PART II: THE ANIMAL MIND

CHAPTER 2

FROM SENTIENCE TO SENSATION: MENTAL EVOLUTION FROM PROTOZOA TO AGNATHANS

2.1. Prologue: The Origins of Life, Behavior, and Sentience .................................................. 54
   2.1.1. The Concept of Emergent Evolution ........................................................................ 54
   2.1.2. From Inorganic Molecules to the RNA World ....................................................... 54
   2.1.3. The Quasi-Living Viruses ..................................................................................... 56
   2.1.4. The Simplest Organisms: The Prokaryotes ............................................................. 56
   2.1.5. The Insentient, Stochastic Behavior of Flagellated Bacteria .................................. 57

2.2. The Evolution of the Behavioral Apparatus and Protopathic Sentience in Amoebas and Ciliates: Our Protozoan Legacy .......................................................... 60
   2.2.1. The Unicellular Eukaryotes: Protophyta and Protozoa ......................................... 60
   2.2.2. The Transient Behavioral Apparatus of Amoebas .................................................. 61
   2.2.3. The Behavioral Apparatus of a Ciliate: Paramecium ............................................. 62
   2.2.4. The Behavioral Apparatus of a Flagellate: Euglena ............................................. 65
   2.2.5. The Teleological Behavior of Protozoans ............................................................... 67
   2.2.6. The Putative Protopathic Sentience of Protozoans .............................................. 68
   2.2.7. The Limitations of Protozoan Sentience and Behavior ........................................ 71
   2.2.8. Our Protozoan Legacy .......................................................................................... 74

2.3. The Evolution of the Peripheral Nervous System and the Emergence of Sensation in Headless Radiata: Our Cnidarian Legacy ......................................................... 75
   2.3.1. Porifera: From Protozoans to Metazoans .............................................................. 75
   2.3.2. Cnidarians: Hydra and Jellyfishes ......................................................................... 75
   2.3.3. The Sense Organs and Sensory Discrimination in Cnidarians ............................... 77
   2.3.4. The Neurons and Peripheral Nervous System of Cnidarians ............................... 79
   2.3.5. The Behavior of Cnidarians .................................................................................. 80
   2.3.6. Cnidarian Mentation: From Protopathic Sentience to Qualeous Sensation ........ 82
   2.3.7. Our Cnidarian Legacy ......................................................................................... 83

2.4. From Radial to Bilateral Body Organization, Emergence of the Head and the Brain, and Mental Advances in Planarians ......................................................... 84
   2.4.1. The Evolution of Animals with a Bilateral Body and a Head ................................ 84
   2.4.2. The Behavioral Apparatus of Planarians ............................................................ 84
   2.4.3. Behavioral Advances in Planarians ...................................................................... 87
   2.4.4. The Mental Status of Planarians .......................................................................... 89

2.5. The Emergence of the Vertebrate Head, Neuroepithelium, and the Paleoccephalon: Our Chordate and Agnathan Legacies ......................................................... 90
   2.5.1. The Invertebrate and Vertebrate Divide .............................................................. 90
   2.5.2. Extant Primitive Chordates: The Ascidians and Amphioxus ............................... 91
   2.5.3. Extinct Chordates with a Head and a Brain: Yunnanozoon and Haikouella .... 95
   2.5.4. Extinct and Extant Jawless Fishes: The Agnathans ............................................. 96
   2.5.5. The Origins of the Vertebrate Neuroepithelium ................................................ 104
   2.5.6. The Origins of the Vertebrate Head and the Paleoccephalon ............................ 109
   2.5.7. Our Chordate and Agnathan Legacies ................................................................ 111
2.1. Prologue: The Origins of Life, Behavior, and Sentience

2.1.1. The Concept of Emergent Evolution. Facing the enigma how matter can generate mind, we have to remind ourselves that a similar (though perhaps less daunting) problem has existed until recently in trying to explain how living beings arose from nonliving matter. Considering the great chasm between the living and the nonliving, some biologists attributed the origin of life to a supernatural vital force, Aristotle’s entelechy (e.g., Driesch, 1908). An alternative, naturalistic view has been the extension of evolution theory to life’s emergence from inanimate matter. The concept of emergence originated in the realization that qualitatively new properties arise as matter changes from simpler units to more complex ones (Goldstein, 1939; Novikoff, 1945; Wimsatt, 1976). The classical example is water which, as a molecule, has quite different properties than the hydrogen and oxygen atoms of which it is composed. That is, the properties of water cannot be described simply as the sum-total of the atoms of which it is composed. The water molecule, it is argued, is not a static aggregate or assembly of atoms but a new dynamic unit: the holistic qualities of chemical molecules are due to the dynamic transformation of the constituent physical atoms rather than their static summation. A similar transformation, it has been argued, occurs at the next level of holistic organization, as inanimate molecular constituents become organized into living systems. As various carbonaceous molecules become integrated to form organic substances—amino acids turning into functional proteins, and protein complexes into organelles—qualitatively new properties emerge. In contrast to the entropy of physical and chemical aggregates, whose potential energy tends to dissipate in time (water flowing downhill and mountains eroding), the energy level of organisms increases as their constituents multiply, grow and differentiate. The extrinsic supernatural force of the vitalists is reinterpreted as an intrinsic natural force, the enigmatic drive or urge of living organisms that goads them to survive. (The Darwinian concept of “struggle for survival” takes for granted this emergent biological property). What do we currently know about the mechanisms and processes that make possible the emergence of living entities?

2.1.2. From Inorganic Molecules to the RNA World. The transformation of inorganic substances into organic matter is a perennial phenomenon on our planet as photosynthetic organisms capture solar energy and transform energy-poor carbon dioxide into energy-rich carbohydrates. But can that occur de novo, in the absence of already existing living things? The question has a long history. What we have learned recently is that the transformation of inorganic carbon molecules into simple and complex organic molecules might have taken place several billion years ago on our planet when conditions were quite different from those prevailing now.

THE ORIGINS OF ORGANIC MATTER. In a pioneering experiment, Miller (1953) showed that passing electric sparks through a flask containing a mixture of inorganic methane, ammonia, hydrogen, and water vapor leads to the formation of organic substances, such as amino acids. Subsequent work (Oró and Kimball, 1961; Miller and Orgel, 1974; Kobayashi et al., 1998; Miyakawa et al., 2000, 2002), showed that irradiation of such a mixture of inorganic substances with X-ray and γ-rays, and some other treatments that mimic presumed early-Earth conditions,
leads to the spontaneous formation of such complex organic molecules as lipids, sugars and nucleotides (adenine, guanine, uracil, and cytosine). Moreover, it has also been shown that amino acids polymerize into peptides and protein-like macromolecules when subjected to various experimental treatments, and that these macromolecules may form encapsulated microspheres when boiled in water (Fox et al., 1974; Fox, 1984). These findings suggest the possibility that organic matter was produced de novo from inorganic precursors early in our planet’s history. Alternatively, life on our planet may have been initiated by extraterrestrial “seeding.” It is known that meteorites and comets, which are believed to be remnants of asteroids that formed the solar system about 4.6 billion years ago (b.y.a.), contain an appreciable concentration of organic carbon compounds. The Murchison meteorite that fell in Australia in 1969, and other carbonaceous chondrites, contain organic compounds, such as amino acids, alcohols, sugars, aldehydes, ketones, purines and pyrimidines that are similar to those produced by living cells and tissues (Miller, 1992; Cooper et al., 2001; Pizzarello, 2004). Importantly, in contrast to the amino acids catalytically synthesized by living cells, which mostly have an L (levorotary) configuration, the amino acids found in carbonaceous chondrites come in equal amounts as L and D (dextrorotary) isomers, suggesting inorganic origin. The local synthesis of some organic compounds under early-Earth conditions and the extraterrestrial origin of other organic compounds are not mutually exclusive scenarios; they both could have served as abiotic raw materials of later-forming biotic units.

THE PREBIOTIC RNA WORLD. The Earth solidified about 4.5 b.y.a. and the earliest oceans may have formed 4.3 b.y.a. (Halliday, 2001; Mojzsis et al, 2001). A heavy meteoric bombardment of the Earth, a possible source of extraterrestrial organic seeding, is believed to have occurred about 3.9-3.8 b.y.a. (Line 2002). How might the earliest living organisms have formed in the cooling oceans, seas and lakes during the violent times of this so-called Archean era? The extensive oxygenation of the hydrosphere and atmosphere, which is attributed to the photosynthetic activity of cyanobacteria, began much later, at the beginning of the Proterozoic era about 2.4-2.2 b.y.a. (Catling et al., 2001). Hence, the earliest organisms must have derived their energy from some source other than photosynthesis. The absence of oxygen, and of a shielding ozone layer to absorb ultraviolet radiation during the Archean era, has given rise to the idea that these living organisms originated in hydrothermal vents (submarine chimneys) as heat-energized archaebacteria (Holland, 2002; Foustoukos and Seyfried, 2004). These unicellular prokaryotes are presumed to have preceded the familiar eubacteria that came into being later under milder climatic conditions.

It has been hypothesized that the archaebacteria were preceded in the cooling Earth by simpler metabolizing entities that, lacking RNA and DNA, assembled their essential ingredients by some catalytic process from abiotic organic substrates that were available in the “primeval soup.” That is, organic metabolism and growth of a primitive sort originally antedated genetic reproduction (“the chicken came before the egg”). If this is correct, the next momentous event was the advance from the adventitious synthesis of organic compounds under early-Earth conditions to their programmed biochemical synthesis under genetic guidance (de Duve, 2003). How could these genes have arisen? In most extant living cells, DNA segments serve as self-duplicating genetic templates that are copied and transcribed by a series of RNA molecules, known as intermediary, transfer, messenger, and ribosomal RNAs. Which came
first in the evolution of this self-duplicating system, RNA or DNA? The discovery that short stretches of RNA, called ribozymes, can operate like catalytic enzymes (Cech and Bass, 1986) gave rise to the idea that before the evolution of DNA, prebiotic entities existed in which RNA molecules served both as catalytic templates for protein synthesis, metabolism and growth, and as genetic templates for reproduction. This hypothetical epoch is known as the RNA world (Orgel, 1986, 2004; Gesteland and Atkins, 1993; Line, 2002; Fedorov and Fedorova, 2004). It has been suggested that in the aquatic niches of the early Earth, microspheres (Fox, 1984) or hydrogels (Trevors and Pollack, 2005) formed that contained ribozymes that catalyzed the available abundant organic substrates, thus maintaining themselves and perpetuating their kind. If this scenario is correct, these early organisms without a dedicated genetic machinery for reproduction could not have endured once the propitious early-Earth conditions ended.

2.1.3. The Quasi-Living Viruses. The perpetuation of an organic species requires that its members are endowed with three organic mechanisms: (i) a metabolic apparatus that enables them to use available raw materials to grow and repair themselves; (ii) a coding and transcription apparatus that provides them with a blueprint for the regulation of their metabolic, morphogenetic and regenerative processes; and (iii) a genetic apparatus that enables some individuals to pass on their heritage to their descendants and thus assure the survival of the species. To maintain the integrity of an individual organism, it must be encased in a unique envelope (membrane, skin, capsule) that separates and shields it from harmful environmental influences but which, at the same time, allows it to interact with the outside world by promoting the inward flow of needed raw materials and the outward flow of waste products. The inhabitants of the hypothetical RNA world apparently did not possess all these properties. Ribozymes or single-stranded RNA molecules cannot reliably serve two functions, i.e., regulate the hectic processes of daily life as well as ensure the perpetuation of the species over generations. That required the emergence of entities furnished with both DNA and RNA. The least complex of these entities are viruses that contain both RNA and DNA as well as some proteins (including enzymes) that aid their self-assembly and self-replication. However, viruses are not true living entities because they require the host’s or victim’s metabolic machinery to propagate themselves, and their relation to the world of living beings is unclear (Balter, 2000). The fact that viruses infect all living organisms—archaeabacteria, eubacteria, and eukaryotes—suggests that they evolved early in the history of life on this planet (Forterre, 2006; Koonin et al., 2006). However, while all viruses have many genes and gene products in common, they share very few with even the simplest single-celled organisms, the prokaryotes. This suggests that viruses are not part of our evolutionary heritage but evolved in parallel with the cellular world (Koonin et al., 2006).

2.1.4. The Simplest Organisms: The Prokaryotes. True living beings came into existence when unicellular organisms evolved with a membrane-enclosed cytoplasm rich in organic substrates, double-stranded DNA molecules as genetic templates, and complementary RNA molecules as transcription mechanisms. The simplest of these unicellular organisms, the prokaryotes, lack a nucleus, a separate cellular compartment for the sheltering of the chromosomes composed of DNA strands. (The more advanced unicellular organisms, the eukaryotes, have a membrane bound nucleus inside the cytoplasm.) It is uncertain when prokaryotes emerged on our planet because of the scarcity of reliable microfossils (Altermann
and Kazmierczak, 2003). The more primitive prokaryotes, the archaebacteria, may have emerged during the Hadean era of the Precambrian 4.1-3.1 b.y.a., the more advanced eubacteria during the Archean period 3.2-2.5 b.y.a. (Schopf and Walter, 1983; Schopf, 1993; Mojzsis et al., 1996; Battistuzzi et al., 2004). The archaebacteria are believed to have been chemoautotrophs that obtained substrates and energy from abiotic organic resources of the early Earth and depended mainly on anaerobic metabolism. The proliferation of photoautotrophs, the cyanobacteria (blue-green algae), which were responsible for the oxidation of the planet, is assumed to have been a later event that took place about 2.3 b.y.a. (Catling et al., 2001; Holland, 2002).

Although both prokaryotes are generally smaller (1-10 µm) than eukaryotes (10-100 µm), and have a less advanced cytoplasmic organization, they are, in fact, extremely complex both structurally and functionally. The prokaryotic cell is not an amorphous protoplasm as previously believed. It has cytoskeletal elements—microtubules and intermediate filaments—that are distantly related to those found in eukaryotes (Carballido-Lopez and Errington, 2003; Moller-Jensen and Lowe, 2005). And it is of great evolutionary significance that extant eukaryotes share with prokaryotes most of the DNA codes (codons) for the synthesis of the 20 amino acids that are the raw materials of the myriad of proteins that organisms contain (Freeland et al., 2000; Line 2002). Hence, this canonical genetic code is a conserved feature of all organisms, man included, suggesting a monophyletic origin of life on Earth. The evolutionary changes that occurred subsequently consisted mainly in the modification, increase and diversification of the genomes that serve as codes for the varied cell constituents of advanced organisms.

2.1.5. The Insentient, Stochastic Behavior of Flagellated Bacteria. We have argued earlier (Section 1.4.7) that sessile, nonbehaving plants are devoid of mental powers but animals that possess the triad system of sensors, neurons and muscles, and display teleological behavior, are sentient beings. Hence the question: are bacteria that lack these animative mechanisms, but display sensitivity and motility, sentient beings? We shall present evidence that the behavioral mechanisms of bacteria are fundamentally different from those of protozoans and, we shall argue, that they are insentient organisms because their behavior is of the stochastic (random) rather than teleological (goal-seeking) kind.

The Motility of Bacteria. Bacterial motility was discovered by van Leeuwenhoek in 1683, and its features were extensively studied in the late 19th century (Lux and Shi, 2004). In many species of bacteria, swimming in an aqueous medium is produced by one or more flagella (Fig. 2-1A). Other bacteria use a gliding or twitching mechanism for locomotion. The machinery of bacterial swimming in Escherichia coli (Fig. 2-1B) consists of the flagellum that works as a screw and a complex motor mechanism (Berg and Anderson, 1973; Silverman and Simon, 1974; Macnab and Aizawa, 1984; Eisenbach et al., 1990; Bren and Eisenbach, 2000; Berg, 2003; Lux and Shi, 2004; Blair, 2006; Thomas et al. 2006). The latter is composed of several protein-based cylindrical structures, including (i) a stationary brake, (ii) a rotor composed of several rings mounted on a central axis with stepping rods, and (iii) a switch. The complex motor mechanism is linked to receptors by signaling molecules (Fig. 2-1B) and is driven by ion fluxes. Over 50 genes have been identified in Escherichia coli and Salmonella enterica that play a role in this mechanism, with about half of them shared as a conserved feature by all bacterial species (Liu and Ochman, 2007). It is important to note, however, that the bacterial
The Sensitivity of Bacteria. Both optical and chemical sensitivity have been demonstrated in bacteria. Light has two principal organic functions. First, photo radiation is the ultimate energy source of life on this planet. Chlorophyll containing plastids are responsible for photosynthesis in many autotrophic organisms, including some bacteria (Albertano et al., 2000; Xu et al., 2001). Second, photo radiation may also be a source of information about visual features of the environment and light is used by behaving heterotrophic organisms for orientation. There is evidence for the latter in many bacteria. For instance, it has been shown that populations of *Halobacterium halobium* avoid regions of damaging blue and near-ultraviolet light (photophobia) and aggregate in red and orange light (Hildebrand and Dencher, 1975). The cell membrane of these flagellated bacteria contains a family of proteins that are structurally similar to rhodopsin, the retinal pigment of higher animals (Oesterhelt and Stoeckenius, 1971; Spudich and Bogomolni, 1984). Hence, our most basic ability to respond to photo stimuli, and perhaps to discriminate some hues, may be a prokaryotic heritage. However, the evidence that changes in membrane potentials are not involved in the photophobic reactions of *H. halobium* is of great significance (Oesterhelt and Marwan, 1987), because that is the basis of the processing of visual information in higher metazoans. More generally, the propagation of bioelectric potentials is the material foundation of all sentient processes.

