

Neural plasticity: a window into the complexity of the brain

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ABSTRACT

The ability of the human nervous system to process information, perform complicated simultaneous mental and physical tasks, and express feelings and emotions is peerless. Because of its complexity, the human brain is the seminal achievement of biological evolution on our planet. This paper focuses on one aspect of brain complexity, neural plasticity, the ability of the nervous system to alter its output in response to changing stimuli. Several examples of neuroplasticity at the molecular, cellular, systems and cognitive levels are presented, all of which have physiological and behavioral consequences. The examples presented provide a basis for the premise that neural complexity arose from the need to perform complex functions. These examples also lend support for the notion that complex adaptive functions are subdivided into separate neural pathways which are oftentimes anatomically distinct.

INTRODUCTION

“Men ought to know that from nothing else but the brain come joys, delights, laughter and sports, and sorrows, griefs, despondency, and lamentations. And by this, in an especial manner, we acquire wisdom and knowledge, and see and hear and know what are foul and what are fair, what are bad and what are good, what are sweet and what are unsavory. ... And by the same organ we become mad and delirious, and fears and terrors assail us. ...All these things we endure from the brain when it is not healthy. ...In these ways I am of the opinion that the brain exercises the greatest power in the man.”

Hippocrates, *On the Sacred Disease* (4th Century B.C.)

The human brain is extraordinary. Its ability to detect and process sensory information, execute complex motor tasks, express emotions and feelings, communicate with others and generate a state of consciousness is without equal. We see and hear clearly in real time; we perform intricate motor behaviors; we feel pain, get angry, express joy; we converse freely; we think. Complex activities such as these are performed constantly by the brain and provide the underpinning for our existence as sentient beings.

The total number of conscious and unconscious behaviors and actions generated by the brain has not been calculated but is likely to be astoundingly large. Even more impressive is that the brain is able to vary the performance of every behavior depending on changes in internal or external conditions. For example, the precise muscular movements underlying running depend on the substrate; running on sand utilizes a slightly different pattern of leg muscle activation than running on a flat track surface (Bartlett *et al.*, 2007). Food and drink ingestion, commonly known as eating and drinking, describe ~250,000 different mouth movements (van der Bilt *et al.*,

2006). The precise body position when seated in a chair is dependent on many variables including level of exhaustion and chair shape (Shenoy & Aruin, 2007). Variations in individual behaviors, known as behavioral plasticity, are not limited only to motor movements. Responses to individual sensory inputs as well as the expression of emotions and feelings are also quite variable. The exact nature of any specific motor, sensory or emotional response depends on a combination of environmental conditions and internal motivational and physiological circumstances.

Behavioral plasticity is possible only because of the ability of the nervous system to modify its output. Minute adjustments in motor behaviors, sensory reactions, and emotional responses are mediated by numerous brain mechanisms. The term “neuroplasticity” is often used to characterize the neural adaptations that enable the central nervous system (CNS) to generate variations in individual behaviors. Neuroplasticity occurs at all levels of the nervous system including molecular, cellular, systems and cognitive levels. This paper presents an example of neuroplasticity at each of these levels. The premise underlying each example is that neural plasticity arose in each case due to the need to perform complex functions. Evidence is also presented for the notion that complex adaptive functions are frequently subdivided into separate neural pathways.

MOLECULAR PLASTICITY: EVOLUTION OF VOLTAGE-GATED SODIUM CHANNELS IN EUKARYOTES

The vast diversity of life forms on our planet is thought to have evolved from one simple organism. This means that seemingly unrelated organisms such as blue-green algae, fireflies, and orangutans are distantly related and share a common ancestor. The process of gradual divergence by which more complex organisms arose from simpler ones was first described by Charles Darwin as “descent with modification” or evolution. Evolutionary change is driven by modifications at the genetic level. Random genetic alterations occasionally generate positive adaptations that, over time, lead to new, increasingly complex species (*e.g.*, Barton, 2008). An example of a molecular level change that impacted organismal complexity is the evolution of voltage-gated sodium channels in nerve cells.

Individual nerve cells have four functional regions: dendrites, which receive an input from other neurons; the cell body, which integrates all the dendritic inputs and provides other cellular functions; the axon, which connects the input and output regions of the cell; and the terminal, which sends an output signal to the next neuron (Kandel, Schwartz & Jessell, 2000). Nerve cell inputs and outputs are usually mediated by chemical neurotransmitters. To transfer the transmitter-mediated input signal from the dendrites to the terminals, a distance of 1 meter or more in some neurons, each nerve cell converts the neurotransmitter chemical message into an electrical signal which very rapidly travels down the nerve axon to the terminal (Kandel, Schwartz & Jessell, 2000). Once the electrical signal, known as an action potential, reaches the

terminal, it is converted back into a transmitter-mediated chemical message that is released from the cell, crosses the synaptic cleft and is detected by the downstream neuron.

The action potential of a nerve cell is generated by a rapid influx of sodium ions across the cell membrane followed closely in time by an efflux of potassium ions. Sodium and potassium each pass across the cell membrane through separate, proteinaceous pores or channels that selectively pass only one type of ion. The pores passing sodium and potassium each open and close in response to changes in voltage (Hille, 1989; 2001). When the neuron is at rest, these channels are closed. However, when the voltage changes across the cell membrane, the potassium and sodium channels open, albeit with different kinetics. Because they are activated by changes in membrane voltage, these ion-passing membrane pores are known as voltage-gated sodium and voltage-gated potassium channels, respectively (Hille, 1989; 2001).

Voltage-gated sodium channels have their origin in potassium channels from prokaryotes such as bacteria (*N.B.*, prokaryotes are organisms whose cells lack a nucleus). Bacteria typically have cellular requirements for potassium but not for sodium. Consistent with their needs, bacteria do not usually express sodium channels but do contain potassium-specific channels in their membranes to allow passage of potassium ions into and/or out of the cell (Milkman, 1994). Ranganathan (1994) suggested that the primordial prokaryotic ion channel was a potassium channel with a gate on the cytoplasmic or inside face of the channel that opens or closes it. Other researchers have suggested that other types of potassium channels with a cytoplasmic gate, such as mechanosensory or cyclic-nucleotide-gated channels, may be the ancestor of all voltage sensitive channels (Jan & Jan, 1994; Anderson & Greenberg, 2001). The general consensus,

however, is that the first channels in bacteria were simple, non-voltage-gated potassium channels with a cytoplasmic gate controlling ion flow through the channel (Hille, 1989).

