visual arrays and touch the array with the smaller numerical value (without counting the dots), their performance reliably yields the pattern shown in Fig. 16.1: accuracy decreases as the ratio between the numerical values in the two arrays approaches 1 [Cantlon and Brannon (2006); see Dehaene (1992) and Gallistel and Gelman (1992) for review]. The explanation of this performance pattern is that both groups are representing the numerical values in an analog format (Fig. 16.2).

In an analog format, number is represented only approximately, and it is systematically noisy (Dehaene, 1992; Gallistel and Gelman, 1992]. More precisely, the probability of noise (i.e., the spread of the distributions) in the subjective representation of a number increases with the objective number of items that are coded by that representation. Consequently, the probability of confusion (i.e., the overlap between distributions) between any two objective numbers increases as their value increases. This means that the probability of having an accurate subjective representation of a numerical value decreases with its objective value. This relationship can be succinctly quantified by the ratio between the numerical values being

FIGURE 16.1 Accuracy on a numerical discrimination task for monkeys and humans plotted by the numerical ratio between the stimuli. From Cantlon and Brannon (2006).
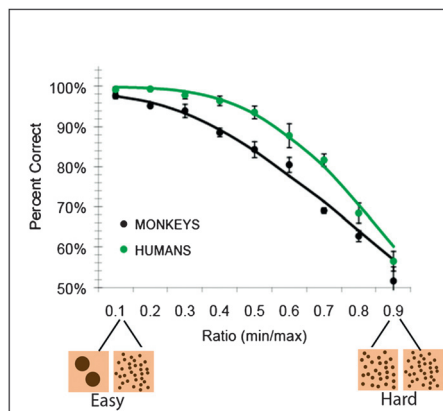
compared. Two different pairs of numerical values that have the same ratio (e.g., 2 and 4, 4 and 8) have the same amount of overlap, or the same probability of confusion. As numerical pairs get larger and closer together, their ratio increases and so does the probability that they will be confused (leading to more errors). For example, one might be 80% accurate at choosing the larger number when the numerical choices are 45 vs. 70 (45/70 = a 0.64 ratio) but might perform at chance when the choices are 45 vs. 50 (45/50 = a 0.9 ratio). This effect is known as Weber's law. The curves in Fig. 16.1 [from Cantlon et al. (2009c)] represent predicted data from a model of number representation under Weber's law (Pica et al., 2004), and they show that the predictions of this analog numerical model fit the data well.

FIGURE 16.2 An analog representation of numerical value represents an objective numerical value with a probability distribution that scales with the size of the objective numerical value. From Cantlon et al. (2009a). Reprinted with permission from the American Association for the Advancement of Science.
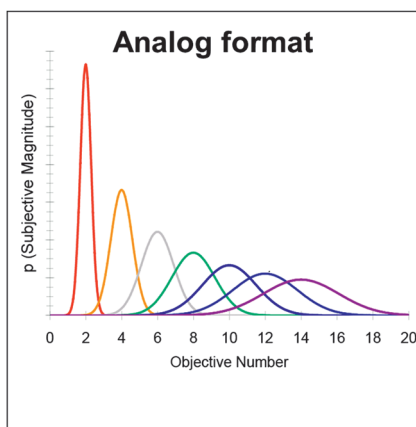
The empirical data from monkeys and humans and the fit of the analog model demonstrate that although humans have a means of representing numerical values precisely using words and Arabic numerals, they still have an approximate, analog numerical system that functions essentially in the same way as in monkeys.

Comparison

The ratio effect, described by Weber's law, indicates that numerical values can be represented in an analog format. However, that does not tell us anything about the process by which two numerical values are compared. We have identified a signature of mental comparison in monkeys that is commonly observed when adult humans make judgments of magnitudes: the semantic congruity effect (Cantlon and Brannon, 2005; Holyoak, 1977). The semantic congruity effect is a response time effect that is observed in adult humans' response times whenever they have to compare things along a single dimension. For instance, when people are presented with pairs of animal names and asked to identify the larger or smaller animal from memory, they show a semantic congruity effect in their response time: people are faster to choose the smaller of a small pair of items (e.g., ant vs. rat) than they are to choose the larger of that pair. However, for pairs of large items (e.g., horse vs. cow), people are faster to choose the larger item than the smaller item. This effect suggests that the physical size of the animal interacts with the "size" of the question (whether "Which is larger?" or "Which is smaller?") in subjects' judgments. In humans, the semantic congruity effect is observed for judgments of many dimensions, including judgments of numerical values, from Arabic numerals. We found that this effect is also observed in monkeys when they compare numerical values from arrays of dots. Monkeys performed a task in which they had to choose the larger numerical value from two visual arrays when the background color of the computer screen was blue, but when the screen background was red, they had to choose the smaller numerical value of the two arrays. As shown in Fig. 16.3 [from Cantlon and Brannon (2007a)], both monkeys showed a crossover pattern of faster response times when choosing the smaller of two small values compared with the larger of two small values, and the opposite pattern for large values. The semantic congruity effect is the signature of a mental comparison process wherein context-dependent mental reference points are established (e.g., 1 for "choose smaller" and 9 for "choose larger"), and reaction time is determined by the distance of the test items from the reference points; this has been modeled as the time it takes for evidence to accrue in the comparison of each item to the reference point (Holyoak, 1977). In humans the semantic congruity effect is observed for a variety
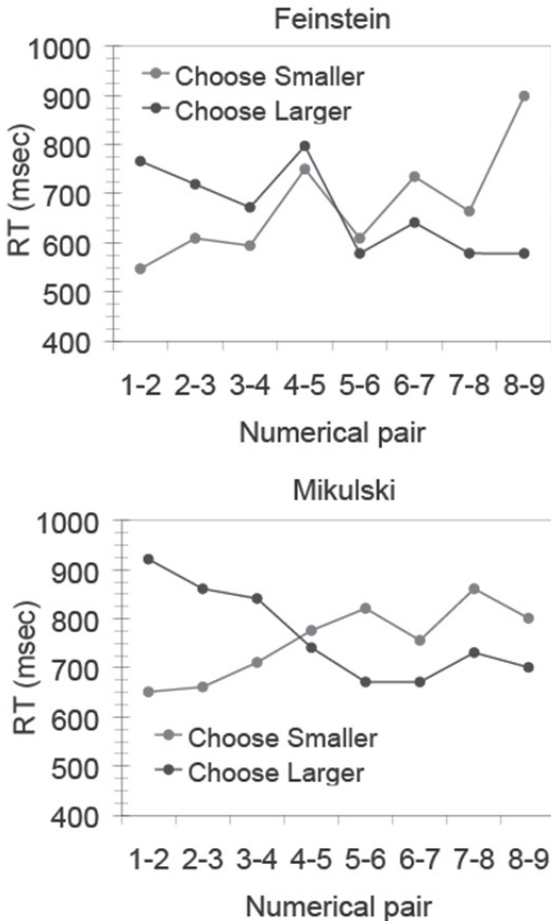
FIGURE 16.3 The semantic congruity effect in the response times of two different monkeys (Feinstein and Mikulski) on a numerical comparison task where they sometimes chose the larger numerical value from two arrays (dark line) and other times chose the smaller value (light line). The cross-over pattern reflects the effect of semantic congruity. From Cantlon and Brannon (2005).

of mental comparisons from both perceptual and conceptual stimuli: brightness, size, distance, temperature, ferocity, numerals, etc. Our data from nonhuman primates indicate that the mental comparison process that yields the semantic congruity effect is a primitive, generalized, non-verbal mental comparison process for judging quantities and other one-dimensional properties.

In fact, the ability to compare quantities, and the proposed algorithm underlying that ability, could be so primitive that it extends to nonprimate animals. A recent study by Scarf et al. (2011) showed that pigeons can compare numerical values, and in doing so they represent an abstract numerical rule that can be applied to novel numerical values. Pigeons' accuracy on that ordinal numerical task is comparable to that of monkeys tested on an identical task (Brannon and Terrace, 1998).

Arithmetic

Arithmetic is the ability to mentally combine values together to create a new value without having directly observed that new value. We have found that monkeys possess a capacity for basic, nonverbal addition that parallels human nonverbal arithmetic in a few key ways (Cantlon and Brannon, 2007a). First, monkeys and humans show a ratio effect when performing rapid nonverbal addition, similar to the ratio effect described earlier. Monkeys' and humans' accuracy during arithmetic depends on the ratio between the values of the choice stimuli. We also observed a classic signature of human arithmetic in monkeys' performance: the problem size effect. Adult humans typically exhibit a problem size effect wherein performance worsens as the problem outcome value increases (Campbell, 2005). Like humans, monkeys exhibited a problem size effect in their addition accuracy (even when controlling for the ratio effect).

However, there are also important and potentially informative differences between the performance of humans and monkeys. Adult humans and young children show a practice effect in their arithmetic performance wherein performance on a specific problem improves the more that it is practiced (Campbell, 2005). Monkeys do not show a practice effect for specific problems. This was the case even over 3 years of practice on a specific problem (Fig. 16.4 shows performance for two monkeys, over 3 years of testing on 1 + 1, 2 + 2, and 4 + 4). Nonhuman primate arithmetic thus parallels human nonverbal arithmetic in the ratio and problem size effects but not the practice effect, which has been observed primarily in symbolic arithmetic performance in humans. Presumably, discrete symbols are necessary for humans to encode arithmetic problems in a format that is amenable to memorization, which is why monkeys do not show a practice effect.

The overarching conclusion from this line of research is that the abilities to represent, compare, and perform arithmetic computations reflect a cognitive system for numerical reasoning that is primitive and based on analog magnitude representations. However, if analog numerical cognition is truly "primitive" and homologous across primate species, then it should be rooted in the same physical (neural) system in monkeys and
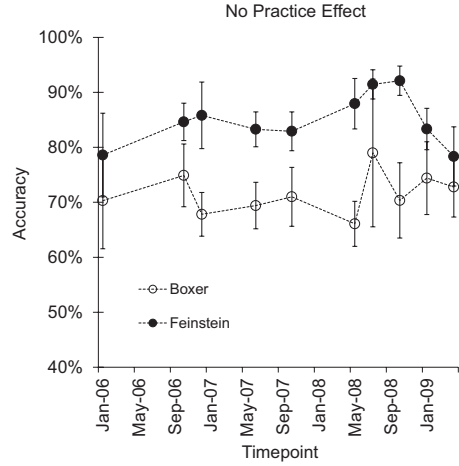
FIGURE 16.4 The lack of a practice effect in monkeys' addition performance over 3 years. Data from Cantlon and Brannon (2007a).

humans. In fact, there is evidence from multiple sources that analog numerical processing recruits a common neural substrate in monkeys, adult humans, and young children (Fig. 16.5).