Much like photo radiation, certain chemicals also serve two organic functions: as sources of calories and substrates, and as signals of the presence and location of nutrients. It has been
known for some time that several bacteria, such as Salmonella typhimurium, Bacillus subtilis, and E. coli, approach or avoid certain chemical stimuli, i.e., display positive or negative chemotaxis (Adler, 1975; Lux and Shi, 2004). The molecular mechanisms that mediate bacterial chemosensitivity and influence flagellar rotation have been analyzed in detail (Eisenbach et al., 1990; Bren and Eisenbach, 2000). The chemical attractants of bacteria are sugars, certain amino acids, and peptides; chemical repellents are organic acids, and a variety of other compounds (Adler, 1975; Schimz and Hildebrand, 1979). Three major chemoreceptors have been identified in E. coli: a serine-binding transducer, a product of the tsr gene; an aspartate- and maltose-binding transducer, a product of the tar gene; and a galactose- and ribose-binding transducer, a product of the trg gene (Eisenbach et al., 1990; Kim et al., 2001; Sourjik and Berg, 2002; Jenal et al., 2005). These receptors influence the level of phosphorylation of diffusible intracellular signalling proteins, CheY and CheZ (Fig. 2-1B). Binding of chemoattractants decreases their phosphorylation level, whereas absence of attractants increases them. These two states, in turn, lead to the switching of flagellar responses. It is noteworthy, however, that bacterial chemosensitive and signalling mechanisms are unrelated to the mechanisms used by higher eukaryotic organisms in smell and taste.

The Stochastic Character of Bacterial Behavior. Flagellar activity is under stimulus control. Under different conditions, flagellar rotation may (i) decrease or increase (up to several hundred Hz), (ii) its direction may be switched clockwise or counterclockwise, and (iii) there may be infrequent or frequent pauses between rotations and their directional change (Larsen et al., 1974; Lapidus et al., 1988; Bren and Eisenbach, 2000; Berg, 2003). Infrequent flagellar switching produces random straight runs, frequent back and forth switching produces random tumbles (Fig. 2-1C). Positive stimuli (attractants) increase switching frequency, which leads to tumbling. Tumbling is the stochastic “foraging mode,” which biases the bacterium to actively move about within the confines of a favorable medium without leaving it. Negative stimuli (repellents) decrease switching frequency. That is the stochastic “escape mode,” which biases the bacterium to make straight runs and thus increase the probability that it will vacate the unfavorable site. This stochastic character of bacterial behavior suggests that it is fundamentally different from the teleological behavior of protozoans and multicellular animals (see below).

Absence of Bioelectric Signaling in Bacteria. It is noteworthy that the membrane-mediated bioelectric signaling that mediates sensitivity and motility in eukaryotes is absent in bacteria. Instead of propagated bioelectric signaling, bacteria use an elaborate intracellular protein-protein mediated cascade for intracellular communication (Fig. 2-1B). Bioelectric signaling in eukaryotes is produced by ion movements through transmembrane channels. Ion channels are also present in bacteria (Saimi et al., 1988), yeast (Gustin et al., 1988), and plants (Tester, 1990). But the basic function of ion movements through transmembrane channels in bacteria (as in plant cells) is the maintenance of cellular homeostasis: the regulation of the type of solutes that can move into the cell interior from the outside and which will leave it. Among the ion channels that have been identified in bacteria are KcsA, a homologue of the eukaryotic potassium channel (Milkman, 1994; Jan and Jan, 1997); MScL, a mechanosensitive ion channel (Hamil and Martinac, 2001; Martinac and Kloda, 2003); and the CIC family of chloride channels (Maduke et al., 2000; Koprowski and Kubalski, 2001). Potassium influx is activated
in bacteria immediately after a rise in the saline concentration of the medium, suggesting that potassium channels play a role in protecting the cell from hyper-osmotic shock (Wood, 1999). A mechanosensitive bacterial channel has also been implicated in osmoregulation (Levina et al., 1999). Bacterial chloride channels play a role in cell-volume regulation (Maduke et al., 2000). And while sodium channels may play a role in bacterial sensitivity and motility (Ito et al., 2004), they do not seem to be involved in the propagation of bioelectric potentials either as stimulus transducers or as intracellular messengers (Saimi et al., 1988). For instance, *E. coli* displays normal chemotactic reactions when the ionic composition of their medium is perturbed or when fluctuations in their membrane potential is experimentally prevented (Eisenbach et al., 1990). The findings suggest that while some of the ionic channels found in prokaryotes may be ancestral to those found in eukaryotes, they do not appear to play a role in bioelectric signal propagation and behavioral control as they do in the latter. As we shall argue, bioelectric signal propagation is a behavioral control mechanisms that has evolved in eukaryotes and it is intimately associated with sentient processes.

**Bacterial Behavior as an Insentient Process.** In summary, we propose that we need not attribute sentience to bacteria because the function of sentience is *goal-seeking* behavior and that is absent in these prokaryotes. Behaving bacteria do not respond to attractants by directly approaching and pursuing them, as protozoans do, nor do they turn around to flee from a repellent (see below). Motile prokaryotes succeed in adaptively responding to environmental stimuli because they are furnished with an ingenious motor mechanisms that makes them tumble when in a favorable milieu and run when in an unfavorable milieu. And while some of the molecular mechanisms of sensitivity (such as rhodopsin as a photon transducer) may be a prokaryotic legacy, the flagellar apparatus that produces bacterial motility has not been conserved in eukaryotes.

2.2. The Evolution of the Behavioral Apparatus and Protopathic Sentience in Amoebas and Ciliates: Our Protozoan Legacy

2.2.1. The Unicellular Eukaryotes: Protophyta and Protozoa. Unicellular eukaryotes evolved about 2.3 b.y.a. in association with the great rise of oxygen levels during the Lower Proterozoic (Catling et al., 2001; Holland, 2002). More advanced eukaryotes, many of which soon evolved into metazoans, became widespread about 1.5-1.2 b.y.a. (Carroll, 2001). Eukaryotes differ from prokaryotes not only by having a nucleus inside the cell but also by the presence of several membrane-bound organelles in their cytoplasm, including mitochondria, endoplasmic reticulum, Golgi apparatus, and some other structures, such as plastids, vacuoles, contractile fibers, and sensory elements. Mitochondria play a major role in energy metabolism; endoplasmic reticulum in protein synthesis; the Golgi apparatus for the assembly of cell membranes and secretory vesicles; and plastids are responsible for photosynthesis in algae. It has been hypothesized that some of these organelles were once free-living organisms that later became incorporated into the cytoplasm of eukaryotes through endosymbiosis and horizontal gene flow (Gray 1995; Margulis, 1995). Supporting this hypothesis is the fact that mitochondria have their own DNA.
PROTOPHYTA AND PROTOZOA. There are two kinds of unicellular eukaryotes: (i) autotrophic protophyta, which produce organic molecules by photosynthesis from ubiquitous inorganic substances, such as carbon and water, and (ii) heterotrophic protozoans, which cannot themselves synthesize many of their essential organic substrates and, therefore, have to feed on organic nutrients to survive. Protozoans did not begin to evolve until the Upper Proterozoic, and became abundant during the Cambrian period of the Paleozoic era about 0.5 b.y.a. Extant protozoans—like amoebas, ciliates and flagellates—exist as free-living organisms and as symbionts, parasites or pathogens of higher organisms (Buchsbaum et al., 1987). Symbiotic amoeboid cells play a major role in the immune system as phagocytes; ciliated epithelial cells move fluid in the trachea; and flagellated cells function as motile sperms. The amoeba Entamoeba histolytica and the ciliated Balantidium coli are parasites that cause dysentery in humans.

Because most protozoans live on scattered particulate nutrients, the procurement and utilization of these requires behavioral transactions, such as searching, pursuing, and ingestion. Being single-celled, protozoans lack specialized cells and tissues that metazoans use for behavior. What they do possess are macromolecular complexes and subcellular organelles that serve the function of sensing, signal propagation, and motility, the phylogenetic progenitors of differentiated sensory cells, nerve cells and muscle cells of multicellular animals. It was in protozoans that ionic transmembrane mechanisms have evolved for the propagation of bioelectric signals, and actin and myosin became incorporated into the cytoplasm to produce fast movement through reversible mechanical contraction and relaxation. We shall refer to these as neuroid and myoid mechanisms.

2.2.2. The Transient Behavioral Apparatus of Amoebas. Amoebas are protozoans without a fixed shape and devoid of permanent sensory and motor organelles (Fig. 2-2). Nevertheless, they are sensitive to various stimuli and respond to them in a coordinated manner (Jennings, 1906; Anderson, 1987). For instance, A. proteus, a naked and free-living freshwater amoeba, crawls over solid surfaces in the water by projecting temporary protoplasmic protrusions, called pseudopods, in the direction of its locomotion. When irritated by mechanical, chemical, thermal, or optical stimuli, it escapes from the irritant by forming pseudopods at the opposite end of its body. Light microscopic observations have established that the body of mobile amoebas has two components: a viscous outer cover, the plasmagel, and a fluid core, the plasmasol (Buchsbaum et al., 1987). The two states are reversible, and the plasmasol streams in the direction of pseudopod formation to produce forward or backward locomotion. Pseudopod formation is also involved in the temporary formation of a gullet that engulfs the captured prey.

Biochemical analyses indicate that the protoplasm of amoebas contains actin and myosin filaments, the contractile protein constituents of metazoan muscle (Pollard and Korn, 1971; Taylor et al., 1980; Brix et al., 1990; Gavin, 2001). It is hypothesized that the temporary polymerization and cross-reaction of actin and myosin in the plasmagel provide the contractile mechanism to produce the hydrostatic pressure for forward plasmasol streaming (Hellewell and Taylor, 1979; Yanai et al., 1996; Uchida et al., 2003; Pomorski et al., 2007). Pseudopod formation in amoebas involves changing ionic fluxes across the cell membrane (Anderson,
and the temporary assembly and disassembly of actin filaments in the cell membrane (Pollard and Borisy, 2003). We consider the assembled actin and myosin complex of amoebas, the precursor of the enduring contractile elements of smooth and striated muscle in metazoans, as a myoid mechanism. It is noteworthy that there is a resting electric potential across the cell membrane in amoebas, the magnitude of which is comparable to the resting potential of neurons in higher animals (Braatz-Schade, 1978). This suggests the presence of some nerve-like, or neuroid, constituent in protozoans. (These constituents, as we describe below, are better developed in a more advanced order of protozoans, the ciliates.)

Cells using the amoeboid mode of locomotion and feeding persist in the bodies of all higher animals, including man, and play an important role in certain housekeeping functions. Through our entire life, we depend on the amoeboid motility of macrophages and neutrophils which locate, engulf, and metabolize pathogenic agents in our body, and on fibroblasts involved in the healing of superficial wounds (Preston et al., 1990). Moreover, amoeboid locomotion, and growth cone (“pseudopod”) formation, are properties displayed by migrating and differentiating cells during embryonic development. This includes the developing nervous system in which young neurons migrate over long distances from their site of production to locations where they form discrete brain structures (Altman and Bayer, 2002).

2.2.3. The Behavioral Apparatus of a Ciliate: Paramecium. The most extensively studied ciliated protozoan is paramecium (Fig. 2-3A,C). Unlike an amoeba, paramecium has a consistent body structure, with a front and back end, a complex cytoskeleton, specialized subcellular organs (oral groove, gullet, digestive vacuoles, osmoregulatory vacuoles, trichocysts), and an integrated motor apparatus consisting of hundreds of cilia over the body surface. Paramecium uses its cilia as oars in a coordinated manner to swim forward, backward and sideways (Fig. 2-3B). Paramecium also has cilia around its gullet which, beating inward or outward, draw food particles into the gut and expel unpalatable or noxious substances. There is some evidence that the cilia are also involved in sensory discrimination (Mitarai and Nakaoka, 2005). Electron microscopic observations have revealed, that each cilium is composed of

![Fig. 2.2.](https://www.helpfulhealthtips.com/Images/A/Amoeba.jpg)
nine outer doublets of microtubules and two central strands, the 9+2 pattern (Fig. 2-3D). The individual cilia originate in a basal body, and the basal bodies are interconnected by a cross-linked network of filamentous proteins to form an infraciliary lattice (Allen, 1988; Cohen and Beisson, 1988). Although lacking specialized receptor organelles, paramecia respond to chemical, photic, mechanical, and gravitational stimuli either positively, by swimming straight forward, or negatively, by turning and then resuming swimming (Eckert et al., 1972; Machemer, 1988; Dryl and Grebecki, 1966; Van Houten and Preston, 1988; Nakaoka et al., 1991; Bell et al., 2007). For instance, *Paramecium tetraurelia* is attracted to the bacterial metabolites acetin and biotin by swimming fast toward higher gradients of these substances and slowing down and turning when entering lower gradients. Extremes of pH, high salt concentrations, and quinine act as strong repellants. Paramecia also respond to gravitational force (Braucker et al., 1998). Reaction to light has been studied in another ciliate, *Stentor coeruleus* (Fabczak et al., 1993; Menzies et al., 2004; Sobierajska et al., 2006). These ciliates are photophobic, they swim away from light sources and gather in shaded areas.

**NEUROID BIOELECTRIC POTENTIALS.** Electrophysiological studies indicate that the responses of paramecia to attractants and repellants are based on changes in membrane polarization (Fig. 2-4). As in the nervous system of metazoans, so also in paramecium, changed ionic fluxes across the cell membrane are responsible for the generation and propagation of two types of bioelectric signals, graded generator potentials and all-or-none action potentials (Eckert et al., 1972; Naitoh and Eckert, 1973; Brehm and Eckert, 1978; Bonini and Nelson, 1988; Saimi et
al., 1988; Machemer, 1988; Hennessey, 1989; Kung, 1989). Decreased membrane negativity makes the cilia beat forward, producing backward swimming (Fig. 2-4A). Increased membrane negativity makes the cilia beat backward, producing forward swimming (Fig. 2-4B). Reversal of ciliary beating and change in movement direction is associated with the discharge of action potentials (Dryl and Grebecki, 1966; see Fig. 2-4C). Intracellular microelectrode recordings in Stentor showed that graded increases in light intensity produced graded membrane potentials, followed by an action potential, and a reversal of ciliary beating (Fabczak et al., 1993).

The ionic channels identified in paramecia in relation to ciliary beating have both similarities and differences with those operating in the nervous system of multicellular animals. For instance, the sodium (Na⁺) channels of paramecia are only distantly related to the Na⁺ channels of metazoan nerves (Jegla and Salkoff, 1995). Possible “neurotransmitters” of paramecium may be acetylcholine (AChE) and gamma aminobutyric acid (GABA) (Delmonte Corrado et al., 2001, 2002). AChE has been identified in paramecium together with its synthetic enzyme, choline acetyltransferase, and its lytic enzyme, acetyl cholinesterase, as well as its nicotinic and muscarinic receptors of AChE. GABA has been implicated in the regulation of swimming behavior of paramecium (Ramoino et al., 2003). Evidently, protozoans devoid of specialized sensors and nerves have made some progress in the assembly of some of the molecular constituents responsible for the propagation of bioelectric signals. We consider these neuroid mechanisms.

**PARAMECIUM BEHAVIOR AND PHYSIOLOGY**

**Fig. 2-4.** A. Anterior stimulation of paramecium makes the cilia beat forward, which results in backward swimming, or retreat. Microelectrode recordings indicate that that reaction is associated with decreased membrane negativity (graded potential) and overshooting (action potential). B. Posterior stimulation has the opposite behavioral and electrophysiological effects. C. Action potentials in synchrony with the reversal of ciliary beating a paramecium. (A and B, modified, from Eckert, 1972; C, from Dryl and Grebecki, 1966.)
MYOID MECHANISMS. In addition to some neuroiud mechanisms, eukaryotic ciliates (and flagellates) also possess myoid mechanisms. The contractile proteins of muscle, myosin and actin, are present in the basal bodies of cilia and flagella (Tiggemann and Plattner, 1981; Gavin, 2001; Sehring et al., 2007). However, the mode of action of actin and myosin appears to be different in protozoans than in the muscle fibrils of metazoans. According to current view, ciliary movement in protozoa is brought about by the sliding of microtubules relative to one another (Satir, 1984; Preston et al., 1990) which is unlike the interaction of actin and myosin in muscle. The same applies to the contractile stalk of Stentor, a trumpet-shaped ciliate, which attaches itself to a solid substrate, such as a plant, and moves sideways or contracts when irritated (Fig. 2-5). The stalk of Stentor contains longitudinal fibrils, the myonemes, but these are apparently composed of microtubules rather than actin and myosin (Huang and Pitelka, 1973). Apparently, the fully differentiated actin/myosin-based contractile metazoan muscle has evolved through a prolonged evolutionary process.

2.2.4. The Behavioral Apparatus of a Flagellate: Euglena. The freshwater Euglena (Fig. 2-6) is a unique unicellular organism that can alternate between an autotrophic and a heterotrophic life style. It relies on chloroplasts for photosynthesis but uses its flagellum to move to well-lit areas (positive phototaxis) or in search of particulate nutrients (Jennings, 1906; Mast, 1911). The eukaryotic flagellum is a complex organelle and works differently than the bacterial flagellum. Like the cilium, it is composed of two basal bodies, the 9+2 configuration of microtubules, and an axial fiber known as the paraflagellar rod (Hyams, 1982; Farmer and Triemer, 1988). Supplied with ATP, this complex mechanism produces the sliding
EUGLENA

Fig. 2-6.
A. Euglena with its eyespot, gullet, chloroplasts, flagellum, contractile fibers, and cell inclusions.
B. Micrograph of Euglena’s body with its myoid pellicles.