Voltage-activated membrane channels first appeared in single celled eukaryotes such as protozoans (*N.B.*, eukaryotes are organisms whose cells possess nuclei and other membrane bound intracellular organelles). Many protozoans express separate voltage-gated potassium and voltage-gated calcium channels (**Figure 1**; Hille, 1989; Anderson & Greenberg, 2001). The latter are used to bring calcium into the cell and as the charge carrier for electrical signaling purposes. There is good evidence supporting the notion that voltage-gated calcium channels evolved in primitive, single-celled eukaryotes after the appearance of voltage-gated potassium channels and before voltage-gated sodium channels. The use of sodium as a charge carrier to change the membrane potential of a cell did not appear in evolution until the advent of multicellularity. Sodium channels were not common until the appearance of cnidarians (*N.B.*, animal phylum with two cellular layers, stinging cells, and radial symmetry, *e.g.*, hydras and jellyfish) and are not found in higher plants, protozoa and algae (Hille, 1989). Thus, voltage-gated sodium channels did not evolve until much later.

These conclusions are supported by molecular data. Voltage-gated potassium channels in both prokaryotes and eukaryotes consist of multiple, independent subunits that associate together using weak chemical bonds to create the ion channel. The predominant class of voltage-activated potassium channels has four quite similar protein subunits (**Figure 1**). Each subunit contains 6 transmembrane spanning segments (S1-S6) plus a loop, called the P- or pore region, between segments S5 and S6 that allows the channel to be selectively permeable only to potassium.

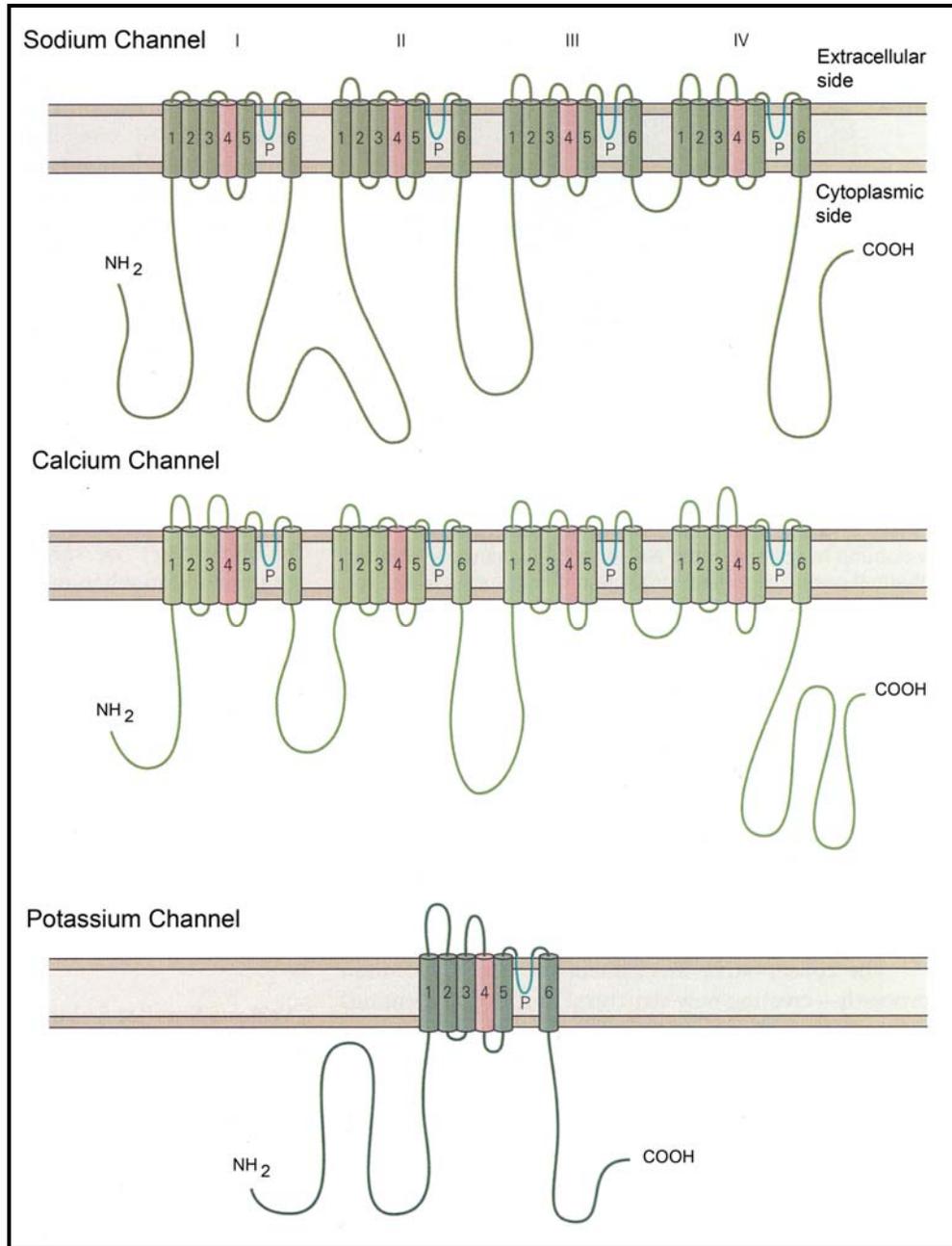


Figure 1: Schematic drawing of voltage-gated sodium, calcium and potassium channels. Sodium and calcium voltage-gated channels consist of 4 similar domains (I-IV). Each domain is composed a single polypeptide chain with 6 α -helical, transmembrane spanning regions (1-6), a pore region (P) between α -helices 5 and 6, and voltage sensor in α -helix 4 (red). The potassium channel is a single subunit containing a single repeat of the 6 α -helices. Four of these potassium subunits assemble together to form the potassium channel. NH₂ and COOH refer to the amino (beginning) and carboxyl (end) terminals of the channel proteins (modified slightly from Kandel, Schwartz & Jessell, 2000).

Studies have determined that the voltage sensor is localized to the S4 transmembrane region of each subunit (**Figure 1**; Hille, 1989; 2001).

In contrast, voltage-gated calcium and sodium channels consist of a single protein with 4 very similar or homologous domains strung together linearly (**Figure 1**). Each domain consists of the same general architecture as that for the voltage-gated potassium subunits, *i.e.*, 6 membrane-spanning regions (S1-S6), a P-region between S5 and S6 segments, and voltage sensitive S4 segment (Goldin, 2002).

On the basis of structural and amino acid sequence data, Strong and colleagues (1993) hypothesized that the single domain, voltage-gated potassium channel subunit is likely to be the ancestral form of all voltage-gated channels. They proposed that voltage-gated calcium channels, with 4 nearly identical domains, evolved from two rounds of gene duplication during the evolution of the early prokaryotes. The first duplication produced a two-domain channel similar to domains I/III and II/IV of a voltage-gated calcium channel. Each of these two-domain channels went through another round of gene duplication to generate the first 4 domain calcium channel.

Molecular analyses of the amino acid structure of the voltage-gated sodium channel indicate that each of its four domains is more similar to its homologous domain in the voltage-gated calcium channel than to each other. These data argue in favor of the notion that voltage-gated sodium channels evolved after the double gene duplication event that created the voltage-gated calcium channel (Hille, 1989; Strong *et al.*, 1993).