In monkeys who are trained to match visual arrays of dots according to number, single neurons along the intraparietal sulcus (IPS) will
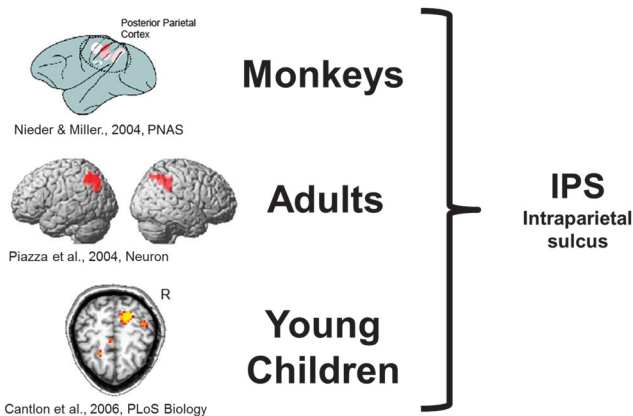
FIGURE 16.5 Monkeys, human adults, and human children exhibit similar activation in the IPS during analog numerical processing. Redrawn from Nieder and Miller (2004), Piazza et al. (2004) (reprinted with permission from Elsevier, Copyright 2004), and Cantlon et al. (2006).

respond maximally to a preferred numerical value, and their firing rate decreases as the number that is presented gets numerically farther from that preferred value (Nieder and Miller, 2004). This neural firing pattern has been linked to the behavioral ratio effect and is thought to reflect analog numerical tuning in the IPS. A similar pattern of numerical tuning has been observed with functional MRI in the human IPS. Manuela Piazza et al. (2004) found a neural adaptation effect for numerical values in the IPS that depended on the ratio between the adapted numerical value and a deviant numerical value. Our group also observed neural adaptation in the IPS for numerical values ranging from 8 to 64 in preschool children who could not yet verbally count to 30 (Cantlon et al., 2006). Together, these studies reflect a common neural source for analog numerical representation that bridges species as well as stages of human development and is thus independent of language and formal mathematics experience. These neural data support the conclusion derived from the behavioral data that there is continuity between humans and nonhuman animals in the mechanisms underlying analog numerical representations.

THEN THERE WERE SYMBOLS

A long history of studies with preverbal human infants has shown that they too possess an ability to quantify objects with approximate, analog representations (Feigenson et al., 2004). Thus, there is general agreement that the analog system for numerical reasoning is primitive in human development. A fundamental question is how a child's developing understanding of numerical symbols interfaces with preverbal analog representations of number. Of particular interest is how children initially map numerical meanings to the first few symbolic number words (Gelman and Gallistel, 1978; Wynn, 1990; Gelman and Butterworth, 2005; Le Corre and Carey, 2007; Piazza, 2010). There is currently a debate over the types of preverbal numerical representations that form the initial basis of children's verbal counting. However, regardless of how this initial mapping transpires, behavioral evidence suggests that as children learn words in the counting sequence, they map them to approximate, analog representations of number (Wynn, 1992; Lipton and Spelke, 2005; Gilmore et al., 2007). Lipton and Spelke (2005) found that 4-year-old children could look at a briefly presented array of 20 dots and, if they could count to 20, they could verbally report (without counting) that there were 20 dots in the array, and their errors were systematically distributed around 20 (i.e., their errors exhibited a numerical ratio effect). If they could not yet count to 20, however, they responded with random number labels. Thus, as soon as children learn a particular verbal count word in the sequence, they know the approximate quantity to which it corresponds without counting, sug-

gesting that number words are attached to the analog numerical code as soon as they are learned. These data have been taken to indicate that analog numerical representations are used to assign semantic meanings to numerical symbols over human development. There is also evidence that children who have learned to count verbally, but have not yet learned to add and subtract, psychologically "piggyback" on analog arithmetic representations as they transition to an understanding of exact symbolic arithmetic (Gilmore et al., 2007). The general conclusion that then emerges is that the cognitive faculties that children initially use for nonsymbolic, analog numerical operations (and which they share with nonhuman animals) provide a scaffolding for verbal counting in early childhood.

IS "NUMBER" ALONE?

The data from the development of counting in early childhood make the case that a primitive numerical system is conceptually transformed into a system for symbolic numbers. However, how do we know that analog *numerical* representations are the sole precursors of formal, symbolic numerical cognition? Currently, we do not. Although numerical reasoning seems to be primitive in the sense that it is shared among primate species, other quantitative abilities are just as widespread. For instance, the abilities to judge nonnumerical intensities such as size, time, brightness, height, weight, velocity, pitch, and loudness are as common among animal species as the ability to judge numerical values. Furthermore, all of these quantities can be discriminated by human infants, and discriminations among instances from those continua bear many of the same properties and signatures as numerical discrimination [e.g., ordinality, Weber's law, the semantic congruity effect, arithmetic transformations; see Feigenson (2007) for review]. In adults, all of these dimensions are effortlessly mapped to numerals. For example, adult humans can represent loudness, handgrip pressure, time, size, and brightness as numerical values. Finally, evidence from the semantic congruity effect (described earlier) suggests that many different quantitative dimensions are mentally compared by a common process. The modularity and taxonomy of analog numerical representations is a central issue for understanding the development and origins of numerical and mathematical cognition. Here I discuss relations between numerical cognition and other quantitative dimensions, such as size, length, duration, brightness, pitch, and loudness.

Until recently, the cognitive and neural mechanisms of numerical cognition were considered to be specialized processes. Neuropsychological and neuroimaging studies of adult humans have shown that numerical knowledge dissociates from other forms of semantic knowledge, and it has been argued from those data that the processes subserving numerical

knowledge are domain specific [see Dehaene et al. (2003) for review]. For example, individuals with semantic dementia, resulting from left temporal lobe atrophy, exhibit severe impairments on picture and word naming tasks but can be spared for number tasks (Cappelletti et al., 2001). The opposite disorder of impaired numerical cognition but spared semantic and linguistic knowledge has also been demonstrated (Warrington, 1982; Cipolotti et al., 1991). Moreover, in cases of developmental dyscalculia, mathematical reasoning can become selectively impaired over development (without impairments to other aspects of reasoning). Furthermore, developmental dyscalculia is coupled with atypical anatomy and functional responses in the IPS (Molko et al., 2003; Price et al., 2007). The fact that focal brain injuries and developmental impairments, perhaps especially to the IPS, specifically impair numerical reasoning indicates that at some level of cognitive and neural processing, numerical computation is independent. However, it remains unclear what aspects of numerical processing operate independently of other psychophysical and conceptual domains. Most previous neuropsychological and neuroimaging studies controlled for many nonnumerical abilities (eye movements, spatial attention, memory, semantic knowledge), but they did not test performance on continuous dimensions other than number (length, area, brightness, etc.). Thus we cannot know whether other quantitative abilities were simultaneously impaired in many of those neuropsychological patients.

Recently, Marco Zorzi et al. (2002) found that representations of spatial and numerical continua can be jointly impaired in patients with right parietal lesions and hemispatial neglect; patients not only neglect the left visual field and place the midpoint of a line right of center in a line bisection task, but they also overestimate the middle value of two numbers in a numerical bisection task. The patients thus neglect both the left side of a line and the left side of their mental representation of the numerical continuum. This finding and several others have led to proposals that concepts of "space" and "number" are interrelated (Walsh, 2003; Pinel et al., 2004).

The degree to which "space" (e.g., size, height, or length) interacts with numerical information is currently being investigated with a range of methods [see Walsh (2003), Cantlon et al. (2009c), and Lourenco and Longo (2011) for reviews]. One view is that space and number have a biologically privileged psychological relationship (Dehaene et al., 2008; de Hevia and Spelke, 2009, 2010). Evidence for this view comes from developmental studies of number and space representation (de Hevia and Spelke, 2009, 2010). In line-bisection tasks, incidental displays of dot arrays presented at the endpoints of the line systematically distort preschoolers' perception of the line's midpoint; subjects bisect the line asymmetrically toward the larger number of dots (de Hevia and Spelke, 2009). In addition, infants spontaneously map number onto space when habituated to positively

correlated number/line-length pairs (de Hevia and Spelke, 2010). The fact that infants map number onto space within the first months of life has been used to argue for an innate bias to relate space and number.

Biologically privileged relations between space and number are also indicated by the universality of their association (Dehaene et al., 2008). The ability to map numbers onto space (number lines) is widespread among human cultures. The Mundurucu, an Amazonian people who lack a rich linguistic system for discrete number words or symbols, can place sets of objects that vary in numerical value onto horizontal lines in numerical order (just as Western subjects do). That finding supports the conclusion that mapping between space and number is not culturally determined by reading and reciting numerical symbols, because Mundurucu do not generally use such symbols. However, this finding does not necessarily indicate the presence of an innate bias to map numbers to space in humans, but may represent an analogical relation between the ordinal properties of the stimuli or the primacy of "space" alone (Cantlon et al., 2009a). In support of those alternatives, there is evidence that a similar mapping to space is made with representations of pitch in typical adults from Western cultures (Rusconi et al., 2006). If pitch shows the same kind of relation to space as number does, then a biologically "privileged" relation between space and number seems less likely. One possibility is that the relationship is ubiquitous among any of a number of dimensions (e.g.., pitch, number, length, loudness, etc.). Alternatively, number and space and pitch and space could be related because of a privileged representation of space alone, which grounds a number of quantitative representations.