Fig. 2-6. A. Euglena with its eyespot, gullet, chloroplasts, flagellum, contractile fibers, and cell inclusions. B. Micrograph of Euglena’s body with its myoid pellicles. (A. Modified, from Chris and Dawn Schur. B. www.pmbio.icbm.de/mikrobiologisschr-garten/pics/euglena-rem.jpg

of microtubules to bend the flagellum. While cilia are short and produce movement by an oar-like action, the flagellum of eukaryotes, like Euglena, is long and produces forward locomotion by a whip-like action. Euglena also has myoid fibers around its body, used to change the shape of its body as it darts about rapidly and with great agility.

VISUAL AND CHEMICAL SENSITIVITY. Euglena is of particular interest in the evolution of the behavioral apparatus because, unlike paramecium, it has a specialized sensory organelle, the eyespot. This photosensitive organelle is located near the base of the flagellum (Fig. 2-6) and contains two types of rhodopsin, one that absorbs blue light, the other green light (James et al., 1992; Gualtieri et al., 1992). Behavioral observations indicate two types of photic reactions in Euglena, the slow directed swimming toward or away from a light source; and the swift turning response and flight when the intensity of a light source is suddenly increased or decreased (Creutz and Diehn, 1976; Iseki et al., 2002; Ntefidou et al., 2003). In addition to controlling its phototactic reactions, the eyespot is also involved in the synchronization of Euglena’s endogenous circadian rhythm with the day/night cycle of its environment (Yee and Bartholomew, 1988). In the absence of light, Euglena swims upward in a water column, displaying negative gravitaxis (Daiker et al., 2011). The gravitaxis of Euglena has been studied under weightlessness, hypergravity, and in density-adjusted media (Hader et al., 2003). Since Euglena lacks otoliths, it is assumed that the entire mass of its cytoplasm acts as a gravity sensor by exerting pressure.
GOAL-SEEKING BEHAVIOR IN AMOEBA

A Pursuing Prey

B Ingesting Prey

Fig. 2-7. The directed prey-pursuit (A) and ingestive (B) behavior of an amoeba.  (Modified, from Jennings, 1906.

on stretch-sensitive ion channels at the base of the body. In addition to its visual and gravitational responses, Euglena also reacts to a select group of chemicals. An interesting aspect of chemical sensitivity in Euglena is the phenomenon called necrotaxis. Euglenas flee from the site where conspecifics have been killed with a laser beam (Ragot, 1993).

2.2.5. The Teleological Behavior of Protozoans. In contrast to the stochastic behavior of bacteria, protozoans display teleological behavior when they directly follow some stimulus sources and flee from others. Such behavior has been documented in amoebas, ciliates and flagellates. Thus, Jennings (1906) found in a pioneering study that a food-deprived amoeba will tenaciously pursue a moving prey, such as a bacterium, until it succeeds in engulfing it (Fig. 2-7). According to a recent study, certain chemicals, such as folic acid and riboflavin, serve as attractants to amoebas (Maeda et al., 2009). This direct pursuit behavior differs fundamentally from the behavior of a flagellated bacterium that locates a favorable milieu by a random-walk process of running and tumbling.

Similarly, Jennings (1906) found that paramecia flee from a site polluted with certain salts and move to a site made slightly acidic. Is this behavior a stochastic process like that of bacteria? Jennings’ observations indicated that it is different. When Oxytricha fallax moves forward and encounters a heated region along its path, its first response is to reverse its ciliary beating and retreat. After a brief backtracking, the protozoan turns, and then resumes its forward swimming. This cycle of retreating, pivoting, and moving forward is repeated several times until finally the ciliate locates a cooler region and relaxes. Jennings made similar observations in paramecia that encounter an obstacle along their path (Fig. 2-8A), and so did Dryl and Grebecki (1966) using chemical stimuli (Fig. 2-8B, C). Paramecia avoided a region of water made alkaline, and aggregated in the region made slightly acidic. This is not stochastic behavior but a coordinated sensory process of sampling the stimulus properties of the environment and triggering a coordinated motor response. According to a recent study, a high proportion of the tracks that led to the avoidance of a lit region by Stentor coerulescens had the character of “smooth turns” rather than that of biased random walk (Menzies et al., 2004).
The mechanisms of locomotion in amoebas and ciliates, as we described earlier, are quite different and so is the coordination of their teleological behavior. The prey-pursuit behavior of amoebas is based on a relatively simple mechanism, the change of the cytoplasm from the gel to the sol state near attractants, resulting in pseudopod formation and streaming in that direction, and vice versa. As such, it may have little in common with how ciliates coordinate their goal-directed behavior. Ciliate behavior is far more complex, and less direct, but has greater bearing on the evolution of teleological behavior in the animal kingdom. The behavior of a paramecium is considered teleological because it is based on active search. However, it is inefficient because, lacking special sensors, ciliates cannot directly pinpoint the source of an attractant or a repellent. According to a recent study, it takes a paramecium about 1 minute to realign its body when the direction of gravitational force is changed (Braucker et al., 1998). That is a long latency when compared to the reaction time of a Euglena furnished with an eyespot. As Jennings (1906) observed, when Euglena swims toward a lighted bulb, the axis of its body rapidly oscillates right and left, as if sampling the illumination gradient, to find the stimulus source. If the position of the light source is then reversed, Euglena turns immediately and swims in a similar zigzag manner toward the new location (Fig. 2-9). We would argue that the difference between the two reactions is that Euglena has a special sensor, the eyespot (Fig. 2-6), to assess gradients in light intensity, while paramecium has neither special tactile nor gravity sensors. The behavior of Euglena is, of course, less efficient then that, for instance, of a fish with paired eyes. A fish can swim in a straight line towards a target by using elaborate central computations to determine its location and distance.

2.2.6. The Putative Protopathic Sentience of Protozoans. To distinguish between insentient (robotic) and sentient (mental) discriminatory behavior, we return to our earlier criterion of teleology. Leaving or staying in a milieu cannot be considered a sentient process if it is manifestly based on a stochastic process (biased running or tumbling), but it may be
sentient if it has features of a teleological action or reaction. As we have argued, the primal organic function of sentience is to instigate and guide an organism to reach a target or achieve a particular end state. By this criterion, protozoans may be sentient beings because they are motivated—display a drive or urge—to reach a stimulus source or flee from it.

If protozoans are sentient beings, what sort of mental processes or states may we attribute to them? Displaying goal-seeking behavior, ought protozoans be considered sentient beings? Jennings argued affirmatively by pointing out that not only does an amoeba manifest sensory discrimination but it also displays a ... pertinacity in the pursuit of food such as we would attribute in a higher animal to a desire for it.

(Jennings, 1906; p. 331)

Indeed, when a food-deprived amoeba persistently follows its prey, tracking it doggedly as the prey turns in different directions, it displays a behavior similar to our own hunger or appetite. Likewise, when it turns away from a harmful stimulus, we may attribute to it something like our own discomfort or suffering. Unlike a robot, the food-depleted amoeba does not become more sluggish as its energy reserves diminish; rather, its food-seeking behavior becomes more vigorous and persistent. But Jennings went beyond attributing feelings (“desires”) to protozoans, he argued that they also possess some intelligence. As he wrote:

It is difficult if not impossible to draw a line separating the regulatory behavior of lower organisms from the so-called intelligent behavior of higher ones; the one grades insensibly into the other.

(Jennings, 1906; p. 335)

However, if intelligence means awareness of what goes on in the outside world, we cannot grant amoebas that faculty because they lack specialized sensory organelles. How could an organism be aware (see, smell, hear, etc.) of what goes on in the outside world when it lacks eyes, nostrils or ears? But while we consider it inconceivable that a protozoan could be cognizant of what goes on its environment, that does not rule out the possibility that they are

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**Fig. 2-9.** Path of a Euglena swimming toward a light source (1), then reversing its direction as the light source (2) is moved. (Modified, from Jennings, 1906.)
sentient beings in the sense of having inchoate emotions or feelings, such as appetite or hunger, likes and dislikes.

THE DIFFERENCE BETWEEN AWARENESS AND SENTIENCE. Higher animals and man are aware of three classes of stimuli through mediation by three types of receptors, what Sherrington (1906) called teleceptors, exteroceptors, and interoceptors. (i) Distal stimuli from outside the body, often far away, are detected by paired cranial teleceptors, like the eyes, nostrils and ears. (ii) Proximal stimuli that directly affect the body surface, like touch and taste, are detected by cutaneous exteroceptors. (iii) Internal stimuli that originate from the body interior, like pain or itch, are detected by visceral interoceptors. This mental ability to project what is sensed or perceived to its source is the foundation of our awareness of an outside world and of our inner self. While it is enigmatic how these subjective experiences are generated, the organic mechanisms mediating them are not. The identity and location of a seen object is made possible by paired eyes with topographically organized retinas which supply monocular and binocular information to dedicated CNS structures carrying out the necessary computations. Likewise, proximal stimuli affecting cutaneous receptors are accurately referred to their source (we do not feel the itch in our head but where the fly has landed on our arm) because this system is also topographically organized. Finally, sensed internal stimuli are referred, often not very accurately, to the body region or viscera affected (the gas pain or “heart burn” is felt in the abdomen). Could an amoeba, or even a paramecium that has a simple eye spot, see the prey it pursues as being out there? We believe that they cannot possibly have such a subjective experience because they lack the requisite sensory and neural processing mechanisms. Indeed, the fact that a paramecium cannot locate and instantly orient itself toward a stimulus source, like a fish does, suggests that they are not aware of something specific being “out there.” That is, they do not possess, what Uexküll (1909) called an Aussenwelt. However, it is conceivable that they possess an inchoate Innenwelt, feelings devoid of awareness. We propose that this mental ability, feelings without sensory or perceptual content, has emerged in protozoans.

PROTOPATHIC SENTIENCE: MIND WITHOUT AWARENESS. We call this inability to perceive what transpires in the external world but being attracted or repulsed by external stimuli, protopathic sentience. The term “protopathic” was coined by the clinical neurologist, Henry Head, in reference to poorly localized human pain and suffering (Head, 1920), and we have attributed that mental state to human neonates whose cerebral cortex is as yet unmyelinated (Altman and Bayer, 2001). Protopathic sentence connotes a hypothetical mental state of inchoate feelings, i.e., subjective experience devoid of sensory or perceptual content: feeling content or miserable or, to use the vernacular, feeling cozy or lousy. We propose that protopathic sentence evolved in protozoans because it operates as a powerful endogenous “stick-and-carrot” mechanism. The feeling of hunger by the food-deprived amoeba goads it to embark on a hunting expedition; the appetizing chemical emanating from the prey lures it to pursue and capture it. Though without discriminatory attributes, protopathic sentence is coupled with something like a passion that mobilizes all the resources of a protozoan to struggle for its individual survival. What might the inchoate sentient state of a protozoan be like in terms of our own awareness and consciousness? Closest to that experience may be our inchoate feeling states, or moods—such as excitement and distress—that occasionally overcome us, feelings that we cannot relate to anything particular within ourselves or the outside world. A
kindred but more esoteric subjective state might be the altered state of consciousness reported by mystics and those under the influence of certain psychoactive drugs—the experience of ineffable rapture and bliss, on the one hand, or utter dread and terror, on the other hand—experiences in which all referential contents disappears and all that the subject is left with are his or her inchoate feeling state.

**SUBJECTIVE SENTIENCE AND THE OBJECTIVE PHYSICAL WORLD.** As we noted before, we do not understand how our mind, defined as subjective (private) experience, is related to matter, defined as the objective (public) physical world. This enigma also applies to the hypothetical inchoate sentience of lower organisms. It is notable, however, that attraction and repulsion are ubiquitous natural processes that govern the dynamics of the physical world much as they do the behavior of animate beings. At the subatomic level, positively charged particles (like protons) attract negatively charged particles (like electrons) and, conversely, particles with like electromagnetic charges repel one another. Atoms with negative valence attract atoms with positive valence and unite to form small molecules which, in turn, depending on their free charges, interact with one another to form larger and ever more elaborate macromolecules and macromolecular complexes. The presocratic philosopher, Empedocles of Acragas (5th century BCE), speculating about the constructive and destructive phenomena operating both in the physical and living world, argued that there are two ultimate universal forces, what he called “Love” and “Strife” (Kirk et al., 1982). Love, as a unifying or centripetal force, produces order; strife, a divisive or centrifugal force, produces chaos. It is not clear whether Empedocles’ “love” and “strife” was an early formulation of our current conception of positive and negative electromagnetic charge, in which case the animistic terms he used could be construed as metaphors, or they had mental connotations, implying that all physical matter had psychic attributes. Indeed, the modern philosopher Lotze (1885) argued that there is continuity between the inanimate world and animate life with the forces of “love” and “hate” governing both of them.

This sort of panpsychism, however, is antithetical to modern scientific thinking. Few scientists would accept the proposition that atoms and molecules feel love and hate just because they attract or repulse one another. They assume, instead, that positive and negative charges and valences are inanimate physicochemical phenomena. On the other hand, we human beings do feel love and hate, and we attribute similar affects to many higher animals that we are intimately associated with, and have a similar sensory and neural constitution, such as cats and dogs. Is there some linkage, as emergent phenomena between physical negativity and positivity and mental love and hate? And if so, at what stage of organic evolution can we start attributing affective experience to living beings? Living itself cannot be the criterion because we are unlikely to attribute the slow and tentative growth of a plant’s roots towards a water source as a “desire to drink,” as we do when we see an overheated dog rapidly and unerringly running to its water dish. We tend to attribute mental powers to behaving beings only. But if self-generated, goal-seeking and goal-directed behavior is a criterion of mentation, there is some justification in attributing protopathic sentience to protozoans.

### 2.2.7. The Limitations of Protozoan Sentience and Behavior

Protopathic sentience does not furnish an animal with awareness or consciousness. It merely provides the individual
with a dichotomous (positive or negative) feeling state, and an impulse to respond adaptively. Protozoans are endowed with innate mechanisms to recognize a limited number of environmental stimuli—certain chemical attractants that signal nutrients they live on; light that they may be attractive or repulsive, depending on wavelength and intensity; touch, gravity, and a few other stimuli—but without any awareness of their features, location and other attributes. Can these inborn behavioral reactions be altered by life experiences? The available evidence suggests that, in contrast to higher animals, the plasticity of protozoans is very limited. Jennings (1906) studied the problem of behavioral modification in Stentor. As we described earlier, Stentor is a trumpet-shaped ciliate whose body contains muscle-like fiber strands that bend or contract its body when irritated (Fig. 2-5). Jennings used a pipette filled with carmine particles and offered that unpalatable substance to a Stentor. It avoided ingesting the particles by bending sideways. As the supply continued, the Stentor responded by reversing its ciliary beat to expel the water. When that failed to rid of the irritant, the Stentor contracted its body and detached itself from the plant and slid away. Jennings interpreted these changing responses as “modifiability of behavior,” arguing that the altered behavior was due to the preceding experience of failure. However, by modern comparative psychological criteria, this demonstration does not represent an instance of true learning. Rather, Jennings’s observations indicate that Stentor has a series of hierarchically organized inborn response dispositions that are sequentially activated as irritation persists or increases. Indeed, Jennings himself found that if, after the lapse of a short interval, Stentor was again stimulated it displayed the initial bending response.

HABITUATION IN PROTOZOANS. The simplest form of animal learning is habituation. Habituation refers to a decrement in response rate, or the cessation of responding, following repeated presentation of an innocuous stimulus. It has been reported that Stentor’s contraction to a mild mechanical stimulus habituates after repeated trials (Wood, 1970). This habituation effect was associated in Stentor by the progressive diminution of the amplitude of the elicited electric potential during repetitive mechanical stimulation (Wood, 1988). Habituation effects were also found in other ciliates (Applewhite and Gardner, 1971; Osborn et al., 1973). However, because cessation of responding can also be brought about by fatigue, that factor has to be ruled out before one concludes that the subject has “learned” or “remembers” not to respond to a harmless stimulus. Indeed, a study in the vermiform ciliate, Spirostomum ambiguum, showed (Applewhite and Gardner, 1971) that after a rest period of 5 minutes, much of the response decrement was abolished, suggesting that the effect was short-lived (Fig. 2-10). But another study in Stentor indicated persistence of habituation.
ASSOCIATIVE LEARNING. Associative learning is a higher and less ambiguous form of learning than habituation. The three traditionally recognized forms of associative learning in higher animals are appetitive, aversive, and instrumental conditioning. In appetitive conditioning, the animal learns to respond to a previously unheeded, or conditional, stimulus if that is paired with an unconditional stimulus, one that triggers a detectable inborn response. A well-known example of appetitive conditioning is the dog’s salivation to a previously ineffective stimulus (say, the ringing of a bell) if that stimulus is repeatedly associated with a food reward (Pavlov, 1906, 1927). In aversive conditioning, a previously neutral stimulus is associated with a shock delivered to the animal; after a few such trials the animal will squeal when presented with the conditional stimulus. In contrast to appetitive and aversive conditioning (which represent the learning to heed novel stimuli), in instrumental conditioning the animal learns to perform a novel motor response to bring about a desired outcome. A well-known example of instrumental conditioning is the hungry cat’s learning to open the latch of a door if that allows it to leave the cage and reach the bait placed outside (Thorndike, 1911).