These data taken together provide strong evidence that the prokaryotic potassium channel was the ancestral form of all voltage-gated ion channels. Soon after the appearance of eukaryotes, the single domain potassium channel developed voltage sensitivity followed by two rounds of gene duplication to produce the four domain, voltage-gated calcium channel. A final duplication event and alteration of ion selectivity led to the appearance of the voltage-gated sodium channel found in all invertebrate and vertebrates including humans. The advent of voltage-gated sodium channels enabled long distance, rapid signaling between cells and clearly resulted in an increase in cellular neuroplasticity and organismal complexity. The evolution of four domain, voltage-gated sodium channels in eukaryotes from single domain, non-voltage-gated potassium channels in prokaryotes is an unambiguous example of increasing biological complexity driven by evolutionary pressures.

CELLULAR NEUROPLASTICITY: FUNCTIONAL PLASTICITY IN MATURE INSECT NEURONS

Structural and functional alterations at the molecular level, as illustrated by the origin of voltage-gated sodium channels discussed in the previous section, have been a primary mechanism used repeatedly throughout evolution to increase nervous system complexity. Molecular modifications, however, are not the only means to achieve the vast neural and behavioral plasticity seen in invertebrates and vertebrates, including mammals and humans. The ability of individual cells to alter their function provides another mechanism to generate

complexity and behavioral plasticity. This section details an unusual example of a set of identified neurons that undergo a dramatic transformation at a specific point in their lifetime and assume a completely different identity and function.

Following their birth from ectodermal cells, newly born nerve cells develop into mature adult neurons with a distinctive set of biochemical, anatomical and physiological characteristics (Kandel, Schwartz & Jessell, 2000). The combination of transmitter identity, dendritic arbor shape, axonal projection pattern, biochemical profile, and electrical properties are often sufficient to differentiate a neuron from its neighbors and identify it individually.

Research on identified neurons has stimulated significant progress in cellular neurobiology. The strength of this approach lies in the ability to repeatedly analyze the same, uniquely defined neuron from different individuals of the same species. The most notable example is the squid giant axon which was used to elucidate the basic cellular properties of neurons (*e.g.*, Hodgkin & Huxley, 1952). With few notable exceptions, invertebrate preparations have provided most of the known individually identifiable neurons because of the simplified nature of their CNS. Insects have long been a favorite model system for studies on identified cells because of their rapid generation time, ease of rearing, wide repertoire of complex behaviors, and significantly fewer neurons compared to vertebrates ($\sim 10^4$ vs. $\sim 10^{11}$ cells). One insect species, the tobacco hawkmoth *Manduca sexta*, is a particularly amenable animal model system because of the wealth of information already known about its physiology, anatomy, development, endocrinology and biochemistry of its individually identifiable neurons (*e.g.*, Dai *et al.*, 2007; Duve *et al.*, 2005).

The *Manduca* nervous system follows the general plan of other arthropods (Chapman, 1991). It consists of a cephalized brain connected to a series of segmentally iterated ganglia lying along the ventral side of the body, the latter of which are collectively known as the ventral nerve cord (**Figure 2**). Each ganglion in the *Manduca* ventral nerve cord generally contains ~1000 individual nerve cell bodies that supply neural information to a single body segment. Some cells in each ganglion are motoneurons or sensory neurons and others are interneurons which interact with motor and sensory cells. A very small percentage of ganglionic neurons are neurosecretory cells, specialized nerve cells with the electrical activity of neurons and which also act like gland cells to release neurohormonal signals into the blood (Maddrell & Nordmann, 1979). One set of identified neurosecretory cells in *Manduca* are the Lateral Neurosecretory Cells (LNCs; **Figure 2**). The LNCs are four pairs of cells in each abdominal ganglion that go through a remarkable makeover during metamorphosis from caterpillar to adult moth (Tublitz, 1993).

The LNCs in caterpillars are involved in regulating cardiac activity (Prier, Hwa & Tublitz, 1994). They release a set of hormones called Cardioacceleratory Peptides at specific times during larval life (Tublitz *et al.*, 1991). For example, the CAPs are released into the blood to control heart rate during the last larval stage when the caterpillar is preparing to enter metamorphosis (Tublitz *et al.*, 1992). During the metamorphic transition from caterpillar to adult moth, the LNCs stop making the CAPs and begin to produce a different neurotransmitter, bursicon, a peptide hormone involved in tanning the skin or cuticle of the adult moth (Loi & Tublitz, 1993; Tublitz & Loi 1993). The transmitter switch from CAPs to bursicon is triggered by the insect