Several researchers have suggested deep psychological interactions not just between number and space but among many quantitative dimensions. In their review of behavioral data from humans and other animals, Gallistel and Gelman (2000) argued that although number is objectively a discrete property, it should be represented with an analog magnitude code. They argued that animals must combine discrete number with continuous quantities in making decisions. For example, they observed that animals need to combine estimated time and amount of potential food in making foraging decisions (i.e., for "rate"). Because natural numbers are discrete and time is continuous, combining information from these incompatible formats necessitates conversion to a common analog format. The same argument could be applied to "density," which integrates information about number and surface area. This idea implicates the possibility of common representations and shared computations for multiple quantities.

Studies in young children provide evidence that different quantitative representations have a common foundation, in the sense that they develop together. As described earlier, numerical discriminations are modulated by the ratio between the values, as per Weber's law. In human infants,

the ratio effects for judgments of size, time, and number are refined at a similar rate of development (Brannon et al., 2006; vanMarle and Wynn, 2006; Feigenson, 2007). Infants' discriminations of size, time, and number improve by approximately 30% between 6 and 9 mo of age. Similarly, in children, the precision of numerical discrimination improves from ages 6 to 8 years, and the discrimination of luminance, duration, and length systematically follow the same developmental trajectory (Holloway and Ansari, 2008; Volet et al., 2008). Because they develop at the same rate, it is likely that either the same mechanism underlies the different abilities or that different mechanisms are subject to the same constraints. The developmental trajectories of the discrimination of other quantities, such as loudness, pitch, pressure, temperature, density, motion, and saturation, have not been tested. However, there is evidence that young children and even infants can form compatible representations across many of these different dimensions (Smith and Sera, 1992; Gentner and Medina, 1998; Mondloch and Maurer, 2004; Walker et al., 2010).

As mentioned earlier, the dimensions of space and number can be related to one another already in infancy (de Hevia and Spelke, 2010). One recent study showed that 9-mo-olds were equally likely to transfer an arbitrary, experimentally learned magnitude-to-texture association from one dimension (e.g., number) to another dimension (size or duration) (Lourenco and Longo, 2010). In addition, 9-mo-olds can readily learn pairs of positively (but not negatively) correlated line lengths and tone durations (Srinivasan and Carey, 2010), suggesting that infants at least can represent an abstract "more-than" and "less-than" representation that applies to both dimensions. However, 9-mo-old infants do not show equal sensitivity to monotonic pairings between the dimensions of loudness and space as they do for pairing of space and time (Srinivasan and Carey, 2010). Those findings suggest that there may be an asymmetry between magnitudes in their intrinsic ordinal associations. It is important to note, however, that asymmetries in relations between magnitudes could arise either through a biologically privileged psychological mapping (de Hevia and Spelke, 2009) or through correlational and statistical learning [see Bonn and Cantlon (2012) for discussion].

Perhaps the best evidence for early-developing psychological relations among quantities is that infants at 4 mo of age spontaneously prefer to look at a ball that is bouncing congruently with the pitch of an auditory stimulus (the ball goes up when the pitch goes up) compared with a ball that is bouncing incongruently with pitch. In addition, they prefer to look at a shape that is getting sharper as the pitch of the auditory stimulus gets higher than the reverse (Walker et al., 2010). Infants are thus capable of aligning the dimensions of pitch and space (height) as well as pitch and shape (sharpness) early in development. Similarly, 3-year-olds reliably

match high-pitched sounds to smaller and brighter balls in a categoriza-tion task (Mondloch and Maurer, 2004). Those data show that magnitude dimensions beyond the canonical "privileged" dimensions of space and number can be mapped onto each other early in development.

Relations among different quantities also have been found at the neural level in adult humans and nonhuman primates. As mentioned above, individuals with spatial neglect resulting from damage to parietal cortex can exhibit impaired numerical processing. Single-neuron data from neurophysiology studies of monkeys broadly indicate that regions of parietal cortex represent space, time, and number (Tudusciuc and Nieder, 2007). Moreover, some data even suggest that a single parietal neuron can represent more than one type of magnitude. In one study (Tudusciuc and Nieder, 2007), monkeys were trained to perform a line-length matching task and a numerical matching task. During stimulus presentation as well as a subsequent delay, single neurons in the IPS responded selectively to visual stimuli according to their numerosity or length. Although some neurons responded only to numerosity and others only to line length, a subset of cells (~20%) responded to both magnitudes of line length and numerical value. These and other studies, including functional MRI stud-ies of adults, have led some researchers to argue for a "distributed but overlapping" representation of different magnitudes at the neural level (Pinel et al., 2004; Tudusciuc and Nieder, 2007; Cantlon et al., 2009c). Sim-ply put, different types of magnitude representation, including size, num-ber, and time (and possibly others such as brightness), share *some* neural resources in parietal cortex but not others. The next section discusses some possible explanations of the origin of the relationship between number and other quantitative dimensions.

HOW IS NUMBER LINKED TO OTHER QUANTITIES?

How do different quantitative dimensions become related in the mind and brain in the first place? We have recently reviewed existing theoreti-cal frameworks for how quantitative relations might originate (Bonn and Cantlon, 2012). Here, I briefly sketch five mechanisms for how different quantities could become related in the mind. These hypotheses are not mutually exclusive and may even be complementary.

Correlational and Statistical Associations

Learning via association and correlation is the classic developmen-tal account of the origins of abstract percepts and concepts [e.g., Piaget (1952)]. On this view, integrated representations of information coming from separate senses, modalities, or cognitive domains arise from expo-

sure to correlations in the environment. Under this account, relations among magnitudes would arise from the strength of their correlations in the natural environment. For example, it takes a long time to walk a great distance (time and space are correlated), and a large number of a particular object tends to take up more surface area than a small number of that object (number and space are correlated). In this way, empirical correlations between different quantities can be absorbed through experience.

Analogical Reasoning

Another possibility is that conceptual alignment of relational information, termed "structural similarity," mediates mapping among magnitude dimensions (Gentner and Medina, 1998). On this view, cross-dimensional mapping could be a form of analogy. Relations between magnitudes could develop through conceptual knowledge of how those dimensions are structured (Srinivasan and Carey, 2010). For example, knowledge of the conceptual fact that time and number are ordinal and monotonic dimensions (they are organized from small/short to large/long) could serve as the cognitive basis for identifying relations among those dimensions.

Amodal Representations

A third conceptual framework that could be useful for understanding relations among magnitudes derives from the literature on cross-modal sensory perception. Gibson (1969) argued that an abstract, amodal representation of intensity or amount of stimulation is present from birth or very early in infancy. On her view, amodal representations can take one of two forms: (*i*) intersensory redundancy (e.g., timing information about hammer strikes can be sampled from both the auditory and visual modalities), and (*ii*) relative intensity [e.g., "sharpness, bluntness, and jerkiness"; Gibson (1969, p. 219)]. Under a conceptualization of magnitude representation within this framework, redundancy of information would be the main source of representational overlap. For example, a bright light could be mapped to a loud tone because they both evoke an amodal representation of relatively high intensity.

Automatic Cross-Activation

A fourth hypothesis is suggested by evidence that infants experience something akin to synesthesia of sensory representations near birth [reviewed in Spector and Maurer (2009)]. A strong version of this hypothesis claims that a percept experienced in one modality automatically

stimulates a percept in another modality. Over the course of the first year of life, these associated percepts become weaker as overabundant neural connections between different functional areas of the brain become pruned or inhibited. Magnitudes, under a similar conceptualization, might be related via automatic cross-activation of dimension representations. This could imply that patterns of associations (mappings) between many magnitudes are initially strong in infancy, then get weaker during the first year(s), and then return to a strong state later in development. Generally speaking, the developmental data from cross-modal perception indicate that patterns of associations among magnitudes might not strengthen straightforwardly over development.

Evolutionary History

A final possibility is that relations among magnitudes derive from their evolutionary history rather than solely from developmental processes that unfold within an individual lifespan. On this view, one quantitative dimension evolved from another, inheriting functional similarities and potentially mutual dependencies in neural and computational operations. For example, many magnitude representations could have emerged from descent with modification of the functional substrates that code for space, resulting in a common psychological and neural code for dimensions such as space, number, time, loudness, brightness, and pitch (Bonn and Cantlon, 2012).

Clearly there is a dense set of possibilities for how different quantities could come to be related in the mind and brain. The five hypotheses sketched above address different levels of influence ranging from ontogeny to phylogeny. They also address different levels of psychological functioning ranging from basic representations of psychophysical values to abstract perceptual and conceptual relations. Different levels of analysis will be important for understanding the full taxonomy of numerical cognition in humans. However, although questions remain as to how primitive numerical representations are organized with respect to other types of quantities (e.g., size, time, loudness), it is clear that human children use those primitive numerical representations to learn the process of verbal counting early in development. Verbal counting (discussed earlier) is the first formal cognitive step toward acquiring the uniquely human capacity for complex symbolic math. In the next section we discuss how the "primitive" analog numerical abilities are related to symbolic math in humans.

ORIGINS OF MATH IQ

A further issue central to understanding the taxonomy of primitive numerical cognition is the extent to which analog numerical abilities bear
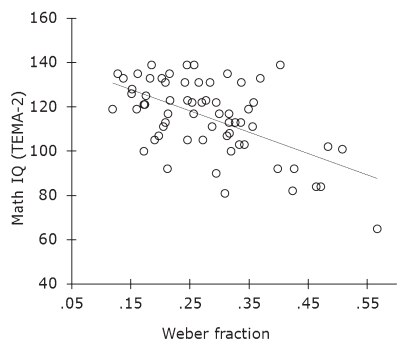
FIGURE 16.6 Childhood math IQ (measured by the TEMA-2) is correlated with the precision of analog numerical discrimination (measured by subjects' Weber fractions). A higher Weber fraction reflects worse discrimination. Redrawn from Halberda et al. (2008).

a neural relationship with full-blown formal mathematics IQ. Researchers have begun to examine, in humans, how formal math intelligence may be modulated by developments in the "primitive" analog numerical system that is shared by nonhuman primates, adult humans, and children. These studies have largely hinged on analyses of individual differences in numerical and mathematical abilities.