Although there has been several claims that paramecia are capable of associative learning (Alverdes, 1937; Bramstedt, 1939; French, 1940), it is now widely accepted that the positive results reported may be based on processes that do not involve true learning (Grabowski, 1939; Best, 1954). This is illustrated by Gelber’s (1952) experiment. When a clean platinum wire is lowered into a glass tube containing paramecia, they tend to avoid the wire. When the wire was baited with bacteria, the paramecia tended to aggregate around the wire. After repeated trials with the baited wire, a clean wire was then lowered into the tube, and the paramecia now clung to it. This was taken to indicate that paramecia are capable of associative learning (appetitive conditioning). Katz and Deterline (1959) repeated this experiment with one change. They stirred the glass tube vigorously to disperse the bacteria before the clear wire was inserted and found that, under this condition, the paramecia no longer aggregated around the clean wire. Although this controversy has not been finally resolved (Gelber, 1965), it is generally assumed that there is currently no convincing evidence that ciliates are capable of associative learning for food reward (Dryl, 1974; Corning and Von Burg, 1976). With regard to avoidance learning, it has been reported that if ciliates are placed into a narrow capillary tube connected to a larger compartment at its bottom, they tend to reach the large bottom compartment faster over successive trials. Observations indicated that this enhanced escape efficiency was due to a decrease in the time spent in futile upward swimming. Is this an instance of avoidance conditioning? Reinvestigating this finding, Hinkle and Wood (1994) found that this decrement also took place when the task was altered so that the paramecia could reach the large compartment by swimming upward. It appears, therefore, that instead of being a manifestation of avoidance learning, the reduction of upward swimming in the original observation was due to some other factor than motor fatigue, perhaps reduced negative gravitaxis.
2.2.8. Our Protozoan Legacy. The hypothetical construct of “legacy” that we shall keep referring to throughout this work (cnidarian, chordate, agnathan, piscine, amphibian, reptilian, simian, etc.) is based on the assumption that most of our biochemical, morphological and physiological traits are an evolutionary heritage. This is not a reiteration of Haeckel’s (1894) categorical dictum that “ontogeny recapitulates phylogeny.” Rather, we assume, in line with current evidence, that the genes of many of our organic traits, and the morphogenetic processes that lead to the development and maturation of those traits, are part of our phylogenetic heritage. Accordingly, we will try to identify among close extant relations of our putative ancestors, from protozoans to primates, the particular taxa to which we may owe these traits. We will examine, for instance, the phylogenetic history of the formation of neurons with transmitter vesicles; neurons with synapses; neurons organized as circuits and networks; and the emergence of specific brain structures, such as the spinal cord or the neocortex. Similarly, we shall attempt to reconstruct the putative ancestry of the sequential emergence of our different mental faculties, such as our ability to see, smell and hear; our perceptual ability to distinguish objects by their texture, shape and other features; our advancement from slow associative learning to rapid learning by recollection; and our ability to think, reason and form abstract ideas.

In one sense, protozoans are very unlike ourselves. They are microscopic in size; in comparison, we are gigantic. They are single-celled; our body is composed of myriads of cells. We have special tissues, organs, and organ systems to perform different vegetative functions (liver, kidney, spleen, gastrointestinal system, etc.); they have submicroscopic organelles for that end. Our behavioral apparatus consists of various sense organs, a complex brain, and a large skeletomuscular system, none of which is present in protozoans. Notwithstanding these differences, much of our fundamental organic traits are a protozoan legacy. Like them, we are eukaryotes. The cells which compose our body, and our basic metabolic, growth and reproductive processes are much like those in the single cell of a protozoan. Our cells contain the same genomic elements, amino acids, and many of the same or similar proteins. They contain similar subcellular building blocks and organelles, such as the bilayered cell membranes with various ion channels; microtubules that serve as cytoskeletal elements; mitochondria that are the powerhouse of cells; ribosomes that serve as a machinery for protein synthesis; and actin and myosin as contractile elements, and so forth. With respect to our behavioral apparatus, it is notable that most of our sense organs contain ciliated cells, either in developed or vestigial form, for stimulus transduction—as exemplified by the hair cells of the auditory, vestibular and olfactory apparatus, and a component of the rods and cones of our retina. Moreover, our body contains ciliated cells to move fluids, and amoeboïd cells to scavenge pathogens and related functions. Of course, our mental life is altogether different from the presumptive inchoate sentience of an amoeba or a ciliate. However, our evolved mental and behavioral apparatus shares some basic properties with protozoans. All our mental functions depend on electrical signaling—analogue generator potentials and digital action potentials propagated by nerve cells that form our peripheral and central nervous systems—and these basic membrane-mediated mechanisms are present in rudimentary form in ciliates.
2.3. The Evolution of the Peripheral Nervous System and the Emergence of Sensation in Headless Radiata: Our Cnidarian Legacy

2.3.1. Porifera: From Protozoans to Metazoans. Most of the metazoans found in 650-550 million year old Precambrian and Cambrian deposits had either an irregular tubular shape (like sponges) or a radial form (like polyps and medusae). The sedentary sponges (Porifera), which are the most primitive extant quasi-metazoans, are without specialized tissues and organs, including sensors, muscles and nerves (Buchsbaum et al., 1987; Satterlie and Spencer, 1987; Woolacott, 1993). Instead, the tubular, single-layered body of sponges is composed of an assembly of amoeboid and flagellated cells, bound together by a network of fibers and excreted minerals (Fig. 2-11). Pore cells pull water into the sponge’s body cavity by flagellar action; the water flows through the porocytes of the body wall; the nutrients in the water are trapped, ingested and digested by amoeboid mesenchyme cells in the body interior; and, finally, the filtered water is expelled through the large excurrent pore at the top, the osculum. The osculum, which comes closest to an organized tissue in sponges, is composed of myocytes that resemble invertebrate smooth muscle cells and contain thick and thin filaments, presumably composed of the contractile proteins, actin and myosin (Pavans de Cecatty, 1986). Free-swimming larvae of some sponges use ciliated cells for propulsion (Warburton, 1966; Woolacott, 1993). It has been argued, based on genetic analyses (phylogenomics), that sponges are not in the line of metazoan evolution that has led to the evolution of Bilateria, like vertebrates (Philippe et al., 2009; Srivastava et al. 2010). Moreover, as noted, sponges display none of the advanced traits of the metazoan behavioral apparatus, i.e., the differentiation of sensory, neural and muscle cells. That advance was accomplished by cnidarians.

2.3.2. Cnidarians: Hydra and Jellyfishes. Cnidarians may have emerged during the
late Precambrian or early Cambrian about 600 m.y.a. (Chen et al., 2002). Together with Ctenophora (comb jellies), Cnidaria belong to a phylum previously classified as coelenterates. Four extant cnidaria are currently distinguished: Hydrozoa (such as Hydra), Scyphozoa (common jellyfishes), Cubozoa (box jellyfishes), and Anthozoa (sea anemones and corals) (Grimmelikhuijzen et al., 2002). They are of great interest from the perspective of the evolution of the behavioral apparatus because they represent the most primitive metazoans in which sensory cells, neurons, and muscle cells have emerged in phylogeny (Buchsbaum, 1948; Bullock and Horridge, 1965; Mackie, 1976, 1990; Satterlie and Spencer, 1987). There are two basic types of cnidarians, the mobile jellyfishes (medusas) and the sedentary hydras (polyps). In medusas, the prey-catching tentacles are situated beneath the pulsating bell, which is used for propulsion. In sedentary hydras, the body is inverted and the waving tentacles are pointed upward or sideways (Fig. 2-12A). In some cnidarian species, medusas and polyps represent two stages in the life cycle of the same individual.

Cnidarians have a more complex body organization than sponges. Their body wall is composed of two tissue layers, the external ectoderm and the internal endoderm, the two being separated by a gelatinous extracellular matrix, the mesoglia (Fig. 2-12A, 2-13A). The ectoderm contains sensory cells, nematocysts, primitive muscle cells, and neurons at its base. The latter form a peripheral network (Fig. 2-12B). The cells of the endoderm are mostly an assemblage of amoeboid and flagellar cells, which perform such internal functions as digestion and excretion. While cnidarians lack specialized organs devoted to circulation and respiration or hard skeletal elements for muscle attachment, they have some specialized organs of behavioral significance, such as the waving tentacles that capture food, and a mouth that opens and closes as the tentacles bring food to it. Cnidarians are in an evolutionary line to the higher metazoans. Cnidarian

**HYDRA**

![Fig. 2-12. A. Structure of the body wall and tentacles of *hydra* with an ectoderm and endoderm. B. The peripheral nerve net of *hydra*. (Modified, from Buchsbaum et al., 1987.)](image-url)
tissues express various genes and transcription factors that are implicated in invertebrates and vertebrates in antero-posterior body patterning, the development of epithelial cells, muscle cells, neurons, and the nervous system. These include members of the *Pax* (paired box) family of genes (Miller et al., 2000; Sun et al., 2001; Galliot and Schmid, 2002;), the *Lhx* homeobox genes (Srivastava et al., 2010), and some others (Galliot et al., 2009; Chapman et al., 2010).

### 2.3.3. The Sense Organs and Sensory Discrimination in Cnidarians

The typical cnidarian sensors are primitive nerve cells with outward projecting cilia (Fig. 2-13C) and an inward directed longer process, the neurite (Fig. 2-13B). The cilia represent the input pole of these cells, the primitive dendrite; the neurite its output line, the primordial axon. The cnidarian sensory cells resemble the bipolar neurons of the vertebrate olfactory epithelium that, likewise, perform the dual function of sensory transduction and impulse propagation.

Although the sedentary hydra displays a marked photosensitivity, it does not possess identifiable photoreceptor cells, rather its photosensitivity is mediated by a rhodopsin-like protein scattered in the ectodermal layer (Musio et al., 2001). In contrast, mobile jellyfish have specialized photoreceptors, ranging from simple eyespots (ocelli) to differentiated eyes (Singla, 1974; Arkett, 1989; Piatigorsky et al., 1989; Martin, 2002). The simplest ocelli are flat patches of tissue consisting of photoreceptor cells interspersed with pigmented ectodermal cells. Complex ocelli are cup-shaped and the photosensitive cells project into the pigment cells that form the base of the cup (Fig. 2-14A). More elaborate jellyfish eyes are camera-like, with a crystalline lens, a pigment layer, and a layer of photoreceptor cells that form a primitive retina (Fig. 2-14B). These eyes, together with ocelli and gravity receptors (statolith) are embedded in structures called rhopalia (Fig. 2-14C). There are several of these in some species of jellyfish (see below).
The jellyfish photoreceptor cells are bipolar neurons, with their ciliated apical pole serving as photon transducers and afferents, and their basal process as an efferent nerve fiber (neurite, or primitive axon). The latter synapse with second-order neurons (Westfall, 1982; Martin, 2002). The cilia consist of the 9+2 assembly of microtubules and in some cnidarians, such as cubomedusae, there are stacks of membranes at the tip of the cilia (Martin, 2002). These apical membranes contain opsin-like proteins (Burr, 1984; Arkett, 1989; Martin, 2002). Cubomedusae can swim relatively fast and have rapid responses to visual stimuli. Some species have 24 eyes of four morphologically different types, assembled in rhopallia (Garm et al., 2007; Gray et al., 2009). Eight of these eyes are camera-like. Some of the eyes are directed upwards, others sideways and downward. Unlike the rhabdomeric opsins found in invertebrates, the ciliary opsins of cnidarian photoreceptors are more like the opsins of vertebrates (Eakin, 1982; Koizmik et al., 2008; Suga 2008). There may be different types of opsins that selectively respond to different wavelengths (Goldsmith, 1990; Martin, 2002). The photoreceptor cells have neuronal features with different types of synapses (Gray et al., 2009) and may use amino acids and peptides as chemical transmitters (Lam et al., 1982; Grimmelikhuijzen et al., 1992, 1995). The photoreceptor cells respond proportionally to changes in light intensity with graded electric potentials (Ohtsu and Yoshida, 1983).

**CHEMICAL AND MECHANICAL SENSORS.** While special chemical sensors have not been identified in hydra, binding sites for glutathione have been demonstrated in membrane fractions (Grosvenor et al., 1992; Ohta et al., 1992). Glutathione is a potent activator of feeding behavior in hydra (Grosvenor et al., 1996). While some jellyfish are drifting predators, others use chemical stimuli to locate and pursue prey (Tamburri et al., 2000). The statocysts are specialized sense organs of jellyfish, which exist in simple and complex forms (Bullock and Horridge, 1965). The complex statocyst is a closed vesicle that contains a suspended otolith (a granule composed of calcium carbonate), which exerts pressure upon the underlying sensory cells furnished with long cilia. As mechanoreceptors, statocysts respond to gravity and to
acceleration or deceleration. They regulate the righting responses of swimming medusas and may be closely linked with eyes (Fig. 2-14B).

2.3.4 The Neurons and the Peripheral Nervous System of Cnidarians. Cnidarians are the most primitive metazoans with specialized neurons. The cnidarian neurons form a peripheral network consisting of a subepidermal nerve plexus and a nerve ring beneath the tentacles (Fig. 2-12B). They lack a central nervous system.

Cnidarian Neurons. The typical neurons of coelenterates are bipolar cells with short or long neurites that form a mesh-like peripheral nerve net throughout the whole body (Pantin, 1952; Bullock and Horridge, 1965; Westfall, 1987; Grimmelikhuijzen and Westfall, 1995). These neurons are typically unpolarized. That is, they are not like the typical neurons of higher metazoans that have arborizing dendrites at one pole, serving as receivers and afferent lines, and a long axon at the other pole, serving as output or efferent lines. Some cnidarians also have giant axons (Meech and Mackie, 1993) and a few larger ganglion cells (Koizumi et al., 1992; Miljkovic-Licina et al., 2004). The cnidarian nerve cells contain all the organelles of vertebrate neurons, including microtubules, neurofilaments, and synapses (Fig. 2-15). They also have synaptic membranes and synaptic vesicles, which resemble but are not as clearly defined as those of more advanced metazoans (Westfall, 1987; Spencer, 1989). While many of the cnidarian synapses are of the standard one-way kind (vesicles are limited to the presynaptic region), two-way synapses (with vesicles on both sides of the synaptic cleft) are more common. The synapses contact other nerve cells, nematocysts, epitheliomuscular cells, or differentiated muscle cells (Bilbaut et al., 1989; Meech and Mackie, 1993; Westfall et al., 2002).

Fig. 2-15. Translucent and dense core vesicles in the hydra nerve net. (Modified, from Lentz.)

Organization of the Peripheral Nervous System. The cnidarian peripheral nervous system has several components (Bullock and Horridge, 1965; Satterlie and Spencer, 1987; Satterlie, 2002; Westfall et al., 2002). (i) A slow conducting, diffuse plexus implicated in the coordination of routine swimming. (ii) A local conducting system responsible for the asymmetrical local motility of body parts, such as body turning and bending of the tentacles. (iii) A fast, through-conducting system of nerve rings and nerve cords that mediate the rapid withdrawal of the tentacles and a fast escape response when irritated. (iv) A nerve plexus that regulates digestive tract motility (Shimizu et al., 2004). Closest to a tight aggregate of neurons are the cell...
bodies embedded in the circumferential nerve ring of hydra’s mouth (hypostome) and in the nerve rings and longitudinal nerve cords underneath the bell of jellyfish (Bullock and Horridge, 1965; Koizumi et al., 1992; Grimmelikhuijzen and Westfall, 1995). The lack of centralization (ganglion formation) is a notable feature of the cnidarian nervous system.

**SYNAPTIC TRANSMITTERS.** There are two basic types of chemical synaptic transmitters in the nervous system of advanced metazoa, the fast-acting acetylcholine, glutamate, glycine and GABA, and slow-acting serotonin, catecholamines, and various peptides. Ultrastructural and biochemical studies indicate that both kinds of transmitters, as well as their receptors, are present in the cnidarian nervous system (Lentz, 1968; Chung and Spencer, 1991; Martin and Spencer, 1983; Takeda and Svendsen, 1991; Grimmelikhuijzen et al., 1992, 2002; Concas et al., 1998; Pierobon et al., 1998, 2004; Morishita et al., 2003). The fast transmitters are associated with the translucent synaptic vesicles, the slow transmitters with the dense core vesicles (Westfall, 1987; Grimmelikhuijzen et al., 2002; Kass-Simon and Pierobon, 2007).

**BIOELECTRIC SIGNAL TRANSMISSION.** The basic mechanisms of neural signal transmission found in higher metazoa, including the generation of excitatory and inhibitory postsynaptic potentials, have been identified in the cnidarian nervous system (Bullock and Horridge, 1965; Spencer, 1989; Meech and Mackie, 1993; Satterlie, 2002). The small amplitude generator potentials, attributed to the opening of calcium channels, may be responsible for the two-way spread of bioelectric potentials in the nerve plexus (Anderson, 1985; Anderson and Grünert, 1988). This mechanism may function as the pacemaker of hydra’s body swaying and tentacle movement. The through-conducting system of medusas may control bell pulsation as there is a one-to-one relationship between pulsation and the frequency of nerve discharges (Satterlie and Spencer, 1987). The larger action potentials, presumably based on the opening of voltage-gated sodium channels, trigger their fast escape responses. These voltage-gated sodium channels, which are responsible for the non-decaying, long-distance propagation of action potentials in advanced metazoa, may have begun to evolve in cnidarians.

### 2.3.5. The Behavior of Cnidarians.

Cnidarian motility is produced by primitive epitheliomuscular cells that contain myofilaments, but in some jellyfish both smooth and striated muscle tissues are present in the mesoglia (Singla, 1978; Bilbaut et al., 1989). As we noted, the behavior of hydras and jellyfishes differ considerably. Hydras spend most of their time attached to a solid substrate, whereas jellyfishes actively forage, propelling themselves by the pulsating action of their bell in search of prey.