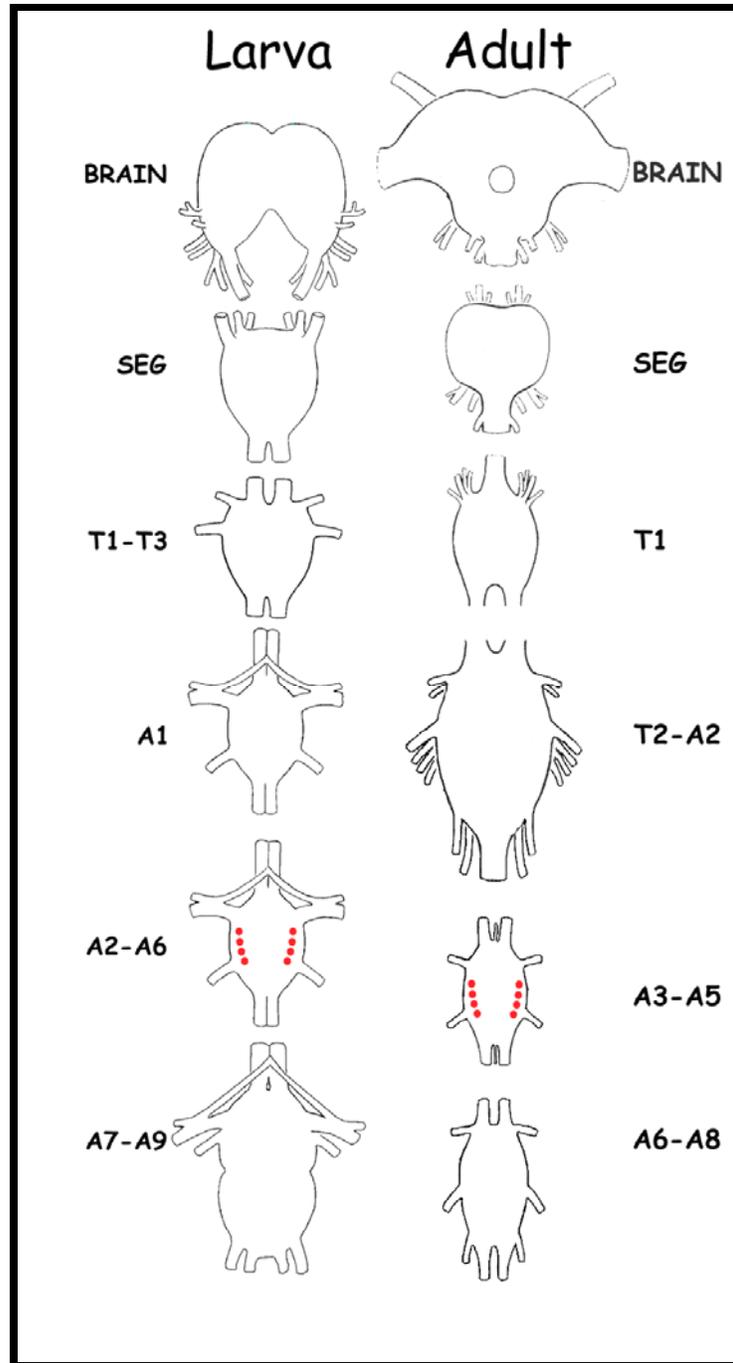


Figure 2: Schematic drawing of the *Manduca* nervous system in larvae and adults highlighting the Lateral Neurosecretory Cells (LNCs; in red). The *Manduca* nervous system consists of a cephalized brain plus a nerve cord with individual or fused ganglia. The nerve cord contains one subesophageal ganglion (SEG), 3 thoracic ganglia (T1-T3) and 8-9 abdominal ganglia (A1-A9) which may or may not be partially fused depending on the developmental stage.

steroid hormone 20-hydroxyecdysone, which mediates the down-regulation of CAP production and up-regulation of bursicon expression (Tublitz, 1993).

The LNCs alter other properties during metamorphosis in addition to changing its transmitter profile. The dendritic arbors of the LNCs expand their penetration of the abdominal ganglion by nearly three fold during the transformation from larva to adult (**Figure 3**; McGraw *et al.*, 1998). Physiological properties of the LNCs also change at this time. Two electrical measures, action potential threshold and input resistance, each decline significantly in the LNCs during metamorphosis (Tublitz & Prier, unpublished data). Like the transmitter switch, these other changes are triggered by 20-hydroxyecdysone. These data demonstrate that the LNCs alter their physiology, biochemistry and morphology in response to steroid hormone exposure.

The changes at the cellular level exhibited by the LNCs during metamorphosis underlie a major switch in function. As described above, the primary purpose of the LNCs in larvae is to regulate heart rate via a local release of the CAPs in the neighborhood of the heart (Tublitz *et al.*, 1993). In contrast, the same LNCs perform a very different function in adult moths, releasing the neurohormone bursicon into the blood to induce tanning of the cuticle. Thus, the LNCs act as local cardiomodulatory neurons in larvae yet serve a neurohormonal function in adults.

The plasticity exhibited by the LNCs is part of a major overhaul of the *Manduca* central nervous system during metamorphosis. Some neurons die, others arise *de novo*, and still others undergo a respecification of targets and/or function (*e.g.*, Levine, 1984; Dulcis & Levine, 2004). The LNCs fall into this latter category, changing their function from local modulator in

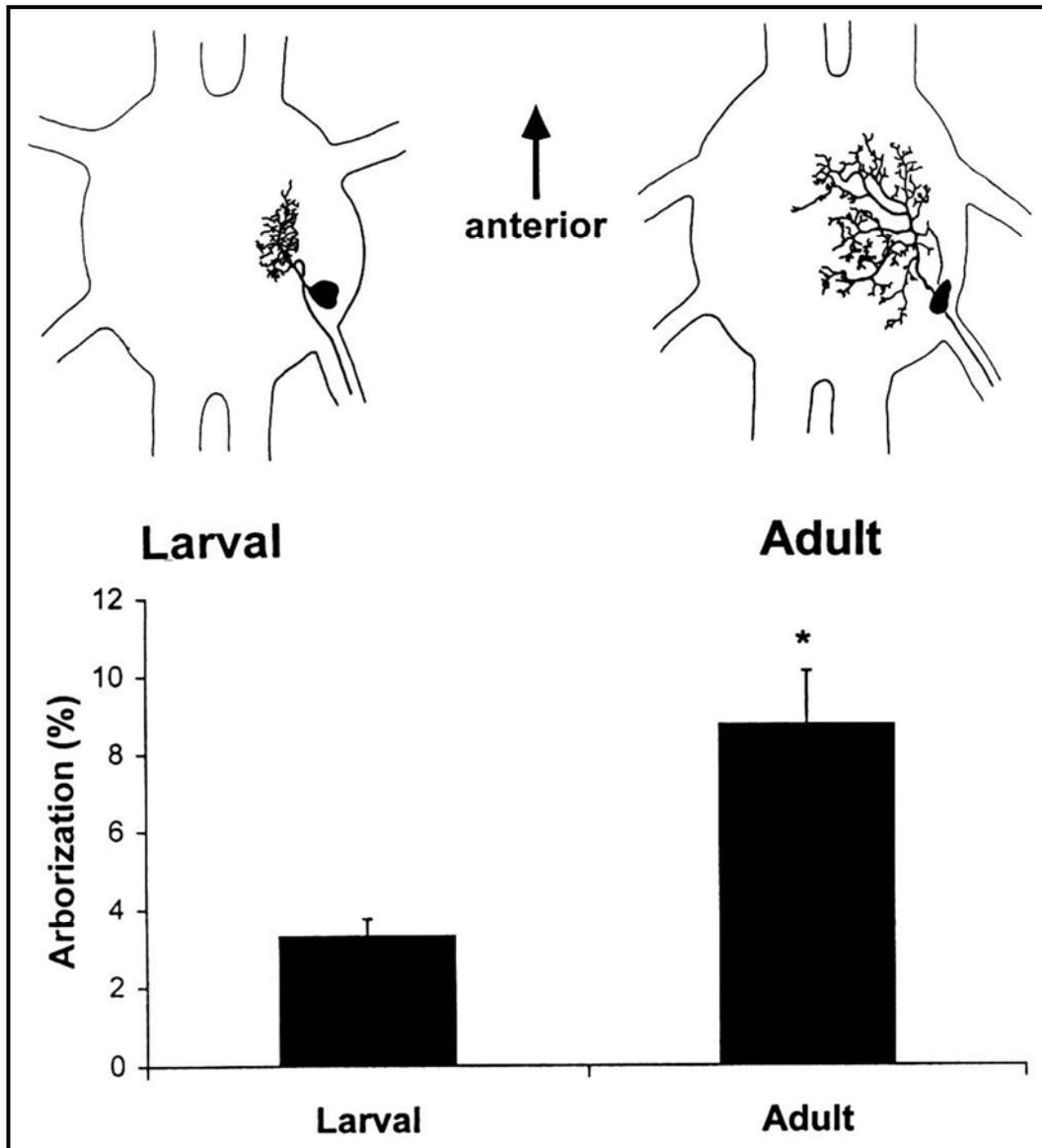


Figure 3. A comparison of the total extent of arborization in larval and adult lateral neurosecretory cells (LNCs) in the tobacco hawkmoth *Manduca sexta*. (A) Camera lucida drawing of the central processes of an individual LNC taken from a larva (left) and an adult (right). (B) Extent of arborization of larval and adult LNCs expressed as a percentage of the total ganglionic volume containing dendritic and axonal processes. Values are mean + S.E.M (N=5 for each data set). *The mean extent of arborization in the adult was significantly greater than that in the larva, using a one-tailed Mann-Whitney U-test, $P < 0.005$ (taken from McGraw *et al.*, 1998).

caterpillars to neurohormonal cells in adult moths. The functional alterations seen in many *Manduca* neurons during metamorphosis correlate well with the massive changes in anatomy and behavior during this period. As the animal goes from the larva to pupa and finally to the adult, it undergoes a massive conversion from a feeding and crawling animal to one that flies and mates. The complexity of the anatomical and behavioral transition in *Manduca* is mirrored by an equally complex neural reorganization (Truman & Riddiford, 2007).