Individual differences in math IQ are predicted by differences in analog numerical sensitivity (Bull and Scerif, 2001; Halberda et al., 2008; Holloway and Ansari, 2009). Studies with children indicate that analog numerical ability correlates with performance on math IQ tests and that formal math ability is more closely correlated with analog numerical abilities than it is with other formal abilities, such as reading. For example, in Fig. 16.6, adolescents' analog numerical ability (measured by the Numerical Weber Fraction) correlates with their math IQ from early childhood [measured by the Test of Early Mathematics Ability (TEMA)-2 test score]. This and similar findings indicate that the "primitive" ability to estimate numerical values from sets of objects is related to the development of full-blown math skills. Other studies highlight the role of executive function and working memory in the development of formal mathematical reasoning (Bull and Scerif, 2001; Mazzocco et al., 2006; Mazzocco and Kover, 2007). Together, these studies indicate a need to understand the relative contributions of domain-specific and domain-general processes to formal mathematical skill.

Behavioral data, like those described earlier, provide evidence of a relationship between the skills required for analog numerical processing and those that are used in formal mathematics by children. Neuroimaging studies of children can provide an independent source of data on whether there is a common foundation for analog numerical abilities and formal math by testing whether a common neural substrate underlies both faculties. As described above, analog quantity judgments recruit regions of

the IPS in adult humans, human children, and nonhuman primates. One issue is whether the same neural patterns that are evoked during analog numerical processing are observed when children and adults process the symbolic numbers that are unique to human culture (e.g., numerals, number words). Several studies suggest that they do: regions of the IPS exhibit activity that is greater for numerical symbols compared with control stimuli, and those IPS regions also exhibit the numerical distance and ratio effects in their neural responses (Cohen Kadosh et al., 2007; Piazza et al., 2007; Ansari, 2008; Cantlon et al., 2009b; Holloway and Ansari, 2010). Research further suggests that the same neural response patterns are elicited for both symbolic and nonsymbolic (analog) numbers in the same subjects (Piazza et al., 2007). Together, these results implicate neural overlap in the substrates underlying symbolic and nonsymbolic (analog) numerical representations in humans.

In humans, a second brain region is often recruited during symbolic numerical tasks: the prefrontal cortex, particularly the inferior frontal gyrus, bordering insular cortex (Ansari et al., 2005; Piazza et al., 2007; Cantlon et al., 2009b; Emerson and Cantlon, 2012). Structurally, the prefrontal cortex is thought to be unique in primates compared with other mammals (Preuss, 2007). In humans the prefrontal cortex responds during many types of abstract judgments (Miller et al., 2002), and several studies have noted a unique involvement of the prefrontal cortex in the development of semantic representations, symbols, and rules [see Nieder (2009) for review]. A pattern of greater activation of prefrontal sites in children compared with adults has also been observed for numerical and basic mathematical tasks (Ansari et al., 2005; Rivera et al., 2005; Cantlon et al., 2009b). The role of prefrontal cortex in children's symbolic numerical processing is related to performance factors such as response time, or "time on task" [Emerson and Cantlon (2012); see also Schlaggar et al. (2002)], which could reflect the nascent state of children's abstract, symbolic numerical representations. Studies with nonhuman primates have suggested that they too engage prefrontal cortex during numerical processing [see Nieder (2009) for review] and that prefrontal regions play a unique role in associating analog numerical values with arbitrary symbols at the level of single neurons in monkeys (Diester and Nieder, 2007).

Findings that highlight mutual involvement of the IPS and prefrontal cortex in basic numerical tasks have led to the hypothesis that interactions between frontal and parietal regions are important for the development of uniquely human numerical cognition, such as symbolic coding. Specifically, it has been proposed that the IPS computes "primitive" analog numerical representations and the prefrontal cortex facilitates links between those analog numerical computations and symbolic number representations in humans (Cantlon et al., 2009b; Nieder, 2009). If this

hypothesis is correct then network-level neural synchrony between frontal and parietal regions should predict formal mathematics development in humans. That is, individual variability in the strength of correlations between neural responses in frontal and parietal regions, or "functional connectivity," should be related to individual variability in mathematics performance. We have recently tested this hypothesis and found that number-specific functional connectivity of the fronto-parietal network does predict children's math IQ test scores (independently of their verbal IQ test scores) (Emerson and Cantlon, 2012). The implication is that number-specific changes in the interactions between frontal and parietal regions are related to the development of symbolic, formal math concepts in children. This general conclusion is in line with the hypothesis that interactions between the "primitive" numerical operations of the IPS and the abstract, symbolic operations of frontal cortex give rise to formal mathematics concepts in humans.

CONCLUSION

The goal of this review has been to examine the origins and organization of numerical abilities ranging from analog quantification to formal arithmetic. The general hypothesis is that the uniquely human ability to perform complex and sophisticated mathematics can be traced back to a simpler computational system that is shared among many animals: the analog numerical system. Humans and nonhuman animals possess a common system for making numerical judgments via analog representations. Throughout development, analog numerical representations interact with the uniquely human ability to represent numerical values symbolically, suggesting a relationship between "primitive" and modern numerical systems in humans. Data from neural analyses of numerical processing support this conclusion and provide independent confirmation that these are in fact related systems. Questions remain regarding the precise taxonomy of the development and organization of numerical information, and its relationship to other domains, such as "space." However, the general nature of the relationship between "primitive" and modern numbers seems to derive from evolutionary constraints on the structure of numerical concepts in the mind and brain as well as the conceptual and neural foundation that evolution has provided for the development of numerical thinking in humans.

ACKNOWLEDGMENTS

17

A Hierarchical Model of the Evolution of Human Brain Specializations

H. CLARK BARRETT

The study of information-processing adaptations in the brain is controversial, in part because of disputes about the form such adaptations might take. Many psychologists assume that adaptations come in two kinds, specialized and general-purpose. Specialized mechanisms are typically thought of as innate, domain-specific, and isolated from other brain systems, whereas generalized mechanisms are developmentally plastic, domain-general, and interactive. However, if brain mechanisms evolve through processes of descent with modification, they are likely to be heterogeneous, rather than coming in just two kinds. They are likely to be hierarchically organized, with some design features widely shared across brain systems and others specific to particular processes. Also, they are likely to be largely developmentally plastic and interactive with other brain systems, rather than canalized and isolated. This chapter presents a hierarchical model of brain specialization, reviewing evidence for the model from evolutionary developmental biology, genetics, brain mapping, and comparative studies. Implications for the search for uniquely human traits are discussed, along with ways in which conventional views of modularity in psychology may need to be revised.

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What is the nature of the brain mechanisms that give rise to human cognition, and how do these mechanisms evolve? Although it is clear that human cognition, like all organismal traits, must be accounted for by some combination of ancestral and derived brain processes, attempts to decompose human mental processes into functional components whose features have been shaped by the process of natural selection—that is, adaptations—have been highly contested and controversial (Buller, 2005). The controversy centers on the difficulty of establishing whether a particular aspect of cognition or behavior is the result of an adaptation or adaptations, and in what way. Is a given cognitive ability in humans or any other species—for example, the ability to discriminate between different quantities of objects, to navigate spatially, or to learn to speak a language—the product of an adaptation specifically for that ability? Or is it just a specific instantiation of a more general ability, such as associative learning, or the general computational properties of neural networks? Or is it not the result of adaptations at all?

Proposals about functional specialization have long been a source of debate in psychology and the brain sciences. In particular, there is little agreement over whether cognitive processes other than perceptual and motor processes—that is, so-called higher-level processes—are specialized, and if so, how (Mahon and Cantlon, 2011). At stake are both theoretical and empirical issues. Theoretically, although it is clear that the brain is the product of evolutionary processes, including natural selection, we cannot move past this simple truism if we are unable to answer the question of what adaptations it contains, or to distinguish the results of natural selection from the results of other processes. Empirically, a variety of methods have been developed for studying brain specializations, including studies of developmental disorders and brain lesions, brain mapping techniques, experimental psychology tasks, comparative studies of brain anatomy and development, and more recently, studies of gene expression and gene regulation in the brain. However, controversy surrounds virtually all these methods and how they can be used to make inferences about functional specialization (Uttal, 2001). Even when brain researchers agree that specialization in the mature adult brain exists, they often cannot agree whether it is a result of selection specifically for that outcome, or is produced by more general developmental processes (Elman et al., 1996). As a result, there is little or no consensus about the nature of adaptations in the brain or even how to study them, especially for "higher-level" cognitive abilities such as language and reasoning (Mahon and Cantlon, 2011).

Although some of the reasons for this slow progress may be methodological, the impasse may also stem from a lack of biologically plausible models of what adaptations in the brain might be like (Barrett and

Kurzban, 2006). In psychology and neuroscience, it is common to think of brain mechanisms as falling into two categories: specialized and general-purpose. Specialized mechanisms are frequently associated with the idea of cognitive "modules," which are in turn associated with several kinds of property (Fodor, 1983). Modules are often held to be "innate" in the sense that they develop similarly or identically across individuals, regardless of environmental input (i.e., they are canalized). They are "domain-specific," that is, tailored to specific tasks or types of information. In addition, they operate autonomously or "automatically," that is, independently of other systems and processes, including consciousness, and therefore produce the same outcomes regardless of context. Nonmodular processes, on the contrary, are held to be domain-general, developmentally plastic instead of innate, and interactive rather than autonomous. Many psychologists believe that human cognition can be accounted for by some mix of these two types of mechanism. This is sometimes called a "dual systems" view (Stanovich, 2004).

This view, derived from models of perception, equates specialization only with highly local, narrow, and stereotyped processes, and defines general-purpose processes as whatever "modular" processes are not (Fodor, 1983). Empirically, this means that evidence for developmental plasticity, interactivity, or capacity to respond to evolutionarily novel stimuli is typically taken as evidence that a brain region or process is not evolutionarily specialized. Moreover, proximal factors such as plasticity and developmental constraint are sometimes seen as alternatives to explanations invoking selection for particular outcomes (Elman et al., 1996). Biologically speaking, however, these distinctions may be based on false dichotomies. There is no reason why adaptations in the brain (or elsewhere) need to be developmentally canalized as opposed to plastic, isolated from other systems rather than interactive, or tightly locked to specific categories of information regardless of developmental circumstance.