**THE BEHAVIOR OF HYDRAS.** The sedentary hydra feeds by swaying its body in different directions and waving its tentacles in search of prey. Upon locating a prey, such as a crustacean, the hydra extends its body and tentacles, then retracts the tentacle that catches a prey and brings it to the mouth (Fig. 2-16A). Often a hydra will release nematocysts to sting and immobilize its victim. When a prey is caught, the hydra’s mouth opens before the tentacles have reached it (Grosvenor et al., 1996). Observations suggest that both external chemical attractants and internal factors control hydra’s feeding behavior. Particularly potent chemical stimuli are glutathione (Concas et al., 1998) and nitric oxide (Colasanti et al., 1997). The exogenous supply of nitric oxide increases tentacle movement and triggers the recruitment of
neighboring tentacles. Cessation of feeding may be induced by satiety or by a chemical extract from prey. The latter substance—which inhibits the binding of glutathione to its putative receptor—reduces the duration of mouth opening and facilitates the contraction of hydra’s body and its tentacles and the assumption of a resting posture (Grosvenor et al., 1996). There is some evidence for a small repertoire of alternate responses to stimulation. If the prey upon which hydra feeds becomes scarce, the animal detaches itself from its support, sinks to the bottom and feeds on organic detritus (Jennings, 1906). When irritated by mechanical, thermal, optic, or other stimuli, the hydra contracts into a ball or, else, escapes by gliding or using a somersaulting action involving the tentacles (Fig. 2-16B).

**THE BEHAVIOR OF JELLYFISH.** In contrast to the sedentary hydrids, jellyfishes forage by swimming slowly in the water using their pulsating bells, while their trailing tentacles wave rapidly in search of prey. Swimming direction is to a large extent visually guided. Jellyfishes ascend to the surface of the sea at dusk and descend at dawn (Arkett, 1989). Some jellyfish come to the surface when it is cloudy and sink downward when the sky is bright. Sudden change in illumination, such as that produced by a passing shadow, may trigger a sudden escape response and retraction of the tentacles (Singla, 1974). Laboratory tests have shown that the latter response helps jellyfish to evade predators (Meech, 1989). Removal of the ocelli may abolish this response (Arkett, 1989). Most cnidarians are slow swimmers and their reaction to visual stimuli is sluggish. However, cubomedusae, which (as noted earlier) have complex eyes, are faster swimmers, and display more complex visual behavior. They may chase and catch fish and avoid obstacles in their path (Larson, 1976). In an experimental study, Garm et al. (2007) examined the ability of cubomedusae to detect transparent and colored obstacles. Placed in
a tank with flowing water, the jellyfish bump into thin or transparent obstacles as they swim with the current, but swim against the current and avoid thick obstacles that are visible (Fig. 2-17).

2.3.6 Cnidarian Mentation: From Protopathic Sentience to Qualeous Sensation. Cnidarians are among the earliest, relatively large-bodied metazoans that evolved during the pre-Cambrian and early Cambrian era. Largeness has many survival benefits but it poses organizational problems. Being composed of specialized cells and tissues that perform different functions, the metazoan body has to be integrated to behave effectively. This challenge has only been partially solved by cnidarians. Hydra lacks differentiated sense organs, such as eyes, hence it cannot directly detect and locate prey by vision, and its mobility is largely limited to the waving of tentacles. And even the most advanced jellyfishes are sluggish creatures, with limited sensory and motor abilities. And while most jellyfish do have ocelli and some of them have camera-like eyes, they lack a central nervous system to integrate visual information. They can swim but do so quite sluggishly and with little agility. Considering their sensory and motor limitations, what mental life may we attribute to cnidarians? We assume that they are sentient beings because their behavior meets the first criterion of sentience, i.e., they display goal-seeking behavior. The sessile hydra sways its body and waves its tentacles when food deprived, as if motivated by hunger; it opens its mouth when a prey is caught and is carried to it by the tentacles; and when sated, it resumes its resting posture. Hydra may also suffer discomfort when irritated for some length of time and detaching itself moves to some other site. However, lacking specialized sense organs and an integrative central nervous system, we have no reason to assume that a hydra has sensations, i.e., can see, smell or taste. Hence, their sentience may have to be classified as protopathic. In contrast, mobile jellyfish with eyes that actively pursue prey and avoid obstacles may have visual sensations. But since they lack a central nervous system and dedicated sensory processing mechanisms, the question arises what might be the nature of their mental life.

**Fig. 2-17.**

A. Tank design for testing medusa movement

B. OBSTACLE AVOIDANCE BY JELLYFISH

A. Tank with flowing water, used to study visually-guided avoidance of obstacles by box jellyfish.

B. The jellyfish avoid bumping into colored obstacles but bump into or pass between transparent obstacles. (Modified, from Garm et al., 2007.)
THE DAWN OF QUALEOUS SENSATION. The term *qualia* has been used by modern philosophers to refer to the unique and distinctive subjective quality, or “feel,” of raw sensations, such as a red or green color, a sweet or bitter taste, a high or low sound, and so forth (reviewed by Tye, 2007). While it remains an enigma how physical stimuli generate these unique subjective experiences, sensations are not phantoms but mental elements that bear a systematic relationship to stimuli emanating from the external world, known as the laws of psychophysics (Stevens, 1974). That is, qualia of sensations provide veridical information about environmental phenomena but do that not as representations what is out there (pictures, models, sketches), as perceptions do, but as cryptic codes (colors, smells, tastes, etc.) that bear no resemblance to the physical and chemical stimuli that generate them. Do cnidarians have this unique kind of experiential acquaintance with what transpires in the outside world?

We may attribute qualeous sensations to an animal if it meets the following criteria. (i) It possesses modality- and quality-specific transducer mechanisms. For instance, to see *light*, the organism has to have a functioning eye, and to see *color* it has to have certain kinds of pigments or cones in its retina. (ii) It has to have a nervous system with segregated afferent lines, such as an optic nerve, olfactory nerve, etc., to maintain the modality-specificity of the input for central processing. And (iii) it has to be endowed with modality-specific brain stations, such as an optic lobe, olfactory lobe, etc., for processing the gathered information. Hydra does not meet any of these criteria, hence it cannot have qualeous sensations. However, jellyfish meet some of these criteria. As noted, most jellyfish possess eyespots, and some of them camera-like eyes as well. Hence, they possess the transducer mechanisms to discriminate, for instance, between the surface of the sea and its depth by relying on the qualeous sensations of brightness and darkness. This ability would account for their upward and downward movement in the sea in relation to the day/night cycle. Jellyfish may also be able to discriminate by luminance differences an expanding shadow (danger) from a shrinking one (safety). However, since jellyfish do not have a CNS to integrate the information conveyed by their eyes, it is unlikely that they could perceive the shape or texture of the optical stimulus, or judge its distance. The fact that they have multiple optic receptors (ocelli, small, and large eyes) oriented in different directions suggests that instead of central integration, they rely on eyes with different properties to perform specific functions. Moreover, pattern discrimination requires learning, but cnidarians have limited learning ability. We noted earlier that a hydra contracts its body when mechanically stimulated. One study showed that when the same mechanical stimulus is repeated for about an hour, the hydra’s tendency to contract diminishes (Rushforth, 1965). However, this “habitation” effect occurs only when the repetition rate is high and it dissipates after a lapse of 3-4 hours. With regard to associative learning, Ross (1965) reported little success in his attempt to establish aversive conditioning in sea anemones by associating food delivery with an electric pulse.

2.3.7. Our Cnidarian Legacy. Our most important cnidarian legacy is the evolution of the elements of the triad mechanisms of the behavioral apparatus: nerve cells, sensory cells, and muscle cells. While neurons share many properties with other cells, they are unique in that they propagate bioelectric signals as conveyors of information over long distances, a prerequisite property of all behavioral and mental processes. In a hydra or jellyfish, single neurons, and neurons assembled into networks, link sensory cells and sense organs with muscle
cells and tissues, enabling these metazoans to gather information about goings on in the outside world and react to them adaptively. However, these mechanisms are variably developed in different cnidarian species and the behavioral aptitude of the most advanced of them is quite limited. Hydras lack distinctive visual sensory cells; some jellyfish have flat eyespots (ocelli) scattered over their body surface; more advanced jellyfish also have eye cups; and still others, in addition, camera-like eyes with a lens. And while all cnidarians have neurons that conduct bioelectric potentials and a nerve plexus for neural integration, there are differences in the synaptic organization of their nervous systems, and none has a central nervous system. With regard to their motor abilities, hydras lead mainly a sessile existence, and there are great species differences in the ability of mobile medusas to navigate. This variability may reflect the evolutionary “tinkering” that took place in this primitive metazoan phylum. The structural and functional integration of the multicellular behavioral system was evidently in a formative stage. And when it comes to a consideration of the mental abilities of cnidarians, it is difficult to imagine what visual experiences a jellyfish might have when it receives input from a host of different optical receptors distributed over the body surface. How is all that input integrated considering the fact that cnidaria lack dedicated nerve tracts and brain centers to integrate all that information? We must assume that no such integration is taking place and their visual sensations are of rudimentary quality, serving specific rather than global orienting functions. As we describe in the next section, sensory integration has been achieved by primitive bilaterians that possess a head furnished with paired eyes and other cranial sense organs, and an integrated central nervous system.

2.4. From Radial to Bilateral Body Organization, Emergence of the Head and the Brain, and Mental Advances in Planarians

2.4.1. The Evolution of Animals with a Bilateral Body and a Head. The evolution of animals with a bilateral body, and a head furnished with paired special sense organs and a brain (cephalic ganglia) was a momentous event in behavioral and mental evolution. An animal with a head and two topographically organized eyes, and matching brain processing mechanisms, can scan the environment to exactly locate targets in 3D space by using monocular and binocular depth and distance cues. Such an animal has tremendous advantages relative to others lacking these abilities. Unfortunately, we currently know only about a few transitional families that bridge the gap between cnidarians and primitive bilateral chordates to be able to reconstruct the details of that evolutionary advance. Hence, we turn to a primitive invertebrate, the extant planarians, to fill that gap. Planarians (flatworms) are not in the direct line of our ancestry but they seem to be not far removed from the putative “last common ancestors” of two divergent lines of bilaterians, the invertebrates (such as annelids, like the earthworm), and the chordates, like amphioxus and the agnathans (hagfish and lamprey).

2.4.2. The Behavioral Apparatus of Planarians. Planarians (Fig. 2-18) have attracted the attention of zoologists, molecular biologists, neurobiologists and comparative psychologists because they have a rudimentary head furnished with two eyes and a pair of cephalic ganglia that are intermediate in organization between the peripheral nerve nets of cnidarians and the more advanced brains of higher invertebrates and vertebrates (Buchsbaum et al., 1987; Bullock
THE NERVOUS SYSTEM OF PLANARIANS. There is great variability in the organization of the nervous system in different species of flatworms (Cebrià, 2007). Some planarians have only a diffuse peripheral nerve plexus, one that is similar to that of cnidarians. Other planarians have two or more longitudinal nerve cords, as well as a pair of cephalic ganglia that may be closely related to the evolutionary precursors of the paired invertebrate and vertebrate brain (Fig. 2-18). Although the nervous system of planarians is ventrally situated, rather than dorsally as in vertebrates, there are morphological and physiological similarities between the cephalic ganglia of flatworms and the vertebrate brain (Lentz, 1968; Chien and Koopowitz, 1977; Keenan et al., 1981; Reuter and Gustafsson, 1995; Agata et al., 1998; Cebrià, 2007).

Fig. 2-18.
A. Photograph of a living freshwater planarian with a primitive head and two eyes.
B. The peripheral nerve net and paired nerve cords and cephalic ganglia of a planarian.
C. and D. The nerves, and paired nerve cords and cephalic ganglia in two planarian species, immunochemically stained with the marker, synapsin.
Abbreviations: cg, cephalic ganglia; vnc, bilateral ventral nerve cords; ph, pharynx.

(A. From Islandwood.org/planarian2.jpg. B. Modified, from Buchsbaum et al., 1987. C. and D. Modified, from Cebrià, 2007.)
1981; Westfall, 1987 Elvin and Koopowitz, 1994; Reuter and Gustafsson, 1995; Agata et al., 1998). The planarian cephalic ganglia are connected with afferent and efferent nerve cords (Fig. 2-19A), and the cephalic ganglia contain multipolar neurons similar to those found in vertebrates (Fig. 2-19B). Their synapses are, likewise, vertebrate-like (Fig. 2-19C).

Some planarian neurons decrementally conduct graded electric potentials while others propagate all-or-none action potentials (Koopowitz et al., 1979; Keenan et al., 1984). An EEG (electroencephalogram) waveform has also been recorded from the head of a planarian that was inhibited by illumination and abolished by cooling (Aoki et al., 2009). Most of the putative neurotransmitters found in the brains of vertebrates have also been identified in the planarian nervous system. These include acetylcholine, noradrenaline, GABA, histamine, dopamine, serotonin, substance P, beta-endorphin, and leu-enkephalin (Lentz, 1968; Welsh and Williams, 1970; Wickgren and Reuter, 1985; Wickgren et al., 1990; Eriksson and Panula, 1994; Saitoh et al., 1996; Wickgren et al., 1990; Wickgren and Reuter, 1985; Wickgren et al., 1990; Eriksson and Panula, 1994; Saitoh et al., 1996; Fernandes et al., 2003; Farrell et al., 2008; Nishimura et al., 2010). Some progress has been made in tracing the circuitry of the planarian nervous system (Umesono et al., 1999; Okamoto et al., 2005). Visual fibers project to the medial region of the main lobes, and olfactory fibers to the peripheral region of the main lobes. The projection of posterior visual neurons is ipsilateral, that of anterior visual neurons is contralateral. There are also commissural neurons that interconnect corresponding parts of the cephalic ganglia.

Since a small fragment of a planarian, taken from any region, will regenerate a whole animal with a normal nervous system, there must be pluripotent stem cells present throughout the planarian body (Agata and Umesono, 2008). Regeneration studies have implicated Hedgehog signaling and Wnt genes in the anterior-posterior patterning of the body (Yazawa
et al., 2009) and, as in all higher species, Pax genes are involved in the regulation of eye development (Gehring and Ikeo, 1999; Pichaud and Desplan, 2002). The development of different components of the planarian nervous system has been linked to a gene (Nou-darake) that has affinities with neuron-specific genes in higher animals (Umesono et al., 1999; Cebrià et al., 2002; Mineta et al., 2003; Nakazawa et al., 2003; Agata and Umesono, 2008).

THE SENSE ORGANS OF PLANARIANS. There is great variability in the sensory apparatus of different species of flatworms. Most common are single sensory cells scattered throughout the body, containing cilia or microvilli. Some flatworms also have photosensitive epidermal eyespots (ocelli). The coarse epidermis overlying the ocelli is indistinguishable in some species from the epidermis covering the rest of the dorsal body surface (Smales and Blankenspoor, 1978). More advanced planarian species have symmetrically arranged bilateral eyes with a transparent cornea and eye muscles that resemble the oculomotor apparatus of higher species (MacRae, 1966). The eyes of planarians contain two cell types: pigment cells that form the eye cup, and bipolar photoreceptor neurons located outside the eye cup. The receiving pole of these neurons are capped by membranous stacks that contain rhodopsin-like photon transducers (Azuma et al., 1999; Inoue et al., 2004). The axons of the bipolar neurons project to the dorsomedial region of the cephalic ganglia (Agata et al., 1998; Sakai et al., 2000). Removal of the eyes of planarians abolishes their light avoidance (Azuma and Shinozawa, 1998).

2.4.3. Behavioral Advances in Planarians. Higher animals, such as fish, use their paired eyes to directly locate targets in their 3-D environment. There is behavioral evidence that flatworms having paired frontal eyes and a bilobed brain can locate a stimulus source and directly turn towards or away from it. Unlike protozoans, planarians do not need to move in a zigzag manner to sample concentration gradients.

SENSORY ORIENTATION. Planarians tend to avoid light (negative phototaxis). Taliaferro (1920) examined that behavior by successively turning on and off a light source in different corners of a testing apparatus. The planarians responded immediately by turning their head away from the new light source and continued with their avoidance by moving in a new direction (Fig. 2-20A). Freshwater planarians respond to water flow by swimming upstream in a river. To study that behavior, Doflein (1925) used a pipette to stimulate different parts of their bodies with flowing water (Fig. 2-20B). The planarians did not respond when the jet was aimed unilaterally at their flank, but they turned immediately when the water flow was aimed at their head from the side, front or rear. According to a study by Koehler (1932), the reaction of planarians to a chemical attractant is somewhat different. The flatworms initially crawled in a random fashion toward the distant bait. As they got closer to it, they turned toward it and bobbed their head as if sampling the chemical gradient in the water. When they got still closer, head bobbing stopped and the animals directly proceeded to the nutrient.

BRAIN AND BEHAVIOR. Is the planarian brain involved in the ability to localize a stimulus source? It has been reported that while decapitation does not abolish spontaneous motility in planarians, it does interfere with their ability to locate food (Bullock and Horridge, 1965). However, if the food is placed near the proboscis of the decapitated animal it promptly swallows it; and when food is placed near the margin of the decapitated animal, the margin of the body
grips the food and conveys it to the mouth (Koopowitz and Keenan, 1982). These findings suggest that the spared longitudinal nerve cords can mediate both locomotor and ingestive behavior but the direct orienting response depends on head sensors and the brain. Furthermore, while normal animals stop feeding after ingesting a certain amount of food, decapitated animals keep passing food to their mouth even after their gut is completely filled. The brain evidently plays a role in the integration of planarian behavior.