The LNCs are arguably the best examples of neuronal plasticity at the cellular level in the animal kingdom, however they are not the only neurons to exhibit significant alterations in function. Functional changes by nerve cells have been described in many organisms from invertebrates to mammals (*e.g.*, Glantzman, 2006; Kampa *et al.*, 2007; Neves *et al.*, 2008; Nikitin, 2007). Sensory cortex cells that have lost their sensory inputs due to lesions or injuries replace those inputs with other sensory signals, usually of the same sensory modality (*e.g.*, Kral & Eggermont, 2007). Motor neurons innervate another muscle when their original target muscles are removed (*e.g.*, Purves & Hadley, 1985). It is not known what percentage of neurons alter their function but the number of studies detailing cellular plasticity has certainly been on the rise. It is likely that this type of cellular neuroplasticity underlies the variation in the production of complex behaviors in many organisms.

SYSTEMS NEUROPLASTICITY: BODY PATTERNING BEHAVIOR IN CUTTLEFISH

Changes at the molecular and cellular levels explain many neurally mediated functions including sensory processing, sensorimotor integration, learning and memory, and motor behaviors. Some cases of behavioral plasticity, however, depend on groups of neurons working together as an ensemble. One example of neuroplasticity at the systems level is the neuroregulation of body patterning behavior in cephalopods molluscs, a taxonomic group that includes octopus, squid, cuttlefish and nautilus (Hanlon & Messenger, 1988). The unique combination of properties found in this group of organisms make them excellent models for studies on the neural control of behavioral plasticity at the systems level.

Of the many fascinating behaviors in cephalopods, perhaps the most remarkable is their ability to rapidly produce highly detailed coloration patterns extending across their entire body. Although body patterning behavior is exhibited by all cephalopods except for the shelled nautiloids, cuttlefish are generally thought to display the largest and most complex repertoire of patterns (Hanlon, 1982; Hanlon & Messenger, 1988; Holmes, 1940; Packard & Hochberg, 1977). Most studies on body patterning behavior have been performed on one species, the European cuttlefish *Sepia officinalis*, which like other cephalopods, are capable of adjusting their body coloration to match numerous different substrates, including many that are visually complex (**Figure 4**). *Sepia* also display specific body patterns in response to the appearance of predators, prey and conspecifics (*e.g.*, during courtship) as well as to local environmental disturbances. Many body patterns are stunningly dynamic; for example *Sepia* display a set of



Figure 4. Photograph of a juvenile European cuttlefish *Sepia officinalis* producing a camouflage pattern while resting on top of pebbles.

several dark and light transverse bands across the body that move anteriorly at 5-10 Hz, commonly known as the 'passing cloud' display (Packard & Hochberg, 1977). The complexity of these displays reflects a CNS origin, and most are visually mediated because blinded cephalopods exhibit significantly fewer body patterns (Sanders & Young, 1974). Cephalopod body patterns are generated by a suite of chromatic elements, including iridiphores, leucophores and chromatophores, the latter of which are responsible for the amazing ability of cuttlefish and other cephalopods to generate intricate displays in less than a second, much faster than any other species in the animal kingdom (Messenger, 2001).

The ability of cuttlefish and other unshelled cephalopods to generate complex patterns so quickly is due to the unique structure of their chromatophore system. A cephalopod chromatophore is a true multicellular organ (**Figure 5**); at its core is the chromatophore cell, a pigment-containing cell with a highly elastic plasmalemma. Attached to and radiating from each chromatophore cell are 6-20 striated muscle cells, the chromatophore muscles, which emanate from the chromatophore cell like the spokes of a bicycle wheel (**Figure 5**; Cloney & Florey, 1968). Contraction or relaxation of the chromatophore muscles results in expansion or retraction, respectively, of the chromatophore cell. Because chromatophore muscles produce graded contractions, many intermediate expansion states of the chromatophore cell are possible. Individual chromatophore cells also exhibit dynamic responses, e.g., "flickering" behavior produced by rapid mini-contraction/relaxation cycles of the chromatophore muscles. Ultimate control of body patterning in unshelled cephalopods lies within the CNS since most if not all chromatophore muscles are innervated by motoneurons (Reed, 1995).