If adaptations in the brain resemble other organismal adaptations—for example, tissue types, limbs, organs, and the molecular machinery of cells—they are likely to be both heterogeneous and hierarchical. Heterogeneity arises from the fact of form-function fit: adaptations have different histories and have evolved to do different things, so they are likely to have diverse properties rather than coming in just two kinds. Hierarchical organization, in turn, is characteristic of systems that evolve via descent with modification. Because new structures evolve from older structures, adaptations frequently share a mix of ancestral and derived features, with relatively ancient features (e.g., properties of neurons in general) shared more widely across organismal structures, and relatively recent ones (e.g., properties of specialized brain regions) more narrowly distributed, in a hierarchically organized fashion (Carroll et al., 2005).

If this is true of adaptations in the brain, it has important implications for current debates about them. Here I outline features of a hierarchical specialization model of brain evolution and show how it may require rethinking some commonly held assumptions about brain adaptations in psychology and the social sciences.

EVOLUTION AND DEVELOPMENT OF BRAIN ARCHITECTURE

If adaptations in the brain exist, they are likely to be built during ontogeny by developmental systems that orchestrate interactions between external inputs (e.g., sensory information), internal inputs (e.g., interactions within and between brain regions), and genetic regulatory machinery to shape phenotypic structure, including the computational properties of developed brain networks. Natural selection acts on these systems based on the phenotypes they produce, and newer developmental systems and mechanisms evolve from older ones via descent with modification. These points have several implications for what specialization means in the context of the brain.

Type and Token Outcomes of Developmental Processes

Because natural selection acts on phenotypes, developmental processes are selected based on the phenotypic outcomes they produce. However, the plastic nature of mammalian brain development means that actual phenotypic outcomes may vary substantially between individuals along some dimensions, while exhibiting similarities along others. For example, the brains of some mammals and birds may contain adaptations for developing cognitive maps of their local environments, but presumably the actual content of those maps varies widely across individuals (Jacobs and Schenk, 2003). Similarly, if human brains contain adaptations for learning language (still a controversial proposal), then the content of the developed phenotypes of linguistic knowledge must vary across individuals in all the ways that human languages vary (Evans and Levinson, 2009). Thus, developmental processes can be described functionally in terms of the *type* of outcomes they produce (e.g., cognitive maps, linguistic knowledge), although the instantiated *tokens* in individually developed brains vary in their phenotypic details (e.g., French, Quechua). This is presumably the norm rather than the exception for much of the brain (Buonomano and Merzenich, 1998).

Reaction Norms in the Brain

Plastic developmental systems can produce different phenotypes when placed into different environments. The mapping functions between
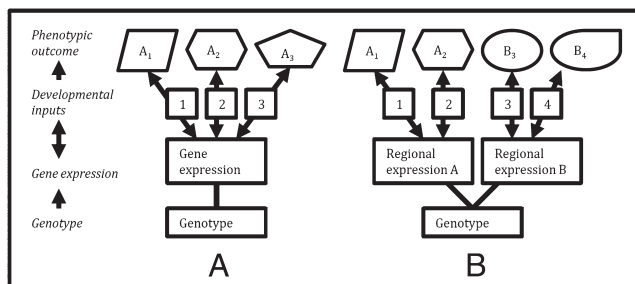
FIGURE 17.1 A reaction norm describes the mapping relationship between genotype, developmental inputs, and phenotypic outcomes (*A*). Brain regions may exhibit different reaction norms as a result of differential patterns of gene expression (*B*).

genotype, environment, and phenotype are known as reaction norms (Schlichting and Pigliucci, 1998) (Fig. 17.1). Because human brains contain multiple developmental processes, they are likely to contain different reaction norms for different functional regions and processes. For example, the reaction norms of motor cortex, which is partly organized around coordinated motor routines such as grasping and defense, may differ from those of somatosensory cortex, which is typically organized around body topology (Stepniewska et al., 2011). Moreover, tissue may be induced to adopt different reaction norms depending on the kinds of input it receives, both external (e.g., sensory) and internal (e.g., from other brain regions) (Sur and Rubenstein, 2005). Because reaction norms are the products of inherited developmental machinery, and because that machinery can be modified by selection based on the phenotypic outcomes it produces—that is, the brain structure that develops in response to external and internal inputs during development—reaction norms themselves evolve through processes of descent with modification. Thus, the developmental components of brain specializations may be thought of as a set of reaction norms, and their phenotypic components as the developed neural structures that they produce.

Ontogenetic Tuning and Module Spawning

As is the case for morphological development more generally, brain development is likely to proceed through processes of serial differentiation, subdividing into progressively finer elements whose neural and computational properties are fine-tuned based on the inputs they receive, interacting with whatever developmental processes are locally active (Sur

and Rubenstein, 2005; Rash and Grove, 2006). As development proceeds, brain tissue can become increasingly dedicated to the function that it will serve, with its computational properties becoming progressively tuned to carry out that function. This process is sometimes known as modularization (Karmiloff-Smith, 1992; Meunier et al., 2009). At least two factors must play a role in this modularization process: the inputs that the tissue receives (including patterns of neural firing) and the developmental procedures (i.e., reaction norms) that shape development as a function of inputs. These developmental procedures may include processes that fine-tune the computational properties of tissue based on inputs, such as long-term potentiation, pruning, and cell–cell signaling (Quartz and Sejnowski, 1997; Redies and Puelles, 2001; Hua and Smith, 2004; O'Leary et al., 2007). They may also include "module spawning" processes that give rise to new modules under certain developmental circumstances. For example, an initially undifferentiated region receiving two heterogeneous types of input might bifurcate into two new modules, each becoming progressively tuned to handle one of the two input types (Jacobs, 1997).

Often it is assumed that neural inputs alone play the most important role in ontogenetic differentiation of this kind: that is, that adult cortical organization is largely a function of where inputs are sent, what neural firing patterns they contain, and other properties such as granularity of receptive fields (Quartz and Sejnowski, 1997). However, analogy with morphological specialization elsewhere in animal bodies suggests that contingently activated developmental procedures, themselves potentially stimulated by inputs, may also play a role. Increasing evidence suggests that local patterns of gene expression may influence the developmental reaction norm that an area of tissue adopts, that is, how it self-organizes in response to inputs (Rash and Grove, 2006; O'Leary et al., 2007). If the topological organization of inputs to different brain regions is consistent across generations, then locally contingent developmental procedures can begin to evolve via descent with modification.

Descent with Modification of Reaction Norms

During evolution of the brain, the developmental properties of brain tissue are subject to evolutionary modification based on the effects they have on brain phenotypes. This can be initiated by initial changes in developmental systems (e.g., via mutation), changes in the environment in which they develop, or both. For example, as organisms' environments change (including changes in the social environment), developmental outcomes that were theoretically part of the brain's reaction norm, but rarely or never produced, can become more strongly acted upon by selection (Price et al., 2003). For example, if a previously non–language-speaking

species begins to evolve language capacities, developmental processes that were not previously involved in language may come under selection specifically because of their effects in language acquisition, resulting in modification of older adaptations.

Descent with modification results in patterns of specialization that have a hierarchical character (Fig. 17.2). As brain specializations evolve through descent with modification, they inherit ancestral design features—including underlying genomic building blocks and regulatory machinery—that were present before recently derived changes. This means that adaptations usually exhibit a mix of ancestral and derived features, which interact in their contribution to the adaptation's function. Ancestral features may in turn be shared across homologous specializations within or between taxa, meaning that derived specializations may be tokens of homologous types within the same organism, and across organisms.

For example, the evolution of limb specialization in animals exhibits this hierarchical character. Within taxa, diverse limb types evolve through processes of serial homology, descending from ancestral limb types via processes of diverging specialization. The result is that the distinct limb types of a given species of animal, such as a crustacean, an insect, or a mammal, exhibit many shared design features and shared developmental machinery, but nested within this shared specialization at the level of limbs are divergent specializations for each limb type (Carroll et al., 2005). In this sense, limb specialization is hierarchical. It exhibits substantial evolutionary conservation of developmental machinery, meaning that "new" specializations are composed largely of "old" design features, rearranged and modified. Brain specializations are likely to exhibit these properties as well.
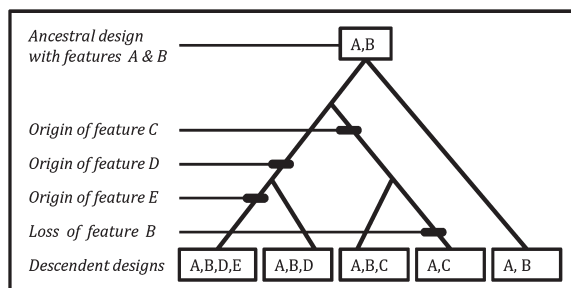
FIGURE 17.2 Descent with modification of organismal traits can lead to hierarchically organized design features. In this example, descendent versions (*Lower*) of an ancestral design (*Upper*) exhibit a mix of ancestral and derived features.

Implications for Conventional Models in Psychology

Psychologists typically assume a choice between domain-specific and domain-general mechanisms: a given psychological process must be handled by one or the other, or perhaps a mix of the two. However, if brain specializations contain a mix of general and specialized features within the same adaptation, this has important implications for efforts to empirically test between domain-specific and domain-general accounts in psychology, because the assumption that domain-specific and domain-general aspects of processing represent distinct mechanisms may be false.