**THE LEARNING ABILITY OF PLANARIANS.** When a flatworm’s body is touched, it contracts. After continued repetition of the tactile stimulus, the response ceases (Applewhite, 1971). Moreover, this habituation effect transfers from one region of the body to another after a few trials. This evidence of transfer suggests that the cessation of responding is not due to local sensory adaptation or fatigue but is an instance of genuine habituation. Habituation has also been obtained with low-intensity optical stimuli, and the effect has been shown to persist for
Chapter 2: From Protozoans to Agnathans

several weeks (Westerman, 1963). We may recall that long-term habituation could not be demonstrated in cnidarians. The persistence of habituation in planarians suggests the formation of an enduring memory trace. There is also evidence from conditioning studies that planarians are capable of associative learning, including aversive, spatial, and instrumental learning. Thompson and McConnell (1955) paired an innocuous optical stimulus (the conditional stimulus) with an electric shock (the unconditioned stimulus) that triggers head turning and body contraction. After 150 repeated trials, the planarians responded to the optical stimulus before the shock was turned on, indicating that after a very large number of pairings they learned to associate that conditioned stimulus with imminent punishment. Aversive conditioning in planarians was confirmed with improved methods by other investigators (Baxter and Kimmel, 1963; Vattano and Hullett, 1964). If the pairing of the optical stimulus and the electric shock was not repeated (reinforced), the conditioning effect extinguished (Baxter and Kimmel, 1963). This is unlike aversive conditioning in higher animals, which persists for a long time without reinforcement. Planarians are also able to master a simple spatial learning task for a reward; i.e., turn in a T-maze either toward the dark or lit arm if one of the alleys consistently leads to water (Best and Rubenstein, 1962). Similarly, planarians can be trained to acquire the habit of turning to the right or to the left by rewarding them with access to their home bowl (Corning, 1964). However, as the trials proceeded the animals’ behavior spontaneously deteriorated. Apparently, the learned response was not stable. The most complex form of learning hitherto reported in planarians is instrumental learning. Planarians could be trained to move to a particular location of the maze to interrupt a weak beam of light and thus trigger a photocell that turned off a noxious bright light (Lee, 1963). After a seven-hour training session, this learned avoidance response was retained for at least 40 hours (Best, 1965). Finally, it has been recently shown that a dopamine agonist, methamphetamine acts as a reinforcer in a conditioned place preference task (Kusayama and Watanabe, 2000). This suggests that learning in planarians may be motivated by a hedonic mechanism.

2.4.4. The Mental Status of Planarians. The evolutionary advances of flatworms relative to cnidarians include: a bilateral body structure; the possession of paired eyes and a primitive bilobed brain; the ability to orient themselves instantly toward or away from a stimulus; and some ability to modify their behavior through learning. Given these traits, what mental status may we attribute to planarians? Possessing two eyes with a lens, and a retina consisting of a small assembly of photoreceptors, provides planarians with the mechanism to behold more than an amorphous expanse of ambient light—what psychologists call a diffuse Ganzfeld (Wallach, 1976). They may be able to detect some coarse features of the stimulus source, perhaps their size, location and texture. They may be able to appreciate whether a looming shadow is large or small, approaching or receding, situated to the right or to the left, and so forth. And since they have optic fibers that target the bilobed cephalic ganglia, and there are commissural fibers between the ganglia, planarians may possess the neural mechanism to project what they see to its location in the outside world. This advance from sensing a bright or dim ambience (something like what we see when immersed in a deep pool of water with eyes open) to perceiving a heterogeneous field, may have been the first step in the evolutionary process of becoming acquainted with the structure of the outside world. This does not mean that a planarian has the perceptual ability to discriminate different objects on the basis of their configuration, precise location in 3D space, and fine details. The primitive retina of even the
most advanced flatworm contains too few photoreceptors to permit pattern discrimination. In our ancestry, this ability emerged in more advanced animals like fish.

2.5. The Emergence of the Vertebrate Head, Neuroepithelium, and the Paleocephalon: Our Chordate and Agnathan Legacies

2.5.1. The Invertebrate and Vertebrate Divide. Some unknown primitive bilaterians gave rise to two advanced phyla with complex sense organs and complex brains, the invertebrates and vertebrates. There are profound differences in the evolutionary course taken by these two lines. The trend among many higher invertebrates (such as arthropods) was toward corporal segmentation, exoskeletization, and the effective miniaturization of body and brain. The trend among vertebrates was toward corporal unification, endoskeletization, and the enlargement of both body and brain.

THE METAMERISM AND NEUROMERISM OF INVERTEBRATES. In many lower invertebrates (such as annelids), the reiterated segments of the body are much alike, with each segment having its own muscular frame (myomeres), body cavity, excretory organs, gonads, and limbs. Matching this corporal metamerism, each body segment has paired neural ganglia, or neuromeres, for the coordination of its activities. The advantage of metamerism is that it permits appreciable body growth through elongation without the need of a unified respiratory, circulatory, and excretory system, and a fully integrated CNS. The obvious disadvantage is the consequent tardiness of behavior, as nerve impulses have to be propagated serially from one segment to the next to produce coordinated locomotion and behavior. Due to this disadvantage, extreme metamerism has persisted only in phyla that occupy sheltered niches, such as underground burrows. In higher invertebrates, such as arthropods (lobsters, scorpions, beetles, spiders, flies, wasps, bees, ants), the number of body segments is greatly reduced and each body segment has its special function, coordinated by local ganglia. Further integration is achieved by a tough and inert chitinous cuticle that provides the animal not only with a protective armor but also with strength and agility as solid attachment sites for the jointed limbs, wings, jaws, and other appendages that they have. The exoskeleton of arthropods has undoubtedly contributed to their great success in colonizing virtually all microniches throughout the globe. However, an exoskeleton has disadvantages. Most importantly, it makes the growth of a large body difficult (many species have to cast off their dead exoskeleton and molt to be able to grow) and that precludes metabolic support for a large brain. Most arthropods are small-brained animals and they achieve behavioral complexity by operating largely with preprogrammed neural circuits and task-specific learning ability. Metamerism is not altogether absent in vertebrates. It is manifest in the reiterated, segmentally arranged sensory ganglia, spinal nerves, and muscle blocks. However, the trend in vertebrates has been to evolve unified functional mechanisms. This is exemplified not only in the evolution of a single set of internal organs (the respiratory, circulatory, gastrointestinal, and urogenital systems) but also in the formation of a trans-segmental notochord, a continuous system of sensory and motor columns in the spinal cord within the segmented trunk, and the evolution of a complex system of suprasegmental integrative brain centers in the head.
CORPORAL AND NEURAL UNIFICATION IN VERTEBRATES. Unlike invertebrates, vertebrates have a unified body with a central nervous system (CNS). Unification of the myomeric body (a vermiform legacy) emerged in primitive chordates, such as the extant amphioxus, with the development of a single elastic rod, the notochord, along the entire body axis. The unified CNS, the forerunner of the spinal cord, traversed by long axons from one end of the body to the other. The chordate nervous system arises developmentally from a continuous antero-posterior germ layer, the neuroepithelium. This is a unique tubular proliferative matrix composed of neural-specific precursor cells and filled with and surrounded by its own fluid milieu. This is unlike the precursor cells of the invertebrate nervous system, which are either scattered among other cellular elements of the developing body or form small aggregates within the formative body segments (see below). Corporal and neural unification is only partially achieved in amphioxus, as it lacks a differentiated head and a developed brain. That came about in fishes in which the notochord became transformed into a cartilaginous or bony axial structure, the vertebral column, and an endoskeleton that supports specialized appendages, such as the head with its sense organs, and fins and a tail for propulsion and postural support. This transformation endowed vertebrates with improved orienting abilities, agility, and speed. Passing from fish to terrestrial vertebrates, the vertebral column came to support a complex appendicular skeleton, including limbs with digits for climbing, running and grasping, a movable head to scan the environment, and jaws with specialized teeth (incisors, molars) to bite and ingest nutrients. Having an endoskeleton lent vertebrates two major advantages: it made possible an immense growth in body size and that, in turn, allowed metabolic support for an expanding brain. It is due mainly to their internal skeleton that some vertebrates (sharks, crocodiles, dinosaurs, mammoths, whales) became the largest animals on this planet, and, in the case of our own ancestry, small insectivores and prosimians gave rise to larger monkeys and apes with ever expanding brains. A large body mass confers strength and invulnerability to the individual, and so does a large brain with its increased computing powers, flexible programmability, and large memory storage capacity.

2.5.2. Extant Primitive Chordates: The Ascidians and Amphioxus. Two extensively studied extant primitive chordates are the ascidians (tunicates) and amphioxus (lancelet). These protochordates share several distinctive traits with vertebrates. Notable among these traits are the notochord, the primordium of the vertebral column, a segmented muscular body, and a dorsally situated CNS with a lumen, the forerunner of the cerebrospinal fluid-filled ventricles (Barrington, 1965; Alexander, 1975). Whereas amphioxus keeps its fish-like body shape, CNS and swimming ability throughout life, the tunicates keep these traits only for a short period as they pass through a brief larval stage.

ASCIDIANS. Also known as tunicates or sea squirts, the ascidians are distant relations of vertebrates. They are filter feeders, permanently attached to a solid substratum in the water. They are covered with a thick, cellulose-like tunic, and feed by drawing water through one upward directed tube, the oral siphon, trap food particles in their large pharynx, and then expel the filtered water through another tube, the atrial siphon (Romer, 1970). Unlike amphioxus, adult tunicates, such as Ciona, manifest virtually no chordate traits and, except for a small ganglion, they lack a nervous system. However, they are of great interest from an evolutionary perspective because they resemble vertebrates during their brief larval stage of development,
with a notochord and a simple tubular nervous system (Katz, 1983; Nicol and Meinertzhagen, 1988a, b). Moreover, genetic studies suggest some similarities in the development of the ascidian larval nervous system and the development of the vertebrate CNS (Mazet et al., 2005). The miniature larval CNS allows the free swimming ascidian larva to locate a suitable site. But once it settles down, it metamorphoses into a sessile animal, and loses most of its nerve cells.

**AMPHIOXUS.** Although shaped like a fish with a streamlined body, lancelet leads a sedentary life as a filter-feeder, either floating in a vertical position in shallow water or buried in gravel at the bottom of the sea. Amphioxus is of great evolutionary interest because it displays several vertebrate-like traits (Bone, 1960). (i) It has gill slits, which are used by amphioxus for filtering food particles from the water (Fig. 2-21A, B). In fishes that have a mouth for food ingestion, the gill slits persist by being transformed into an aquatic respiratory apparatus; and in terrestrial vertebrates that use lungs for breathing, the gill arches play a role during embryonic development. (ii) It has a notochord along its entire body axis. The notochord is the phylogenetic precursor of the vertebral column, and it is present in the trunk and tail of all vertebrate embryos, man included, before the vertebral column develops. (iii) The body wall of amphioxus is composed of segmentally arranged striated muscle blocks (myotomes) that enable it to swim in an undulatory fashion. This is the primordium of the vertebrate skeletal muscular system. (iv) Amphioxus has a post-anal tail with a fin that helps it to maintain its posture when swimming. (v) Most importantly from the perspective of neural evolution, amphioxus has a continuous dorsally situated tubular CNS, much like fish and all other vertebrates (Fig. 2-21C, D).

However, there are differences between the morphology of lancelet and fish (Bone, 1960; Ekhart et al., 2005; Wicht and Lacalli, 2005). (i) Amphioxus does not have a cartilaginous or bony endoskeleton (ribcage) as fish do. (ii) It lacks a unified circulatory system with a pumping heart. (iii) It does not have dorsal or lateral fins, which enable fish to maneuver while swimming. (iv) The organization the amphioxus nerve cord has many similarities with the spinal cord of vertebrates but also differs from it in many respects. The sensory neurons of amphioxus are located centrally rather than in the spinal ganglia peripherally and its motor neurons are located near the spinal canal rather than in the ventral horn. Finally, (v) amphioxus lacks a differentiated head with paired eyes and nostrils, and a differentiated brain.

**THE “BRAIN” OF AMPHIOXUS.** The lumen of the neural tube widens anteriorly. This is the rudimentary brain of amphioxus. This region has been subdivided into three small collections of neurons, called the anterior, middle and posterior cerebral vesicles (Ekhart et al., 2003; Satoh, 2006; Lacalli, 2008). They may serve, respectively, sensory, visceral and neural functions (Fig. 2-22A). (i) The anterior vesicle is thought to be the rudiment of the archaic forebrain. As its neurons are affiliated with the frontal eye, it is more likely that it is the primordium of the retina. (The retina is ontogenetically a CNS derivative.) However, it is unlikely that the anterior vesicle mediates true visual discrimination. First, the axon terminals form large varicosities in this region, instead of synaptic connections with other neurons (Lacalli, 2008). This suggests some diffuse modulatory function. Because some of these neurons express a gene associated with circadian (24 hr) rhythmic oscillation (Wicht et al., 2009), they may be primordia of the circadian pacemaker neurons of the supraoptic nucleus of the vertebrate diencephalon (Fig.
(ii) The middle visceral vesicle is associated with the dorsal lamellar body, which is believed to be homologous with the epiphysis (dorsal eye or pineal gland), and with Hatchek’s pit ventrally, a structure that has been related to the hypophysis (pituitary gland). Hence, the neurons of the middle ventricle may represent the primordia of two ancient diencephalic structures in vertebrates, the dorsal epithalamus (habenular nucleus) and a component of the hypothalamus. Presumably, this neuroendocrine system regulates feeding and reproductive behavior in amphioxus in relation to diurnal and seasonal changes in their environment.
(iii) The posterior motor vesicle is composed of large motor neurons. They may be forerunners of the reticulospinal neurons of the brainstem, neurons well suited to control a fast escape response when amphioxus is disturbed. While synaptic membrane specializations are rare in these neurons, they contain different neurotransmitters and neurohormones, such as dopamine and serotonin (Moret et al., 2004), progesterone (Takeda et al., 2003), and neuropeptide Y (Castro et al., 2003).

THE BEHAVIORAL AND MENTAL LIMITATIONS OF AMPHIOXUS. Having only a single primitive eye, an amphioxus cannot possibly recognize objects in its environment by their shape, location, or other spatial features. But it does respond to changing light intensity, mechanical stimulation, rise in water temperature, or being stimulated by certain chemicals (Parker, 1908). When undisturbed, amphioxus uses its cilia to hover in one place, filter feeding (Stokes and Holland, 1995). Since it is negatively phototropic, it tends to stay in dark areas or feed at night. But amphioxus can also swim fast when irritated (Parker, 1908; Arey, 1915; Reeves, 1931). Its head and tail are more sensitive than the trunk: when its rear is stimulated by negative stimuli, it darts forward; when its front is stimulated, it swims backwards. The details of these sensori-motor reactions are not known but three basic responses have been distinguished. (i) Forward or backward swimming involve a wave of reciprocal contraction and relaxation of myotomes.
on the two sides of its body, progressing from head to tail, or vice versa. (ii) Turning toward an attractant is achieved by ipsilateral contraction of a set of head myotomes. (iii) Turning away from a repellant is achieved by contralateral contraction of a set of head myotomes. Lancelet may arch its body before darting forward. With reference to possible neural control of these responses, it is noteworthy that the neural tube contains two types of efferent neurons: those with axons that synapse with ipsilateral myotomes and those cross over the neural canal and terminate contralaterally (Wicht and Lacalli, 2005). These simple reactions allow lancelet to survive in its favored habitat, intertidal shallow waters.

A recent effort to breed amphioxus for experimental studies, led to an investigation of their spawning behavior. Apparently, males and females do not actively seek each other during the spawning period (Wickstead, 1975). Rather, both swim toward the surface of the water to release their gametes when certain conditions synchronize their reproductive maturation. These include optimal photoperiod and water temperature, and the action of gonadal release of sex hormones (Fang et al., 1992; Fang, 1998; Kubokawa et al., 2003; Mizuta and Kubokawa, 2007). In the wild, European amphioxus spawning occurs from mid-May to early-July, and, in the laboratory, upon increasing the water temperature by 3-4°C (Fuentes et al., 2007).

2.5.3. Extinct Chordates with a Head and a Brain: Yunnanozoon and Haikouella. There is a large gap between amphioxus and the extinct agnathans (such as Ostracoderms) and related extant cyclostomes (such as lampreys and hagfish), which have a genuine head with paired eyes and a differentiated spinal cord and brain (see below). Since the advantage a notochord is that it provides a unified body with agility, it is difficult to see why it evolved in such sluggish creatures as the tunicates or amphioxus. Hence, the latter may be degenerate survivors of chordates that were active foragers and, perhaps, the earliest predators roaming the seas. This idea is supported by the recent discovery of fossil chordates from Cambrian (520 m.y.a.) deposits in China (Shu et al., 1996, 1999; Chen et al., 1999; Holland and Chen, 2001; Mallatt and Chen, 2003). One of these fossils, *Yunnanozoon*, had gills, a pharynx, a notochord, and myomeres. The other fossil with similar body features, *Haikouella lanceolatum*, appears to have been even more vertebrate-like. *Haikouella* has a distinct head with lateral eyes, a mouth fringed with short tentacles, and a pharynx containing small denticles (Fig. 2-23). The bulky posterior two-thirds of *Haikouella* contains a notochord, its muscular body is segmentally organized, and it has a median fin, and a short tail. Dorsal to the notochord, *Haikouella* has a spinal cord, which expands anteriorly into a small bilobed brain. Hence, *Haikouella*, or some closely related chordate, may have been ancestral to such primitive vertebrates as the jawless extinct agnathans and the surviving cyclostomes, the hagfish and the lamprey.