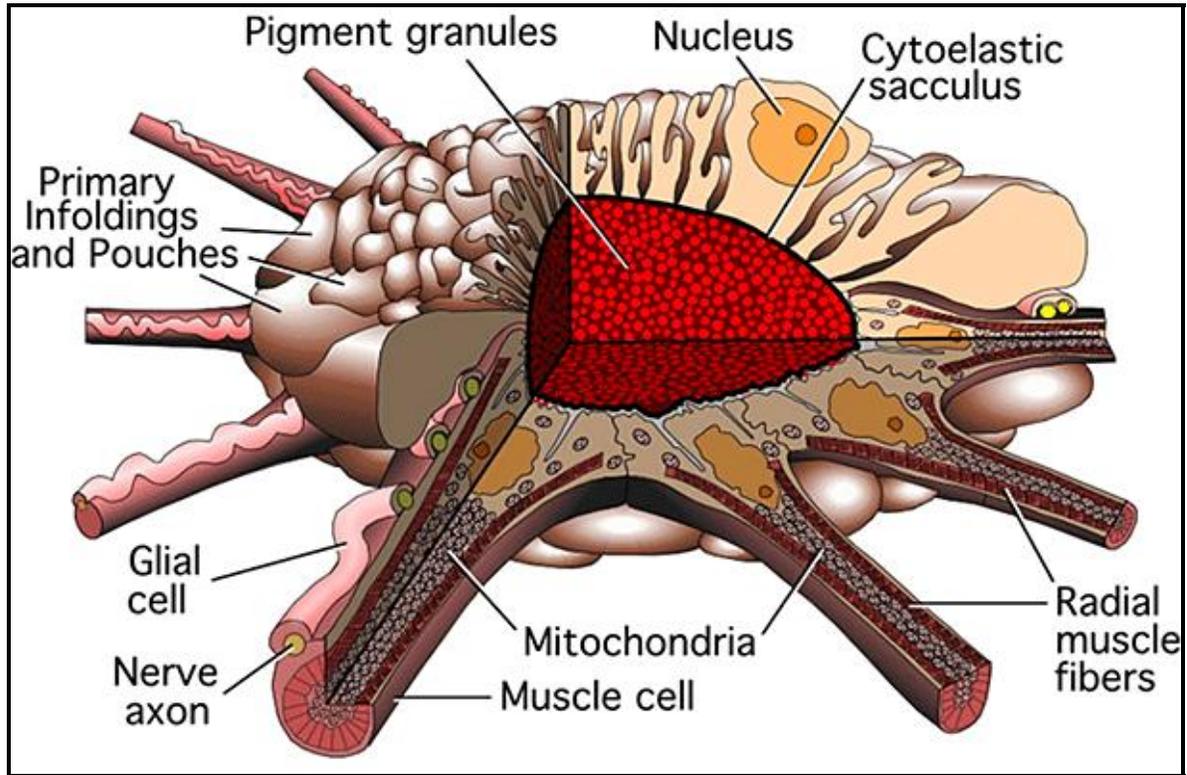


Figure 5. Diagram of the ultrastructure of a retracted cephalopod chromatophore organ. The sheath cells covering the chromatophore and the muscle fibers are not shown (slightly modified from Cloney & Florey, 1968).

Like the body patterning behavior it mediates, CNS control of chromatophore activity is elaborate. Invertebrate striated muscles, unlike their vertebrate counterparts, are innervated by multiple types of motoneurons. *Sepia* chromatophore muscles receive both direct excitatory and inhibitory motor input (Loi & Tublitz, 2000). There are two different types of excitatory motoneurons, one which causes a fast contraction of the chromatophore muscles and the other which induces a slower muscular excitation. These “fast” and “slow” motoneurons are distinguished by their neurotransmitters. The fast motoneurons use the amino acid glutamate and the slow motoneurons release multiple peptides all within the FMRFamide peptide family (**Figure 6**; Loi & Tublitz, 1997; 2000). The third input to the chromatophore muscles is inhibitory, mediated by the classic biogenic amine neurotransmitter serotonin (Messenger, 2001). It is the interaction between these three different neural inputs that underlies the complex responses of individual chromatophore organs (Messenger, 2001).

Mature adult *Sepia* each contain approximately 400,000 to 1 million chromatophore organs and approximately 200,000 chromatophore motoneurons (Hanlon & Messenger, 1988; Messenger, 2001). These figures suggest that each chromatophore motoneuron controls on average about 2-5 individual chromatophore organs and innervates 12-100 chromatophore muscles. Although there is some convergence of neural information from motoneuron to chromatophore organ, the number of individual motoneurons is more than sufficient to produce the complex body patterning seen in these amazing organisms.

More information about the control of body patterning behavior has been discerned by the location and distribution of the chromatophore motoneurons within the *Sepia* CNS. The vast

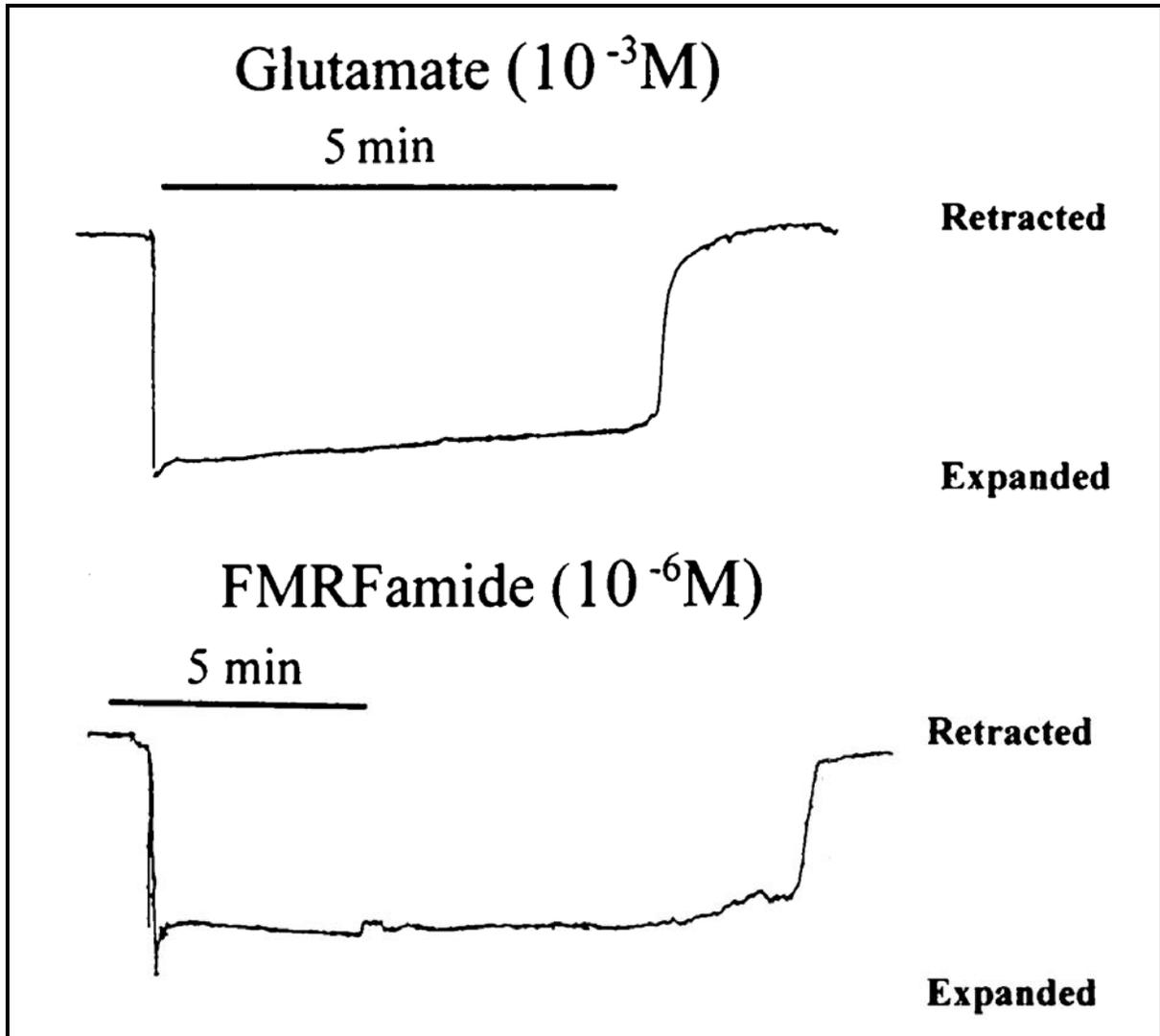


Figure 6. The effect of glutamate (top panel) and the neuropeptide FMRFamide (bottom panel) on an individual *in vitro* chromatophore. A piece of skin from the fin of the cuttlefish *Sepia officinalis* was removed, pinned to a dish and immersed in sea water. A photo-optical system (Loi & Tublitz, 2000) was used to measure the expansion of an individual chromatophore. The bar above each trace indicates the period of transmitter application. Note that glutamate caused immediate expansion of the chromatophore which lasted only for the duration of its application. In contrast, the initial effect of FMRFamide was delayed and the duration of its effect was prolonged for many minutes after FMRFamide removal.

majority of chromatophore motoneurons have been localized to the anterior and posterior chromatophore lobes of the *Sepia* brain (Gaston & Tublitz, 2004). In general, the chromatophore motoneurons innervating the tentacles are found in the anterior chromatophore lobe while the posterior chromatophore lobe houses the motoneurons controlling chromatophores on the rest of the body and the fin (Boycott, 1961; Gaston & Tublitz 2004 & 2006).