As an example, consider the debate over face recognition in cognitive neuroscience. Studies with functional MRI and single-unit recording have shown that humans and other primates possess brain regions that are differentially sensitive to faces, particularly the so-called facial fusiform area (FFA) in the fusiform gyrus (Kanwisher and Yovel, 2006). Impairments to this area can produce deficits in face recognition while leaving other object recognition abilities relatively intact, a condition known as prosopagnosia (Duchaine et al., 2006). Debates have ensued over whether the FFA is an evolved adaptation for face recognition. The domain-specific view holds that it is (Kanwisher and Yovel, 2006). The domain-general or "expertise" view holds that the relevant adaptation is for developing expertise about objects in the local environment, and that faces are simply a type of object that is frequently encountered, leading to ontogenetic specialization of an area highly sensitive to faces without any evolved adaptation for recognizing faces per se (Gauthier and Nelson, 2001). Evidence in favor of this view includes training studies that show that exposure to repeated instances of novel objects can produce cognitive processing signatures similar to those seen with faces (e.g., inversion effects), and activation of the FFA for those stimuli (Gauthier et al., 1999).

Although both positions are cogent, a hierarchical specialization view suggests that they might not be as distinct as the debate suggests. Given the location of the FFA within a larger region known to be active in object recognition more generally, it is likely that face recognition abilities are a specific token within a type category of object recognition procedures, akin to claws as a token of crustacean limbs more generally. Thus, processing signatures characteristic of objects in general are of limited use in testing between the domain-specific and domain-general hypotheses because, like limbs, specialized brain structures are likely to exhibit a combination of specialized and general properties. Moreover, observations suggesting that the FFA becomes progressively tuned to faces during development (Scherf et al., 2007) do not rule out the domain-specific hypothesis, because one would expect module-spawning procedures to use input as part of their ontogenetic differentiation process. The question is whether the ontogenetic specialization of the FFA is something that has been specifi-

cally selected for as a result of its consequences for fitness in ancestral environments—a much more difficult question to answer.

These problems have been viewed as weighing against domain-specific hypotheses, on the assumption that domain-general hypotheses are more parsimonious (i.e., simpler) and therefore more likely to be true. However, analogy with morphological development suggests that this is a problematic assumption. It would be hard to argue that morphological differentiation in animals, for example, proceeds via the simplest possible set of processes, or that parsimony considerations alone would lead us to correctly infer their design. Moreover, the phylogeny and natural history of taxa can shift the burden regarding which account is more parsimonious. Many primates are highly social and can identify individuals in the wild, an ability that likely has fitness benefits (Cheney and Seyfarth, 2007), and social species such as macaques appear to have face recognition areas homologous to those in humans (Rolls, 2000). Thus, the hypothesis that there has been no selection for face recognition in our lineage may be less likely than the hypothesis that there has been.

An implication of the hierarchical specialization view is that signatures of general processing, such as Bayesian updating or statistical learning, may be shared by specialized mechanisms as well. Thus, the common assumption that such signatures weigh against more domain-specific accounts (Elman et al., 1996) should be taken with caution, and other factors should be weighed in mediating between domain-general and domain-specific hypotheses, including phylogeny, natural history, and cognitive form-function analyses akin to those used in functional morphology (Tooby and Cosmides, 1992).

ORIGIN OF NEW BRAIN SPECIALIZATIONS

How do "new" brain specializations—that is, specializations that are derived rather than ancestral in a particular lineage—evolve? If derived brain specializations evolve from ancestral ones via processes of descent with modification, and if these historical processes leave a signature in the design and organization of brain mechanisms, this has implications for the study of human brain architecture and the evolution of so-called uniquely human traits such as language and complex culture.

Varieties of Homology

Homologous traits are traits that descended from a single ancestral trait. Homologies therefore exhibit nested hierarchical relationships that are the signature of phylogenetic processes of descent with modification. Complex brains in humans and other vertebrates likely evolved from

simpler nervous systems through processes of divergent specialization of brain regions and structures, so many (but not all) human brain mechanisms and processes are likely to exhibit relationships of homology (Kaas, 1989; Striedter, 2005).

Several types of homology can be distinguished based on how and when they originate (Fig. 17.3). Orthologous traits are traits in two species that originate from a single ancestral trait in the last shared common ancestor of those species. Paralogous traits, also known as serial homologs, are homologous traits within a single species that have originated through a process of duplication and divergence (Fitch, 1970; Ohno, 1970; Hall, 1995; Koonin, 2005). Outparalogs are traits that arose via duplication and divergence before a speciation event that split two taxa; the descendent taxa will therefore all possess versions of the multiple, paralogous traits. Inparalogs evolved via duplication and divergence within a specific lineage (note that these terms were originally proposed to refer to gene homologies, but are extended here to phenotypic and developmental traits).

Many traits of organisms appear to have arisen through processes of duplication and divergence. Examples include the specialized limb types of vertebrates and invertebrates (Carroll et al., 2005), protein families such as opsins (Dulai et al., 1999), and regulatory gene families such as the Hox cluster (Lemons and McGinnis, 2006). Brain scientists believe that processes
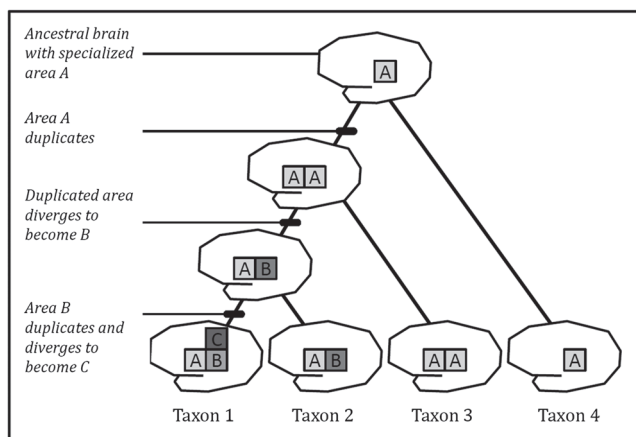
FIGURE 17.3 Varieties of homology. Region A is orthologous across taxa 1 to 4 as a result of shared descent from the ancestral taxon. Regions A and B are paralogs, originating through a duplication event (as are the two copies of A in taxon 3). Regions A and B are outparalogs in taxa 1 and 2, originating through duplication before divergence of the two taxa. Regions B and C are inparalogs in taxon 1.

of duplication and divergence may account for the origin of new brain areas and processes as well (Kaas, 1984, 1989; Striedter, 2005; Marcus, 2006).

Duplication and Divergence in the Brain

There are several possibilities for how new brain structures might evolve through duplication and divergence. One is that an initial change in development, for example, caused by a mutation, duplicates an existing brain area, producing two structures where there had been one. These can then diverge if, for example, one structure retains its initial function while selection then modifies the function of the other (Ohno, 1970; Kaas, 1989). Duplication may also alter selection on both structures, allowing them to carve up what was previously a single functional space, in a process akin to adaptive radiation (Hughes, 1994). Such a process may have driven functional divergence following gene duplication in the evolution of primate digestive enzymes (Zhang et al., 2002b) and color vision (Dulai et al., 1999). It is also possible that divergence could begin without an initial mutation, with an environmental change producing novel phenotypic outcomes, which are then exposed to selection (Price et al., 2003). For example, module-spawning reaction norms might initially bifurcate an area into two as a function of new inputs in the environment (e.g., tools, language), setting the stage for selection to act independently on the two new areas (Krubitzer and Huffman, 2000).

Specialized, category-specific object recognition capacities may have evolved via duplication and divergence from a previously undifferentiated object recognition system. There is evidence for such category-specific capacities in humans and other primates: for example, areas possibly specialized for recognition of faces (Kanwisher and Yovel, 2006), bodies (Downing et al., 2001), places (Epstein and Kanwisher, 1998), and tools (Johnson-Frey, 2004). Such areas could have evolved from a single, primitive, object recognition system in an ancestral mammal, which had not yet been parcellated into specialized regions. In such a scenario, an initial change (i.e., a mutation or environmental change) could have caused developmental subdivision or duplication of this region, allowing selection to favor divergence of the new areas.

Consider a hypothetical scenario for the evolution of a specialized capacity to distinguish between individual conspecifics based on their facial features. In some social species, there may be significant benefits to being able to recognize and distinguish between individual conspecifics (e.g., distinguishing between kin and nonkin, remembering prior cooperative partners), setting the stage for selection to act on variants that might enhance this ability (Cheney and Seyfarth, 2007). One can imagine an ancestral state in which no face-specific ability existed, only

more general object recognition systems that develop expertise through exposure to large samples of within- and between-category variation in objects repeatedly attended to (e.g., predators, conspecifics). Against such a background, any initial change that caused individuals to attend specifically to faces would begin to drive the development of face expertise within the object recognition area, a change that could be favored by selection if it yielded fitness advantages. For example, a mutation or set of mutations that altered perceptual and/or attentional systems to draw more attention to eyes or other facial features (themselves potentially favored for additional reasons, for example, emotion processing) would lead to longer bouts of face input to object systems and in turn greater face expertise. Additionally, any event leading to duplication or bifurcation of the object recognition area—including, perhaps, a module-spawning process triggered by increased face input—could set the stage for further specialization of a dedicated face area via duplication and divergence. In such a scenario, one would expect development of the resulting region to be reliant both on external inputs (i.e., exposure to faces) and mechanisms causing preferential attention to faces during development. There is evidence for attention-orienting mechanisms of this kind in newborn human infants (Johnson et al., 1991) and in other primates (Sugita, 2008).

Similar scenarios could account for the evolution of other specialized capacities from more generalized precursors, including other types of specialized object recognition (e.g., tools, places, body parts) and higher-level skills of language and reasoning as specialized versions of more general primate brain processes. We might expect many new abilities to exhibit relationships of homology to more general-purpose abilities, and relationships of paralogy to their relatives in the duplication and divergence process. If so, this could be evidenced by, among other things, shared network connectivity in the brain, adjacent localization, and shared processing signatures. For example, features of object processing such as inversion effects (i.e., difficulties with recognizing individual objects upside-down) and "holistic" processing effects (i.e., processing of relationships between parts) could be shared partly or fully across distinct object-processing systems (Bukach et al., 2006). More generally, other signatures of neural processing might be widely duplicated across brain mechanisms and regions, for example, Bayesian updating procedures, statistical learning, effects of magnitude such as those described in Weber's law, and others (Kirkham et al., 2002; Nieder and Miller, 2003; Chater et al., 2006).