![Fig. 2-23. The extinct *Haikouella* with lateral eyes, mouth, brain, spinal cord, and notochord. (Modified, from Holland and Chen, 2001.)](image-url)
2.5.4. Extinct and Extant Jawless Fishes: The Agnathans. The jawless agnathans, the chordate ancestors of true vertebrates with jaws and an articulated vertebral column, are of great antiquity. Agnathans have been found in as early as 500 m.y.a. Ordovician geological strata, and more commonly in 450-400 m.y.a. Silurian and Devonian strata (Romer, 1970; Carroll, 1988; Colbert and Morales, 1991; Janvier, 1999; Shu et al., 1999; Donoghue et al., 2000; Xian-guang et al., 2002). Many of the earliest agnathans, such as the Ostracoderms, had an armor of scales or plates that covered their trunk (Fig. 2-24A). They had a distinct head with two eyes and a nostril, a mouth to ingest larger particulate nutrients, a notochord, a powerful tail for fast swimming, and fins for postural balancing. These agnathans dominated the seas for a long time. But they disappear from the fossil record after the Devonian period about 370 m.y.a. Jawless, lamprey-like agnathans without armor, the cyclostomes, first appear in late-Devonian deposits (Gess et al., 2006) and are more common in Carboniferous strata (Janvier, 1999). Their surviving descendants are the Cyclostomes, the hagfish (Myxines) and lampreys (Petromyzons). The cyclostomes have a long and slender body composed of myotomes attached to the elastic notochord, which rebounds as it is pulled successively from side to side by the contracting myotomes. However, cyclostomes lack the internal skeleton of more advanced vertebrates. We discuss briefly the hagfishes (Fig. 2-24B) and in detail the better known lampreys (Fig. 2-24C).

Hagfishes. The widely distributed hagfishes prefer cold marine habitats and spend much of their time buried in the mud at the bottom of the sea (Jorgenson et al., 1998; Martini, 1998). They have a head with single nostril, no eyes or a pair of primitive eyes buried under the skin, and they lack a true braincase. They have a jawless mouth furnished with several sensory barbels, a projecting tongue-like grasping organ, and comb-shaped teeth. The eel-like muscular body of hagfishes is supported by a cartilaginous skeleton but they have only one fin caudally and lack a distinct tail. Hagfishes are principally detritus feeders or scavengers but may also burrow into a live fish through its gills or anus and consume it from the inside. When disturbed, hagfishes protect themselves by excreting a glutinous slime and curling into a knot.

THE NERVOUS SYSTEM OF HAGFISHES. The nervous system of hagfishes is of great evolutionary significance because it displays some, but not all, features of the basic design (Bauplan) of the vertebrate nervous system. The nervous system of all vertebrates has peripheral and central components, constituting the PNS and the CNS. The PNS consists of neurons and nerve plexuses associated with the body’s internal organs (including the autonomic nervous system) and a complex assembly of spinal and cranial sensory and motor nerves that connect, respectively, the sense organs and muscles of the trunk and the head with a complex assembly of CNS neurons in the spinal cord and the brain. With the exception of the first-order neurons of the olfactory and visual systems, which are developmentally of CNS origin, the cell bodies of the primary sensory neurons gathered in the spinal and the cranial nerve ganglia are part of the PNS. These sensory neurons have long bifurcating axons that interconnect distant peripheral sensors with interneurons in the dorsal horn of the gray matter of the spinal cord, and their homologues in the hindbrain. Contrasting with the peripheral origin and location of the primary sensory neurons, the perikarya of the final motor neurons, whose axons synapse with peripheral muscles, are located centrally in the ventral horn of the spinal cord or the cranial motor nuclei.
The basic organization of the hagfish CNS (Jansen, 1930; Wicht and Northcutt 1998) is illustrated in Fig. 2-25. Proceeding from rostral to caudal, there is a well-developed olfactory bulb, which is the target of olfactory nerve fibers (cranial nerve I). The telencephalon is principally the target of second order olfactory fibers, and of a small complement of fibers from the diencephalic thalamus and hypothalamus. The largest component of the diencephalon is the epithalamus (habenula) associated with the pineal gland, and the hypothalamus associated with the pituitary gland. The optic tract (nerve II) is either absent or very small, and the mesencephalic tectum is the target of ascending fibers from the spinal cord. Most prominent of the peripheral input to the brain come from two sources, cranial nerves V and VII (trigeminal and facial), and nerves IX and X (glossopharyngeal and vagal). The large “trigeminal” ganglia and nerve have only two components in the hagfish, presumably the “maxillary” and the “mandibular” divisions, which serve the sensory and motor systems of the jawless upper and lower mouth. (The ophthalmic division serving the eye is presumably absent.) The largest hindbrain structure is the facial lobe, supplied by the trigeminal nerve. Also well developed is

![Fig. 2-24.](image-url)
the vagal lobe, supplied by nerves IX and X. The cerebellum is rudimentary or absent. Absent in most hagfish species are the following cranial nerves: II (visual), III, IV, VI (ocular), VIII (vestibular, auditory), and XI and XII (which control the neck and tongue muscles in higher vertebrates).

While we need more information than what is currently available about the organization of the hagfish CNS, what is known suggests that it is concerned principally with visceral functions. The large olfactory forebrain system is presumably involved in locating nutrient resources; the large gustatory and tactile hindbrain coordinates ingestion and alimentation; and the diencephalon regulates arousal and behavioral mobilization in relation to prevailing external conditions and internal needs. We assume that the voracious appetite of hagfish is mediated by powerful emotions but lacking functional eyes, their mental representation of the external world must be very limited.

Lampreys. Lampreys lead a highly specialized parasitic lifestyle by attaching themselves to live fish with a specialized mouth that sucks blood. They are of great interest from an
evolutionary perspective because they have a head with nostrils, eyes and ears, and their CNS shows many advances relative to hagfish, perhaps reflecting what the brains of the extinct agnathans may have been like (Fig. 2-24A).

**THE SENSE ORGANS OF LAMPREYS.** As in hagfishes, the olfactory sensory cells lining the nasal epithelium are modified bipolar neurons. They have, at their outer pole, cilia and villi as chemoelectrical transducers and an efferent axon that collectively form the olfactory nerve (nerve I). The olfactory axons synapse with large mitral cells and smaller interneurons in the glomeruli of the olfactory bulb (Mellendez-Ferro et al., 2001). The olfactory bulb is well developed in lampreys (Fig. 2-26). The axons of the multipolar mitral cells, in turn, form the olfactory tract, which projects to olfactory component of the telencephalon, what is known in higher vertebrates as the piriform cortex or rhinencephalon (Fig. 2-27). The pair of lamprey eyes are camera-like, furnished with extraocular muscles that move the eyes forward or backward for near or distant vision. The retina is well differentiated, containing several types of photoreceptor cells, bipolar cells, horizontal cells, and ganglion cells (Meyer-Rochow and Stewart, 1996; Collin et al., 1999; Collin and Potter, 2000). As in higher vertebrates, the rods have been implicated in scotopic (nocturnal) vision and the cones in photopic (diurnal) vision.

**LAMPREY BRAIN**

**A. DORSAL VIEW**

![Diagram A. Dorsal View of Lamprey Brain](image1)

**B. SAGITTAL VIEW**

![Diagram B. Sagittal View of Lamprey Brain](image2)

**Fig. 2-26. A. Top view of the lamprey brain, with the rhombencephalon exposed. B. Side view of the lamprey brain. (A. Modified, from Larsell, 1947 and Eisen and Northcutt, 1996. B. From Johnston, 1905 and Ariens Kappers, 1936.)**
The axons of the ganglion cells form the optic tract (nerve II) and terminate contralaterally in the midbrain tectum (Heier, 1948; Iwahori et al., 1999), which functions as a true “optic lobe.” Lampreys also have a pair of middle-ear mediating vestibular and auditory functions but it is less differentiated than in bony fishes or higher vertebrates. There is no external ear, middle ear with ossicles, or a coiled cochlea. Lampreys also have sense organs that have not been preserved in terrestrial vertebrates, such as the lateral line system along the trunk, a superficial canal that is furnished with a variety of sensory cells. These include mechanical sensors that respond to water pressure, current flow and vibration; chemical sensors that respond to chemicals dissolved in the water; and pigmented photosensitive cells that signal changes in ambient light intensity (Deliagina et al., 1995).

*Organization of the Lamprey Brain.* In addition to the olfactory bulb, the lamprey brain contains four structurally and functionally distinctive components (Fig. 2-26). (i) The telencephalon anteriorly with several components, the largest of which is the piriform cortex implicated in olfactory functions. (ii) The diencephalon, with the epithalamus dorsally and the hypothalamus ventrally, and some intercalated regions. The principal connections of the lamprey diencephalon are with the pineal and pituitary glands. (iii) The mesencephalon, with a dorsal component, the optic lobe, which is the target of the optic nerve fibers, and a ventral component, the tegmentum (reticular formation) that has a descending motor outflow to the medulla and spinal cord. (iv) The rhombencephalon, including the facial lobe and the vagal lobe, which control ingestion, alimentation, and some other functions. The lamprey brain, as a whole, works as a higher-level command system that controls swimming by acting upon lower-level pattern generators and efferent neuron pools of the spinal cord.

**THE TELENCEPHALON.** The pallium, the primordial cerebrum, is small and poorly differentiated in lampreys (Fig. 2-27). Unlike in higher vertebrates, in which the paired cerebrum surrounds the lateral ventricles, the lamprey cerebrum flanks a single lumen, called the

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**LAMrey FOREBRAIN**

A. **ANTERIOR**

![Anterior Lamprey Forebrain Diagram]

B. **MIDDLE**

![Middle Lamprey Forebrain Diagram]

B. **POSTERIOR**

![Posterior Lamprey Forebrain Diagram]

Fig. 2-27. One side of the lamprey brain in cross section at three levels. Some of the identifications are controversial. (Modified, from Heier, 1948.)
median ventricle (Ariëns Kappers et al., 1936, Heier, 1948). The lamprey cerebrum has poorly
demarcated (and disputed) pallial and ganglionic components. The lateral pallial component
has been homologized with the piriform cortex (rhinencephalon); the medial pallium with
the primordial hippocampus; the region beneath the latter, with the septum, the basal ganglia
(striatum, nucleus accumbens), and the amygdala. The lamprey pallium, which unlike the
mammalian cerebral cortex is not laminated, receives input from the olfactory bulb, the septum,
and basal ganglia (Polenova and Vesselkin, 1993). Its major output is to the same structures
that it receives input from, as well as to the midbrain tectum and tegmentum (Northcutt and
Wicht, 2002). Although evoked potentials are easily triggered in the lamprey telencephalon by
olfactory stimuli, visual stimulation produces “secondary” or “nonspecific” potentials (rather
than “primary” or “specific” potentials), i.e., shallow potentials with a long latency (Karamian
et al., 1966). The basal ganglia contain neurons with such transmitters as dopamine, substance
P, enkephalin, acetylcholine, serotonin, and GABA (Pombal et al., 1997).

**Diencephalon.** The major components of the lamprey diencephalon are the dorsal
epithalamus and the ventral hypothalamus (Heier, 1948; Matsumoto and Arai, 1992). The
epithalamus (habenular nucleus) is connected with the well-developed pineal gland (dorsal eye).
It has been implicated in lampreys with the synchronization of cyclical reproductive processes
with day-night and seasonal changes (Sower and Kawauchi, 2001). The well-developed lamprey
hypothalamus plays a pivotal role in the control of visceral and endocrine processes controlled
by pituitary hormones. Several hypothalamic and pituitary hormones have been identified
in lampreys (Sower et al., 2000; Bird et al., 2001; Sower and Kawauchi, 2001; Kawauchi
and Sower, 2006) and in hagfishes (Nozaki, 2008). Among these are gonadotropin-releasing
hormones (GTH) that regulate reproductive behavior; a melanin-concentrating hormone that
regulates skin color changes; and adrenocorticotropin (ACTH) and growth hormone (GH)
implicated in homeostatic functions. It is uncertain whether agnathans have a sympathetic
nervous system. There is evidence for a parasympathetic outflow in the vagus nerve but the
effect of vagal stimulation on the heart and gut is unclear (Nicol, 1952; Pick, 1970). The
hypothalamus (together with the tegmentum) also has a descending influence on lower-level
CNS functions mediated by various neurotransmitters, such as excitatory glutamate (Zompa
and Dubuc, 1998b) and serotonin (DiPrisco et al., 1994), inhibitory glycine (Wannier et al.,
1995), and such modulatory peptides as neuropeptide Y, cholecystokinin (Parker, 2000), and
substance P (Brocard et al., 2005).

**The Mesencephalon.** In addition to a retinal projection, the optic lobe receives input from
the octavalateral and trigeminal systems of the rhombencephalon, as well as the diencephalon
and telencephalon (El Manira et al., 1997; Pombal et al., 1997; Zompa and Dubuc, 1998a;
Menard et al., 2007; de Arriba Mdel and Pombal, 2007). Electrical stimulation of different
parts of the optic lobe triggers eye movements and body turning, and locomotion “akin to
struggling behavior” (Saitoh et al., 2007). If the labyrinth is destroyed, the swimming lamprey
rolls toward the affected side; however, illumination of the contralateral eye restores their
postural balance (Deliagina and Pavlova, 2002). There is some evidence that this synergistic
control is exerted by way of the large reticulospinal neurons of the mesencephalon and
rhombencephalon (Ullén et al., 1997; Zompa and Dubuc, 1998; Deliagina and Fagerstedt,
2000). Stimulation of the ventral mesencephalon triggers either locomotion or changes in the
speed or direction of ongoing locomotion (Sirota et al., 2000). The reticulospinal neurons are active in freely swimming lampreys (Zelenin, 2005) and their discharge properties are modified by vestibular stimulation (Deliagina and Pavlova, 2000; Pflieger and Dubuc, 2000) as well as visual stimuli.

**THE RHOMBENCEPHALON.** The afferent fibers from cutaneous sensors (pressure, touch, temperature and pain) distributed over the head and face form the trigeminal and facial nerves (cranial nerves V and VII). The cell bodies of these afferents are located peripherally in the trigeminal and facial ganglia, and their axons terminate in the trigeminal and facial nuclei of the rhombencephalon (Lindström, 1949). Afferents from the inner ear and from the lateral line organs (nerve VIII) project to the lateral line nuclei, and the nuclei of the medullary octavolateralis system (Pflieger and Dubuc, 2000). Gustatory fibers from the mouth are believed to reach the medulla by way of the facial nerve (VII) and those from the body by way of the glossopharyngeal nerve (IX). The final motor neurons of the head and the oculomotor (III), trochlear (IV), and abducens (VI) nerves, which originate in eponymous brainstem nuclei, supply the eye muscles responsible for conjugate eye movements (Pombal et al., 1994). The motor nuclei of the trigeminal nerve have been implicated in the coordination of feeding and swallowing (Huard et al., 1999; Petropoulos et al., 1999). However, the spinal accessory nerve (XI) and the hypoglossal nerve (XII) are absent in lampreys, presumably because they lack a tongue and a hinged neck.

Organization of the Lamprey Spinal Cord. The spinal cord of lampreys resembles that of higher vertebrates but there are some differences (Ariëns Kappers et al., 1936; Nieuwenhuys, 1964; Rovainen, 1967, 1979; Rovainen et al., 1973). There are peripheral sensory ganglia along the spinal cord of lampreys; however, the dorsal roots composed of sensory fibers that enter the spinal cord, and the ventral roots composed of motor fibers that leave it are not present within each spinal cord segment. Rather, much as in amphioxus, the two alternate in adjacent segments (Fig. 2-28A). There is a central gray with a dorsal horn containing small interneurons, and a more pronounced ventral horn with large motor neurons (Fig. 2-28C). The dendrites of motor neurons arborize extensively throughout much of the white matter composed of nerve fibers (Wallén et al., 1985; Fig. 2-28B). However, in contrast to higher vertebrates, none of these fibers are myelinated. Speed of nerve conduction is achieved instead by having cells with large caliber axons. These include the axons of Mauthner, Müller, and other cells located in the mesencephalon and rhombencephalon (Fig. 2-28C). Some of these fibers convey signals from touch and pressure sensors (Martin and Wickelgren, 1971; Christenson et al., 1988). The motor neurons are the source of cholinergic efferents (Pombal et al., 2001) that innervate the myotomes of the trunk and the dorsal fin (Shupliakov et al., 1992; Mentel et al., 2006).

**MOTOR PATTERN GENERATORS.** Forward swimming is produced in the lamprey by passing retrograde waves of sequentially contracting ipsilateral and contralateral muscle segments from front to back (Wallén et al., 1984; Gans et al., 1997). Turning is achieved by bending of the body at a segmental boundary. Experiments with isolated lamprey spinal cord preparations have established that this organized undulatory swimming pattern is triggered by action potentials generated by spinal motor neurons (Wallén and Williams, 1984). The discharge configuration of these command signals, referred to as “fictive locomotion,” is coordinated
Chapter 2: From Protozoans to Agnathans

LAMPREY SPINAL CORD

A. HORIZONTAL
- Spinal ganglionic neurons and axons
- Giant dorsal neurons
- Motoneurons and axons

B. CROSS SECTION
- Dorsal root
- Ventral root
- Mauthner axons
- Central canal
- Spinal ganglionic neurons and axons
- GIANT AXONS IN CROSS SECTIONS

C. GIANT AXONS IN CROSS SECTIONS
- Distance (mm) from the front of the brain
- Axons of:
  - Mesencephalic neurons
  - Isthmal neurons
  - Bulbar neurons
  - Medullary Mauthner neurons
  - Vagal neurons
  - Intrinsic giant neurons

Fig. 2-28. A. Schematic diagram of a horizontal section of the lamprey spinal cord with the dorsal root (afferent) and ventral root (efferent), its intrinsic neurons and traversing axons. B. The dendritic arbor of a single motor neuron in a cross section. C. The distribution of large caliber, identified axons in coronal sections, from rostral (top) to caudal (bottom). (A. Modified, from Nieuwenhuys, 1964. B. Modified, from Wallén et al., 1985. C. Modified, from Rovainen et al., 1973.)
by “central pattern generators” that reciprocally contract muscles on one side of the body and relax them on the opposite side, with a phase lag that spreads from caudal to rostral along the body axis (Fagerstedt et al., 2000). The pattern generator circuits consist of (a) a pool of spinal motor neurons; (b) an aggregate of ipsilaterally-acting glutamatergic excitatory interneurons and contralaterally-acting glycinergic and GABAergic inhibitory interneurons; and (c) some serotonergic neurons (Buchanan 1996; Zhang and Grillner, 2000; Grillner et al., 2001). These intrasegmental and intersegmental spinal cord pattern generators, in turn, are controlled by a complex system of suprasegmental brain centers in the mesencephalon, diencephalon and telencephalon.