Recent brain localization studies on cuttlefish fin chromatophore motoneurons suggest the presence of a somatotopic map in the posterior chromatophore lobe (Gaston & Tublitz, 2006). Somatotopy, the spatial mapping of peripheral body regions onto specific CNS regions, is a well known neural concept in vertebrates including mammals and humans (Kandel, Schwartz & Jessell, 2000). Classic examples of somatotopic mapping include the human sensory and motor cortex and retinal projections to the mammalian lateral geniculate nucleus. Although the cuttlefish data are preliminary, chromatophore motoneuron somatotopy may be present in several brain regions (Gaston & Tublitz, 2006; Tublitz, Loi & Gaston, 2006). There are several reported cases of somatotopy in invertebrate optic lobes, however this report, if confirmed, would be the first example of central somatotopic mapping in an invertebrate brain. Given that the cephalopod CNS is the most complicated and largest among the invertebrates in terms of volume and cell number (Young, 1971), it is therefore not surprising that principle of somatotopic mapping would first arise in these unshelled organisms, which require stealth, camouflage and rapid movement to avoid predation.

The size and intricacy of the cephalopod CNS provide a level of complexity and computational power previously unknown in the invertebrates. Although much is still to be

understood about the neural control of body patterning behavior, it remains the quintessential example of systems level neuroplasticity in invertebrates.

COGNITIVE NEUROPLASTICITY: THE NEURAL BASIS OF LAUGHTER AND HUMOR IN HUMANS

This paper has shown to this point how plasticity at the molecular, cellular and systems levels contributes to neural complexity. Plasticity is also a key feature of the most complicated of human brain activities such as language, learning and memory, perception, thought, and planned action. Each of these cognitive activities involves larger, interconnected networks of neurons. This section focuses on the cognitive processes and brain pathways involved in an intriguing cognitive function, humor, with a specific emphasis on laughter.

Humor is a universal aspect of the human experience (Apte, 1985). It occurs in all cultures and nearly all individuals throughout the world (Lefcourt, 2001). The Oxford English Dictionary (www.oed.com) defines humor as “*that quality of action, speech or writing which excites amusement; the faculty of perceiving what is ludicrous or amusing; or of expressing it in speech, writing or other actions*”. It is evident that humor is a broad term encompassing anything people do or say that is perceived as amusing and which tends to make people laugh. Humor also includes the mental processes that go into creating and perceiving amusing stimuli and the physical responses involved in enjoying humor.

Like all mental and physical processes, humor is a complex series of actions taken by the brain. From detecting a humorous stimulus, to its processing and understanding, and finally to producing a response such as laughter, the brain is involved in every step. It is this final step, laughter and its underlying neural processes, that is the focus of this section.

A frequent behavioral response to a humorous stimulus is laughter. Charles Darwin noted in his 1872 book *The Expression of the Emotions in Man and Animals* that laughter is a mechanism to express a specific emotional reaction to others. Laughter is a distinctive, stereotyped pattern of vocalization that is easily recognizable and quite unmistakable (Provine & Yong, 1991). Although what is funny varies greatly, the sounds of laughter are indistinguishable across cultures. Developmentally, laughter is one of the first social vocalizations after crying emitted by human infants (McGhee, 1979). Infants first laugh in response to the behavior of others at about four months old. Cases of gelastic or laughter producing epilepsy in newborns indicate that the brain mechanisms for laughter are already present at birth (Sher & Brown, 1976). The innateness of laughter is best demonstrated in those born deaf and blind whom are reported to laugh appropriately without ever having perceived the laughter of others (Provine, 2000).

Laughter is a complex physiological response, characterized by a combination of loud oral noises, repetitive diaphragm contractions, open mouth and grimaces caused by facial muscle contractions and flushing of the skin (Ruch & Ekman, 2001). Laughter is also accompanied by a general physiological arousal including increased heart rate, production of tears, loss of strength

in the extremities and flailing body movements (Cacioppo *et al.*, 2000). All of these physiological activities are precisely controlled by the brain.

Studies of patients with brain lesions have identified two distinct functional pathways in the brain that produce smiling and laughter. One pathway produces involuntary, spontaneous and emotional laughter known as genuine laughter. The second pathway mediates forced laughter, which is voluntary and unemotional. The functional separation of these pathways has been demonstrated in several different types of studies.

Some stroke patients who are unable to voluntarily move their facial muscles (*i.e.*, volitional facial paresis), are nonetheless capable of genuine laughter. They are able smile and laugh normally in response to a humorous stimulus (Wild *et al.*, 2003). In contrast, some patients with subcortical brain lesions in the basal ganglia (**Figure 7**) are not able to exhibit spontaneous facial expressions of emotion when they feel amused but are able to smile on command (Wild *et al.*, 2003).

Additional evidence for separate pathways for voluntary and involuntary pathways comes from studies using positron emission tomography (PET), a brain imaging technique that measures changes in regional cerebral blood flow. Iwase and colleagues (2002) used PET to test responses of healthy individuals to humorous or non-humorous videos. Involuntary smiling specifically activated cortical areas such as the left anterior temporal cortex and bilateral occipitotemporal cortices involved in visual processing and integration (**Figure 7**). Limbic system areas involved in emotional reward were also activated. In contrast, voluntary, non-