Role of Evolutionary Feedback

Over evolutionary time, changes in the brain can beget further evolutionary changes through processes of evolutionary feedback, includ-

ing "runaway" or self-catalyzing evolutionary processes (Lehtonen and Kokko, 2012). Changes in one part of the brain can alter how information is routed to or processed by other parts of the brain, potentially altering how natural selection acts on those areas, as in the scenario described earlier in which increased attention to faces might alter selection on object recognition areas. Changes in the brain can also alter the environment itself, setting the stage for further evolutionary change as the new environmental properties in turn alter selection on those same brain regions or others, a process sometimes known as niche construction (Laland et al., 2000). For example, an initial change in the brain that enables slightly more complex communicative abilities—for example, the ability to combine words into more complex utterances in an early protolanguage—changes what is possible for individuals to communicate to each other, potentially leading to further selection when new variants on these communicative skills arise (Jackendoff, 1999). This can lead to a runaway process as brain mechanisms and their behavioral products increase in complexity over evolutionary time.

Similar effects may have obtained throughout human evolution as ancestral hominins developed more sophisticated cultural transmission abilities, leading to environments filled with the products of culture, such as complex languages, tools, and built environments (Richerson and Boyd, 2006). In addition, increasing social complexity may have favored the evolution of new or modified brain mechanisms for social cognition, such as increasingly sophisticated abilities to make inferences about the intentions and mental states of others, known as "mindreading" or "theory of mind" (Saxe, 2006), as well as improved abilities of cooperation and an associated moral sense (Richerson and Boyd, 2006). In all these cases, evolutionary feedback effects could have occurred between brain mechanisms (i.e., evolutionary change of one brain mechanism alters selection on others) and between brain and world (i.e., evolutionary change in the brain alters the species' environment, and vice-versa).

Word Perception as an Example

A useful example of how such evolutionary change might occur comes from studies of how reading occurs in the brain. Converging evidence from brain mapping, behavioral studies, and cases of brain damage point to the existence of an area in the left fusiform gyrus of the visual cortex that is specialized for the processing of written words. This area, called the visual word form area (VFWA), occupies a similar location across individuals literate in different languages and exhibits processing signatures consistent with specialization for identifying whole written words, such as insensitivity to font and word length (Cohen and Dehaene, 2004; Dehaene, 2009).

What does it mean to say that this area is "specialized" for word recognition? That natural selection has shaped this region specifically because of the fitness benefits of reading seems unlikely, as the oldest human writing systems are no more than a few thousand years old. Instead, it seems more likely that this area becomes ontogenetically specialized for words through a process of increasing expertise (Dehaene and Cohen, 2007; Dehaene, 2009; Anderson, 2010). Indeed, the development of this area shares similarities with development of perceptual expertise more generally, including correlations between practice and developmental speed and experience-specific sensitivity to properties of the stimulus class. However, this is not to say that the region in which the VWFA area develops is evolutionarily general-purpose, nor that the VWFA could develop anywhere in the brain. Instead, the location of the VWFA is remarkably similar across individuals literate in diverse languages, and it develops within an area of the visual cortex, the fusiform gyrus, in which other specialized object recognition capacities, such as face recognition, develop (Dehaene, 2009). This is consistent with a hierarchical specialization view: word recognition is a token, albeit an evolutionarily novel one, of an evolutionarily specialized type of brain mechanism, that is, a category-specific object recognition module. It develops when and where it does, in individuals exposed to written language, because written words activate the reaction norm of a specialized developmental system that spawns category-specific modules upon repeated exposure to a recurring class of objects.

Interestingly, there is evidence that written languages themselves have culturally evolved to satisfy the input conditions of object recognition systems. A recent study (Changizi et al., 2006) found that the distribution of junction types in the written letters of diverse world languages closely overlaps the distribution of such junctions in natural scenes, suggesting that processes of cultural evolution have favored retention of letters that are easily processed by human object recognition systems. This appears to be a case of evolutionary feedback in which the design of perceptual systems influences the cultural evolution of written words, which in turn ontogenetically shape a specialized brain area. It may also represent a case of evolution in progress and could be a useful exemplar of how new brain specializations evolve following an initial event such as the appearance of writing.

Effects of Increasing Brain Size

Humans have much larger brains than our closest primate relatives, even relative to body size (Striedter, 2005). When explaining unique aspects of human intelligence and flexibility, increased brain size is some-

times presented as an alternative to the idea that humans possess species-specific brain specializations. However, a hierarchical specialization view suggests that these are not necessarily mutually exclusive alternatives. Indeed, increasing brain size may lead to more specialization, not less, and more specialization may be related to greater, not less, flexibility.

Modularity can be defined in multiple ways. In network theory, modularity refers to the relative amount of within-region vs. between-region connectivity in a network, such as a network of neurons: more modularity means less relative connectivity between regions (Meunier et al., 2009). As brains increase in size, there are simple architectural reasons to expect that modularity, in this sense, will increase (Kaas, 1989; Striedter, 2005). As the number of nodes in a network increases, keeping them all connected to every other node becomes more and more difficult for reasons of space, leading to greater modularity. Comparative brain studies suggest that species with larger brains tend to have greater differentiation of the expanded brain areas, for example, cortical regions (Kaas, 1989, 2000; Striedter, 2005).

If increasing brain size and increasing modularity are linked, there are interesting empirical questions about what selective factors have driven the evolution of large brains in humans. One possibility is that the prime mover in brain expansion was selection for increased neural processing power per se. However, if increased brain size forces increased modularity for architectural reasons, this may set the stage for natural selection to favor further specialization of the resulting brain regions. Another possibility is that selection for specialization itself was the prime mover. If the best way to produce new specialized regions is to increase brain size—including, perhaps, duplicating existing brain areas—then selection for specialization could have favored mutations that increased overall brain volume, thereby increasing modularity. These are not mutually exclusive scenarios, and it may be difficult if not impossible to empirically tease them apart.

In psychology, it is common to assume that increasing modularity is associated with decreasing flexibility, and that undifferentiated, general-purpose systems are more flexible than differentiated, modular ones. However, there are reasons to think that the opposite may be true. In computer science, for example, it is generally recognized that modular software designs yield greater flexibility than nonmodular ones: adding a new modular algorithm to an existing system increases the number of functions it can perform while keeping previously existing functions intact, thereby adding flexibility (as well as robustness, i.e., ability of the system to withstand partial loss of function) (Baldwin and Clark, 2000). Similarly, it may be that the greater modularity seen in larger brains may yield greater behavioral flexibility compared with smaller, less modular brains (Kaas,

1989). The reason is that increasing modularity allows a greater number of interacting parts, yielding more and more complex combinatorial repertoires. If modularity and flexibility are positively rather than negatively related, this may have important implications for understanding the evolution of drastically larger brains in the human lineage.

EXPLAINING HUMAN COGNITION

One of psychology's holy grails is to explain what makes us psychologically unique: different from other apes, primates, mammals, and animals more generally. The facts of descent with modification mean that this will mostly involve modifications to the brain machinery present in the chimpanzee-human common ancestor (CHCA), along with the addition of some truly new, or derived, mechanisms. These changes include modifications to the base pair sequences in our genome (Chimpanzee Sequencing and Analysis Consortium, 2005), modifications to the regulatory machinery that shapes how genes are expressed during development (Khaitovich et al., 2004; Preuss et al., 2004), and changes in the physical and cultural environments in which humans develop, which differ substantially from those of chimpanzees (Richerson and Boyd, 2006).

What We Will Need to Explain

The CHCA was a hominoid ape with a likely brain volume in the range of 300 to 400 cm^3 and the large and complex cortex characteristic of ape brains (Kappelman, 1996). Comparisons with modern chimps and bonobos suggest that the CHCA was likely to be a social species with a relatively long lifespan and a sophisticated cognitive toolkit including social learning of tool use, "Machiavellian" social intelligence, and some elements of theory of mind, such as tracking others' knowledge of food in food competition and sensitivity to intentional communication in contexts such as aggression and reconciliation (Call and Tomasello, 2008; Whiten, 2011). However, although humans and chimps share versions of all of these abilities, most appear to have been substantially elaborated in our lineage, along with some genuinely new abilities not present in chimps.

Modern humans differ from chimps, and probably from the CHCA, in many ways. Humans have spoken languages with complex grammars and arbitrary symbol-meaning mappings (Pinker, 1994). We live and cooperate in larger and more diverse social groups, and are the only species known to have cumulative or "ratcheting" cultural evolution in which the products of culture (e.g., languages, tools, social practices) increase in complexity over generations (Richerson and Boyd, 2006).

Humans are much more rapid social learners than chimps, probably at least in part because of greater sensitivity to others' goals and mental states (Whiten, 2011). There are likely many other differences as well, such as finer-grained motor capacities (Gibson, 2002) and improved "executive" capacities of impulse control and deliberative weighing of behavioral options (Striedter, 2005). Changes in human brains and human lifeways likely coevolved via a feedback processes, involving multiple changes in brain and behavior (Kaplan et al., 2004).

Changes in Genes, Gene Regulation, and Environments

Derived features of human cognition must eventually be accounted for by changes in genes, gene regulation, and human environments (e.g., cultural, linguistic, and artifactual environments). Although we still have a long way to go before understanding these changes, rapid technological advances—including advances in genome sequencing, expression studies, and the sequencing of archaic DNA—are beginning to yield the raw data that can be used to make inferences about how hominin brain architecture has been modified since the CHCA.

Several candidate genes thought to influence brain size (Evans et al., 2005), brain differentiation (Pollard et al., 2006), and other aspects of nervous system development (Dorus et al., 2004) show evidence of selection in the human lineage, although the functional significance of many of these changes is still unknown and they are the subject of active debate and research (Montgomery et al., 2011). In addition to changes in cortical development, there appears to have been selection for increased white matter in humans (Schoenemann et al., 2005), suggesting that modifications in how brain regions communicate with each other may have played an important role in hominin brain evolution.