2.5.5. The Origins of the Vertebrate Neuroepithelium. As we noted earlier (Section 2.4.2), a small piece of a planarian regenerates a whole animal with a normal nervous system. This indicates that planarian neurons do not originate from a discrete neural germinal matrix but from widely distributed pluripotent precursor cells. In more advanced invertebrates, such as insects—whose “brain” consists of supraesophageal and subesophageal ganglia—neurons arise from scattered individual precursor cells that, delaminating from the ectoderm, move into the body interior and form regional clusters. These “neuroblasts” then proliferate regionally to give rise to the differentiated neuron population of the segmental ganglia (Younossi-Hartenstein et al., 1996). Neurogenesis is fundamentally different in vertebrate embryos, including humans, in which neural cells arise from proliferative cells of a distinctive germinal layer, the neuroepithelium (NEP). This important vertebrate developmental property evolved in chordates and agnathans.

THE VERTEBRATE NEUROEPITHELIUM. The NEP first becomes manifest in vertebrate embryos as a mid-dorsal sheet of columnar cells of the ectoderm—the superficially situated, open NEP—traditionally called the neural plate (Fig. 2-29A). The next stage, called neurulation, starts as the neural plate invaginates and forms the neural groove, with the result that the midline neural plate cells come to abut the ventral notochord. This is followed by the apposition and fusion of the lips of the neural groove dorsally, forming the neural tube, or closed NEP. The NEP enclosing the spinal canal caudally becomes the source of neural cells of the spinal cord; the NEP surrounding the ventricles rostrally becomes the source of the neural cells (neurons and neuroglia) of the brain. Before this fusion is completed, germinal cells leave the lateral margins of the neural plate and migrate ventrally. These cells form the neural crest along the trunk, and the preplacodes in the head region (Fig. 2-29B). These two structures are the source of various components of the PNS, such as the spinal and cranial sensory ganglia, and some other tissues of the trunk and the head. After the NEP has closed, its lumen becomes filled with a distinctive aqueous milieu, the cerebrospinal fluid. Henceforth, the germinal potency of proliferating NEP cells becomes restricted to produce region-specific CNS neurons and supporting neuroglia. There is evidence that the embryonic cerebrospinal fluid promotes neuron production (neurogenesis), and that the size of the ventricle is a major factor in sustaining neurogenesis (Altman and Bayer, 2008; Bayer and Altman, 2008; Lowery and Sive, 2009).

EVOLUTION OF THE NEUROEPITHELIUM. Neurulation has been studied in larval ascidians (Nicol and Meinertzhagen, 1988a, b; Langeland et al., 1998) and amphioxus (Romer, 1970; Lacalli, 2000; Meulemans and Bronner-Fraser, 2007). As in vertebrates, the tubular NEP of
larval amphioxus forms above the notochord. But unlike in vertebrates, the NEP consists merely of a layer of a few apposed single cells, and the lumen of the neural tube is little more than a narrow slit (Fig. 2-30). Presumably, the lumen contains very little cerebrospinal fluid and the neuron population generated by this primitive NEP is small.

The NEP is far more advanced in agnathans, the hagfish (Kupffer, 1900; Ota et al., 2007; Ota and Kuratani, 2008) and lampreys (Nakao and Ishizawa, 1984; Sugahara et al., 2011) than in amphioxus. As illustrated in a lamprey embryo (Fig. 2-31), the NEP at both telencephalic and diencephalic levels is composed of a pile of spindle-shaped cells that surround the widened ventricle. These densely packed spindle-shaped cells are surrounded at the diencephalic level by a mantle of less densely packed spherical cells. The spindle-shaped cells have been identified in mammals as proliferative neural precursor cells; the round cells as differentiating young neurons (Altman and Bayer, 2002). The latter leave the NEP to form the different neural components of the maturing brain. The cytology of the lamprey forebrain NEP at this stage is reminiscent of a human embryo at about the third week of gestation (Bayer and Altman, 2008).
The lamprey neural tube is reduced to single-cell thickness posteriorly where the spinal cord develops (Fig. 2-32).

**Ontogeny and Phylogeny of the Vertebrate Brain.** The comparative-anatomical approach of vertebrate origins that we have so far considered is a modern reformulation of the Aristotelean idea of *Scala naturae*: surviving lower forms bear witness to the stages through which higher forms have passed. A related modern approach is the investigation of the temporal order in which different brain structures emerge and mature during embryonic development. This approach assumes that there is qualified validity to Haeckel’s (1894) hypothesis that “ontogeny recapitulates phylogeny,” even though it is not a “biogenetic law,” as he asserted. There are close parallels in several CNS areas in both rat and man between the earlier development (neurogenesis, neuronal migration, neuronal differentiation) and maturation (synaptogenesis, myelogenesis) of phylogenetically older structures, and the later development of phylogenetically younger structures (Altman and Bayer, 1996, 2001, 2002; Bayer and Altman, 2002-2008). For instance, neurogenesis begins and ends earlier in the ancient spinal cord and brainstem than in the more recent six-layered neocortex. Similarly, the myelination of the fiber tracts in spinal cord and brain stem precedes the myelination of neocortical fiber tracts, and within the latter, the phylogenetically older projection areas myelinate before the newer association areas (Flechsig, 1876; Vogt, 1919; Altman and Bayer, 2001). Modern research suggests that the proposition that ontogeny recapitulates phylogeny
Chapter 2: From Protozoans to Agnathans

**EMBRYONIC LAMPREY FOREBRAIN**

A. Telencephalon

B. Diencephalon

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**EMBRYONIC LAMPREY SPINAL CORD**

A. Coronal

B. Horizontal

C. Coronal Electron Micrograph

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**Fig. 2-31.** Photomicrographs of cross sections of the embryonic lamprey forebrain at the telencephalic (A) and diencephalic (B) levels. (Modified, from Sugahara et al., 2011.)

**Fig. 2-32.** The embryonic lamprey spinal cord in coronal (A) and horizontal (B) sections. The NEP and skin ectoderm at higher magnification (C). (Modified, from Nakao and Ishizawa, 1984.)
holds in those instances where conserved ancient genes and transcription factors contribute
to the development of novel organic mechanisms and functions. It does not apply where the
formation of new structures and mechanisms depend on drastically modified or altogether
novel genes. The conservation of some developing central and peripheral nervous system
structures is illustrated in Fig. 2-33 in a comparison the head of a hagfish embryo and a human
embryo.

**THE NEUROEPITHELIUM AS THE FOURTH GERM LAYER OF VERTEBRATES.** Biologists have
traditionally distinguished three germ layers in the early vertebrate embryo: ectoderm, endoderm, and mesoderm (Balinsky, 1965; Carlson, 1981). The germinal cells of the ectoderm,
which form the outer layer of the early embryo, give rise to the epidermis of the skin, the eye,
the sensory ganglia, the spinal cord, and the brain. The endodermal cells, which form the inner
embryonic layer, produce most of the internal organs, including the alimentary canal, the glands
of the digestive tube, the liver and pancreas, and the urinary bladder. The mesodermal cells
are precursors of the skeletal muscles, cartilage, connective tissue, and bones of the body, and
of the heart and the vascular system. Broadly conceived, the ectoderm is the source of organs
that maintain contact with the external world; the endoderm produces the internal organs;
and the mesoderm the body’s motor apparatus. As we saw earlier, the formation of these germ layers, and the tissues and organs they give rise to, have an evolutionary history. The body of sessile sponges is composed of a single layer of primitive cells (Fig. 2-11); the radial body of cnidarians has two layers, the ectoderm and endoderm (Fig. 2-12); the bilaterian body is usually described as having three layers, with the mesoderm situated between ectoderm and endoderm. While that holds for invertebrates, we propose in line with Romer’s (1970, p. 121) distinction between skin ectoderm and neural ectoderm, that the vertebrate body is derived from four germ layers (Fig. 2-34). While it can be argued that the neuroepithelium is part of the ectoderm, it is a distinct germinal matrix from the outset of embryonic development (see below). Indeed, the NEP ceases to be an ectal (outer) layer when it fuses and, supported by its distinctive fluid milieu, becomes dedicated to generate a unique component of the vertebrate body, the neurons and neuroglia of the brain and spinal cord.

2.5.6. The Origins of the Vertebrate Head and the Paleocephalon. Before the neural plate fuses, pluripotent cells leave its lateral edges to form the neural crest and the cranial placodes (Fig. 2-29). The neural crest consists of streams of cells migrating into the interior of the body, giving rise to components of the peripheral nervous system and some associated tissues (His, 1868; Hörstadius, 1950; Le Douarin and Kalcheim, 2009). These include neurons of the spinal ganglia and the autonomic nervous system, and such non-neuronal elements as the Schwann cells that myelinate peripheral nerves, and pigment cells. The placodes, which are limited to the head and the pharyngeal region, generate the cranial ganglia neurons and contribute to the production of various non-neural head structures, such as the lens, nose, ears, palate, and some others (Knouff, 1935; Jacobson, 1963; Noden, 1993). There is currently no cytological evidence for the existence of neural crest cells or placodes in amphioxus larvae, although a few genes that are expressed in vertebrate crest cells have been identified in them.

**Fig. 2-34.** Cell layers of the developing body of sponges (**A**), cnidarians (**B**), primitive bilaterians (**C**), and vertebrates (**D**).
(Northcutt and Gans, 1983; Lacalli, 2001, 2004, 2008; Schlosser, 2005; Shimeld and Holland, 2005; Benito-Guitérrez, 2006; Langeland et al., 1998; Holland, 2009). Nor have cranial placodes been identified in larval chordates, with the possible exception of the hypophyseal placode (Schlosser, 2005). However, neural crest cells, and perhaps some placodes, are present in hagfish and lamprey embryos (McCauley and Bronner-Fraser, 2003; Ota et al., 2007; Ota and Kuratani, 2008; Sauka-Spengler and Bronner-Fraser, 2008). The neural crest cells in agnathans may generate the neurons of sensory ganglia, and the placodes may form some head structures.

**GENOMIC STUDIES.** Ongoing genetic research indicates that the morphogenetic patterning of the bilateral body of chordates and agnathans—the body’s anteroposterior polarization and the dorsoventral differentiation of the neural tube—is dependent on genes, transcription factors, and signaling molecules that are homologous with those found in more advanced vertebrate embryos (Wada et al., 1998; Kishi et al., 2000; Holland et al. 2000; Schlosser and Ahrens, 2004; Mazet et al., 2005; Benito-Gutiérrez, 2006; Shimeld and Holland, 2005; Meulemans and Bronner-Fraser, 2007; Lacalli, 2008; Putnam et al., 2008; Holland, 2009). This implies that these ancient chordate and agnathan genes have been conserved as organizers of somatic and neural development in higher vertebrates. NEP cell specification, beginning at the gastrulation stage, has been associated with the down-regulation of Bmp expression and the up-regulation of Wnt and Sox expression. As neural development proceeds, genomic expression becomes more compartmentalized. Fgf expression has been found both in chordates and primitive vertebrates in the anterior forebrain, perhaps controlling facets of olfactory system development. Otx and Pax expression is associated with forebrain development, Gbx and Irx expression with hindbrain development; and members of the Hox gene family with spinal cord development. This ongoing research suggests that the genomic mechanisms of early NEP development in vertebrates is a chordate and agnathan legacy.

**THE ORIGINS OF THE PALEOCEPHALON.** Comparative neuroanatomists have realized by the end of the nineteenth century that the CNS of all vertebrates, in spite of considerable differences in size and complexity, share a common design (**Bauplan**). The human CNS shares with fishes the following structures: the olfactory bulb with the rhinencephalon; the telencephalon with the pallium and basal ganglia; the diencephalon with the thalamus and hypothalamus; the mesencephalon, with the tectum and tegmentum; the rhombencephalon, with the cerebellum, medulla, and the cranial nerve nuclei; and the spinal cord. What differentiates the brain of mammals from the brain of lower vertebrates is the emergence, expansion and elaboration of a new component of the telencephalon, the laminated neocortex. Edinger (1911) named the phylogenetically older (lower) component of the CNS, the paleoencephalon, and the phylogenetically more recent (upper) component, the neocortex, the neencephalon. Some writers went on to suggest that the paleoencephalon is the neural substrate of instincts and the basic emotions that control the behavior of lower vertebrates, whereas the mammalian neencephalon is the substrate of volitional behavior, memory and intelligence. This theory was later modified by Papez (1937) and MacLean (1949, 1986), who proposed that the forebrain of mammals has an ancient, limbic component (variously referred to as the “emotional,” “visceral,” or “reptilian” brain), composed of the septum, amygdala, hippocampus, and a few other structures. These, by way of the hypothalamus and the tegmentum, control primitive
visceromotor and skeletomotor functions. Based on a different line of research, the brain’s emotional mechanisms has been linked to the “reticular activating system” in the core of the hindbrain (Moruzzi and Magoun, 1949; Lindsley, 1951).

In light of what we currently know about the organization of the agnathan CNS, we illustrate the aforementioned “paleocephalic” or “limbic” structures by embedding them in the brain of a primitive mammal, the newborn rat (Fig. 2-35). Some of these structures—olfactory bulb, rhinencephalon, epithalamus, hypothalamus, tegmentum, and core of the spinal cord—are well developed in hagfish and lampreys. Others are rudimentary, and the identification of still others are controversial and remain to be further investigated. All the structures referred to, of course, have been modified in higher vertebrates as they became integrated into their far more complex CNS.

**CONSTITUENTS OF THE AGNATHAN PALEOCEPHALON IN A MAMMALIAN BRAIN**

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<td>Nucleus accumbens</td>
<td>9</td>
<td>Hypothalamus</td>
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<td>2</td>
<td>Hippocampus</td>
<td>6</td>
<td>Amygdala</td>
<td>10</td>
<td>Optic lobe</td>
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<tr>
<td>3</td>
<td>Rhinencephalon</td>
<td>7</td>
<td>Optic tract</td>
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<td>4</td>
<td>Septum</td>
<td>8</td>
<td>Habenula</td>
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<td>Reticulo-spinal projections</td>
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**Fig. 2-35.** Identified and putative components of the lamprey brain, referred to as the paleocephalon, are shown embedded in a sagittal section of the brain of a developing mammal, the newborn rat. All the structures illustrated have undergone substantial modifications in mammals, hence their placement and size are illustrative.

**2.5.7. Our Chordate and Agnathan Legacies.** The assessment of our chordate legacy is problematic because the extant chordates, the sessile ascidians and the sedentary amphioxus, appear to be poor representatives of a more active ancestral line in which the chordate traits are likely to have evolved. Although amphioxus has a unified fish-like body with an axial notochord and a dorsally situated tubular CNS—which herald the evolution of important vertebrate-like traits—it lacks a head with paired eyes and a true brain, and has a very limited behavioral repertoire. The ancestral chordate line is perhaps better illustrated by the extinct *Haikouella* that had a head with lateral eyes and a brain. Being furnished with cranial sense organs and a brain would have enabled ancient chordates to scan their environment as they searched for nutrients in the open waters, and their streamlined body, strengthened by an axial notochord, would have enabled them to swim about with agility and at a great speed. We have a similar problem with the surviving parasitic hagfishes and lampreys. The fossil evidence indicates that they are not representative of the armored agnathan stock, which had the anatomical features of active
foragers and predators. But while hagfishes and lampreys are poor subjects for the assessment of the behavioral and mental advances achieved by agnathans, the reviewed anatomical, developmental and genetic studies clearly indicate that they made significant contributions to the evolution of our cranial sense organs and components of our brain. Elements of the agnathan visual, olfactory and auditory systems (the retina, olfactory bulb, and inner ear), and most of the cranial nerves that connect them with the CNS, are part of our agnathan legacy. So are core components of our CNS—spinal cord, hindbrain and midbrain, and some elements of our forebrain—what we collectively refer to as the paleocephalon. Reflecting the ancient origin of these paleocephalic structures, they tend to develop and mature earlier in the human CNS during the pre- and postnatal periods than do structures that have evolved in mammals. That is, it is upon this agnathan component of the CNS that the neencephalon is superimposed both in phylogeny and ontogeny. Unfortunately, because hagfish and lampreys are “regressed” representatives of agnathans, we cannot turn to them to study the contribution of jawless fishes to our behavioral and mental evolution. To do that we have to turn to extant cartilaginous and bony fishes. As we shall try to demonstrate in the next chapter, there is ample observational and experimental evidence that fishes with a well developed head, cranial sense organs and a paleocephalon display significant behavioral and mental advances relative to lower metazoans, and many of these advances remain part of our piscine legacy.