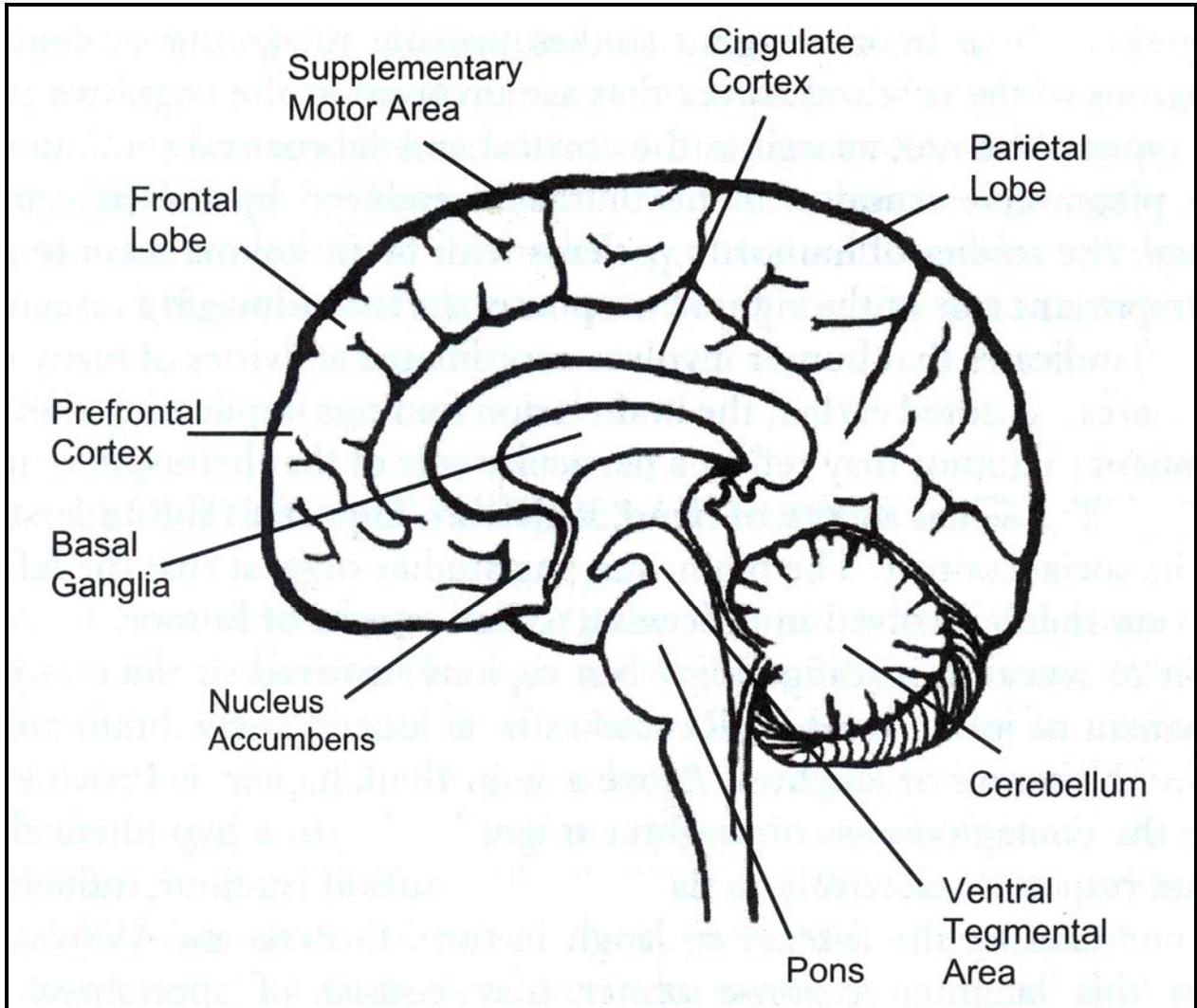


Figure 7. Brain regions involved in cognitive and motor regulation of laughter (modified from Martin, 2007).

emotional smiling was correlated with greater activity in those areas of the frontal cortex involved in voluntary facial movement such as primary and supplementary motor areas (**Figure 7**).

Data from electrical brain stimulation experiments lend further support to the working hypothesis that there are separate brain pathways for voluntary and involuntary laughter. One study described a 16-year-old female patient with epilepsy who, when her supplementary motor cortex was stimulated electrically, consistently laughed even though there was no detectable visual or auditory humorous stimulus (Fried *et al.*, 1998). Her laughter was accompanied by the subjective feelings usually associated with humor such as amusement and mirth. Even more interesting was that every time she laughed due to electrical stimulation, she ascribed her laughter to a specific stimulus in the room. For example, once she claimed to be laughing because of the humorous nature of a horse photo on the wall. It must be noted that this patient's bouts of epilepsy were never accompanied by gelastic laughter.

Based on data from stroke victims, PET and fMRI brain scans, and electrical brain stimulation, it is becoming increasingly clear that the brain has two pathways for laughter that are functionally and anatomically distinct. It is reasonable to postulate that each pathway arose independently to perform its distinctive function and was maintained because it provided a selective advantage to the individual. Although the precise mechanisms and details of the networks involved in humor detection and laughter production remain to be elucidated, the presence of two separate two neural circuits for laughter provides a concrete example of the development of neural complexity at the cognitive level.

CONCLUSIONS

A fundamental tenet of modern evolutionary thought is that complex structures and life forms arose from simpler ones. Biology is rife with microscopic and macroscopic examples of this principle: for example, the origin of energy producing cellular organelles (*i.e.*, chloroplasts and mitochondria) from free-living aerobic bacteria; the advent of multicellularity from single-celled organisms; the appearance of bird feathers from reptilian body scales; the development of the vertebrate camera eye from simple invertebrate photoreceptors; and of course, the evolution of modern day humans from our simian ancestors. The prevailing theme in each case is that these new designs arose from structures already in existence. The basic mechanism underlying this theme is that a structure originally intended to fulfill one role is slowly changed through gradual modification to become adaptive for a different role. It is by this mechanism that simple structures evolve into more complex ones. The principle of gradual adaptation is the bedrock of modern evolution.

Gradual adaptation is the force underlying evolutionary change not only in body structure but also in the form and function of the nervous system. From the simple nerve nets of cnidarians (*i.e.*, sponges, jellyfish and corals) to the complicated interactions of the central and peripheral nervous systems in insects, to the highly cephalized brain of cephalopods with functionally discrete regions, and finally to the remarkable human brain, nervous systems have developed from the simple to the highly complex over evolutionary time.

During the course of evolution, the nervous system of animals has increased in absolute size and in its number of neurons. New functions have also arisen, including the ability to alter one's own behavior in response to changing environmental or internal conditions. Known as behavioral plasticity, this function is an essential adaptation without which individual organisms would neither survive to maturity nor reproduce. All organisms from the simplest unicellular bacteria to humans are endowed with this capacity to a lesser or greater extent. Responsibility for generating variations in behavioral output lies exclusively with the nervous system and its remarkable plasticity.

This paper has presented several molecular, cellular, systems and cognitive examples of neuroplasticity underlying behavioral plasticity. Neuroplasticity arose in each case because of the necessity to perform a complex novel function. At the molecular level, voltage-gated sodium channels appeared in multicellular animals in order to coordinate responses among other cells in the organism. The need to re-specify cellular function in the nervous system of organisms with relatively few neurons is the likely reason behind the major biochemical, physiological and anatomical changes exhibited by the *Manduca* LNCs. The intricate, systems level regulation of cephalopod chromatophores arose because of the necessity to generate precise camouflage patterns and avoid prey. At the cognitive level, the two types of laughter, voluntary and involuntary, evolved to serve different functions by way of separate neural pathways. New functions in each instance resulted from the ability of the nervous system to adapt and adjust to changing environmental and internal conditions. In some cases new pathways were developed to

accommodate these changes. Neuroplasticity at the molecular, cellular, systems and cognitive levels is an essential component for behavioral flexibility and complexity in all animals.

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