If the hierarchical specialization view is correct, we should expect to see selection on genes with different patterns of expression or activity in different parts of the brain. Evidence suggests that some brain areas in humans have expanded differentially with respect to their orthologs in other primates, for example, prefrontal cortex (Rilling and Insel, 1999; Schoenemann et al., 2005; Balsters et al., 2010). Work with other species suggests area-specific gene expression is likely to play an important role in such differential development (Rash and Grove, 2006; O'Leary et al., 2007). Studies of gene expression in the brain have shown substantial differences between humans and chimps (Khaitovich et al., 2004; Preuss et al., 2004), although these studies involve brainwide differences in gene expression measured at the end of life, not ontogenesis. Although detailed studies of regional gene expression in the brain during development must await technological advances, the hierarchical model suggests

several types of changes we might expect to see in humans compared with other primates.

Modified Orthologies

Many derived features of human cognition may be the result of modifications to older brain systems, in the form of modifying the design of those systems per se or modifying how they interface with other and perhaps newer systems. Such modifications are likely to be involved, for example, in the evolution of human language abilities. Although there is debate about exactly how to characterize the uniquely derived features of human linguistic abilities, there is little doubt that these abilities, taken as a whole, are unique among primates and animals more generally (Christiansen and Kirby, 2003). Yet, human abilities to learn, produce, and understand speech appear to mostly or entirely depend on brain regions and processes that have homologs in other primates (Hickok and Poeppel, 2007; Rauschecker and Scott, 2009). This implies that unique aspects of human language may result from derived changes in one or more of these regions, along with, perhaps, changes in how they interface with each other during development and language processing. One example may be the planum temporale, a region associated with language processing that appears to have undergone internal changes in the organization of minicolumns compared with chimpanzees, and specifically in the left hemisphere (Buxhoeveden et al., 2001). Some regions involved in human language processing exhibit substantial laterality (Hickok and Poeppel, 2007), have greater connectivity between them via white matter pathways (Friederici, 2009), and have greater connectivity to other brain areas than do orthologous regions in nonhuman primates (Rilling et al., 2008). This suggests that modifications in how specialized structures interact may play an important role in derived human abilities, in addition to modifications within specialized structures themselves (Balsters et al., 2010). Human language capacities may also rely heavily on interfaces between language areas and other systems, allowing us to, for example, refer to objects in our visual field, talk about things we remember, and use metaphors in the service of reasoning (Jackendoff, 1999; Boroditsky, 2000; Hickok and Poeppel, 2007). Thus, at least some apparently unique aspects of human cognition may result from novel synergies between phylogenetically older mechanisms, enabled by changes in how these mechanisms interact.

Paralogies

Studies of language areas and other cortical regions showing anatomical evidence of microstructural changes within the areas of themselves—

for example, planum temporale (Friederici, 2009) and Brodmann area 10, implicated in executive functioning (Semendeferi et al., 2001; Gilbert et al., 2006)—suggest the possibility of duplication of subunits within those regions. There may be other cases of paralogy at larger scales as well. Some of these might be outparalogies, as in the case of specialized areas in the visual cortex for recognizing faces, bodies, and other kinds of objects. Others might be inparalogies: duplication and divergence events that have occurred within the hominin lineage. One, the VWFA, might be an example of a paralogy in progress. Others might be older.

Consider, for example, areas specialized for tool use. There is considerable evidence for specialized processing of human-made tools in the human brain, involving coordinated links among perceptual, conceptual, and motor systems (Johnson-Frey, 2004). Although studies of tool use in other primates show that homology is clearly involved (Obayashi et al., 2001), specialized regions in temporal cortex and parietal cortex (for tool identification and action knowledge, respectively) may have evolved through processes of differentiation and specialization as use of complex tools became a regular part of the human cognitive and behavioral repertoire from the origins of the genus *Homo* onward. Tool identification regions in the temporal lobes may be paralogous with other specialized object perception regions, for example, for faces, and tool areas in the parietal lobes may represent tool-specific tokens of adaptations for systematizing gestural knowledge.

Of course, these examples are tentative and await further work. There may be other mechanisms of higher-level cognition that have evolved through duplication and divergence—a possibility suggested by expansion of prefrontal cortex in humans—but there remains controversy over how to characterize specializations in this area. Given the many apparently unique aspects of human cognition, including ratcheting cultural evolution, language, the ability to cooperate in large groups, morality, and a unique elaborated theory of mind, there are likely to be many additional examples of derived specializations in humans that we have not yet discovered. However, we should not necessarily expect all or even most of these to have appeared entirely de novo in our lineage, but rather, to have evolved from older precursors through descent with modification.

CONCLUSIONS

If the model presented here is correct, many widely held views in psychology about the nature of brain specializations may need to be rethought, along with the empirical implications of those views. In particular, many of the perceived tensions between specialized and general-purpose mechanisms may not exist, or at least not in the form envisioned

by "dualist" accounts. In addition, many types of evidence widely thought to adjudicate between domain-specific and domain-general accounts—for example, plasticity, which is often held to weigh against specialization—might not.

From a biological point of view, what makes an aspect of brain structure an adaptation is whether it has been selected for, not whether it has a particular set of features such as canalization, narrow targeting of a particular class of stimuli, or isolation from other systems. If the hierarchical specialization model is correct, some brain networks and processes may be minimally different from others, highly plastic, and depend on human-specific environmental factors to develop—and yet may still be the products of selection. If so, the human brain contains adaptations whose empirical signature is quite different from what many psychologists expect to see for a "module."

For example, variation in developmental outcomes across individuals, environments, or cultures—typically interpreted by psychologists as evidence against specialized adaptations—might be standard for many brain adaptations, especially in our highly variable and cultural species. Adaptations for language acquisition, if they exist, would be an example: they must produce highly variable outcomes as part of their evolved design, given the many ways in which the world's languages differ (Evans and Levinson, 2009). Moreover, if new brain specializations evolve through divergent specialization from existing structures, "gene shortage" arguments against the existence of multiple, derived brain specializations in humans—that is, that there are not sufficient genetic and regulatory differences between humans and chimpanzees to account for brain differences—may not hold water (Marcus, 2004).

Although specialist/generalist tradeoffs are likely to be important in shaping brain evolution, they might not always take the form we envision. Many psychologists believe that evolutionary considerations imply a tradeoff between a few generalized processes and many specialized ones, and that the former is more likely because generalized processes yield more flexibility. However, if it turns out that the way evolution creates more flexible brains is by proliferating specialized brain regions that carve up computational problems via specialized division of labor, this widely held assumption may turn out to be wrong.

The hierarchical model presented here poses new challenges for developing and testing hypotheses about evolved specializations in the brain. First, it suggests that the "checklist" of features widely associated with modules does not constitute a checklist for adaptations. Second, it suggests that proximate-level accounts invoking, for example, spatial and temporal interactions between developing brain regions, should not be treated as alternatives to ultimate-level accounts invoking selection; after

all, modification of those interactions is an important way in which developmental outcomes can be selected for. Third, it suggests that domain-general processing signatures may be characteristic of more specialized mechanisms as well.

If these conclusions are true, many current debates about how to interpret data for or against specialization may represent arguments over apples vs. oranges. For example, a particular phenotypic outcome in the brain may be contingent on developmental input, and also the result of a reaction norm selected to produce that phenotype given that input. To properly test evolutionary hypotheses about brain specialization, then, it is important to compare apples against apples and oranges against oranges: to compare hypotheses posed at equivalent levels of the ultimate–proximate continuum of evolutionary causation. Ideally, the most progress will be made when we can compare hypotheses that specify both proximate mechanisms, such as developmental constraints and neural wiring rules, and ultimate reasons for how and why those mechanisms have evolved and been modified in various species, including us, to produce the outcomes we see.

Epilogue

A TANGLED MULTILAYERED WEB

Reviewing the 17 chapters assembled in this volume, we do not see a tightly woven web. Instead, we see diverse perspectives on a much larger nexus that is as yet largely obscure. This larger web is full of interacting molecules, neurons, brain areas, and entire organisms, all changing through development and over evolutionary time. Neuroscience as a field is already complex, but when one adds the evolutionary dimension, the complexity becomes truly awesome and certainly beyond what one can expect to capture in just a few colloquium papers. Nonetheless, some recurring themes emerge.

One idea running through several contributions is that evolution and development are linked. Historically, evolutionary neurobiologists visualized evolutionary changes as transformations between adult forms. This thinking changed with the emergence of evo-devo biology, which was slow to infiltrate neurobiology but is now ascendant (Charvet et al., 2011; Friedrich, 2011; Medina et al., 2011; Sylvester et al., 2011). According to this view, evolutionary changes must involve changes in development, which can be inferred by comparing developmental mechanisms and trajectories between species. Such comparative developmental studies can reveal the mechanistic basis of evolutionary change and thus complement studies that address the ecological and behavioral contexts in which those changes might have been adaptive.

A second theme woven into several of the chapters is that homologies at one level of biological organization may or may not be linked to homologies at higher or lower levels (Brigandt, 2002). For example,

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similarities in the expression patterns of homologous genes are sometimes used to argue for the homology of the structures in which those genes are expressed, but the genes might well have existed before the higher level structures came on the scene. As long as genes can change their functions over evolutionary time, this possibility is not easily dismissed. Even complex networks of interacting genes are, as Jarvis and colleagues argue in Chapter 4, capable of becoming involved in the assembly of novel structures. If similar changes in function occur independently in multiple lineages, then the structures would be nonhomologous, even though the underlying genes are homologous. In such cases, one might say that the structures are "deeply homologous" but "superficially nonhomologous," although this terminology is likely to engender confusion.

Analogous challenges arise in comparative neuroethological studies. One can certainly homologize behaviors, be they swimming in snails or math skills in primates, but those behavioral homologies offer only loose predictions about the homology or nonhomology of the underlying neuronal circuits. If neurons can change their behavioral functions over evolutionary time, then homologous behaviors may involve nonhomologous neurons, and nonhomologous behaviors can involve at least a few homologous neurons. This point has been made before by various authors (Striedter and Northcutt, 1991), but it continues to befuddle the unsuspecting mind. As mentioned earlier, the task of understanding how the tangled bank of molecules, cells, structures, organisms, and behaviors has managed to transform itself in evolutionary time has only just begun. Still, as this volume aims to show, some progress has been made, especially if we compare our current state of knowledge with the knowledge in Darwin's time.

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