Evolution and Function in Serotonergic Systems

Rhanor Gillette[1]

Department of Molecular & Integrative Physiology, 524 Burrill Hall, 407 S. Goodwin Avenue, University of Illinois at Urbana, Urbana, IL 61801, USA

Synopsis Serotonergic systems of invertebrate and vertebrate central nervous systems (CNS) are functionally similar in multiple characters. Serotonin (5-HT) neurons dispersed throughout the CNS of lophotrochozoan invertebrates (molluscs and leeches) are analogous to vertebrate 5-HT neurons concentrated in the raphe nuclei of mid- and hindbrain: they innervate specific central pattern generators and other circuits of the CNS, receive feedback from them, and support general behavioral arousal. In both groups 5-HT regulates excitatory gain of CNS circuitry and uses similarly diverse 5-HT receptors. Marked contrast, however, exists for roles of 5-HT in regulation of appetite. Where invertebrate 5-HT neurons promote an appetitive state, this role is supplanted in the vertebrates by a peptidergic network centered around orexins/hypocretins, to which the role of 5-HT in arousal is subordinate. In the vertebrates, 5-HT has appetite-suppressant properties. This is paralleled by differing complexities of mechanisms that bring about satiety. Lophotrozoans appear to rely on simple stretching of the gut, with no obvious feedback from true nutrient stores. In contrast, vertebrate appetite is regulated by hypothalamic sensitivity to hormonal signals reporting separately on the status of fat cells and digestive activity, and to blood glucose, in addition to gut stretch. The simple satiety mechanism of a mollusc can be used in value-based foraging decisions that integrate hunger state, taste, and experience (Gillette and others 2000). For vertebrates, where appetite and arousal are regulated by signals from long-lived nutrient stores, decisions can be based on resource need going far beyond simple gut content, enabling value estimation and risk assessment in the longer-term. Thus, connection of nutrient storage depots to CNS circuitry mediating appetite may supply critical substrate for evolving complexity in brain and behavior. This hypothesis may be tested in expanded comparative studies of 5-HT and peptidergic functions in appetite and arousal.

Introduction

Both parallels and contrasts can be drawn for the distribution, pharmacology, and physiology of the serotonergic neural networks in the protostomes and mammals. These suggest that considerable serotonergic function has been conserved, despite marked divergence in anatomy and behavior. Basic differences are, however, also evident.

Within a very short period of time serotonin was identified as a neurotransmitter in both molluscs and mammals (Whitaker-Azmitia 1999). Since then, serotonin has been increasingly pinpointed as a critical modulatory factor in behavior in both groups, and appreciable information is now available. For the invertebrates it has been largely the gastropod and lamellibranch molluscs, leeches, insects, and decapod crustaceans that have been intensively studied as accessible model systems for neural bases of behavior. Of those, lophotrochozoans—gastropods and leeches—have been studied in most reductionist depth for the detailed circuitry and interconnections of serotonergic neurons. Knowledge of the chemistry and functions of serotonin in neural networks can be used in a comparative approach to the diverse contemporary roles of 5-HT in the central nervous system (CNS) and behavior.

Table 1 aligns characteristics of 5-HT systems in lophotrochozoans (molluscs and leeches; Satterlie and Norekian 1996; Katz 1998; Straub and Benjamin 2001; Marinesco and others 2004; Kristan and others 2005) and mammals (Jacobs and Azmitia 1992; Marek and Aghajanian 1998). Marked similarities are the dense innervation of the entire CNS by the 5-HT network in both taxa, roles for broad neuromodulatory excitation in the CNS, and prominent roles in neural and behavioral arousal. However, first glance also shows that they seem to contrast in 3 major characteristics. First, 5-HT neuron somas in mammals are concentrated in the raphe nuclei of the midbrain, while those of molluscs, leeches, and arthropods are distributed throughout the nervous system among the various ganglia. Second, the 5-HT neurons of molluscs and leeches are coupled by both electrical and excitatory chemical synapses. The raphe 5-HT neurons are

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[1] E-mail: rhanor@uiuc.edu

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Table 1 Comparing 5-HT systems

Lophotrochozoans	Mammals
5-HT neurons are broadly distributed around the CNS	5-HT neurons are concentrated largely in the raphe nuclei of the brainstem
Central factors in appetite control	Adjunctory to a peptidergic network in appetite control
Broad neuromodulatory system densely innervating the whole CNS and the periphery	Broad neuromodulatory system densely innervating the whole CNS (a separate 5-HT network lies in the enteric system)
Induction of plateau potentials	Induction of plateau potentials
Heterosynaptic facilitation	Heterosynaptic facilitation
Enhanced postinhibitory rebound	Enhanced postinhibitory rebound
Synaptic facilitation	Synaptic facilitation
Major functions in arousal	Major functions in arousal
Coupling through chemical and electrical connections. Generally excitatory	Coupling through afferent connections among the nuclei and by local dendro-dendritic connections. Inhibitory
Embedded in CPG and other motor circuits, providing excitation and receiving input/feedback from the CPGs they innervate	Raphe nuclei innervate CPGs, other motor circuits, and integrating neural circuitry of the brain and are known to receive feedback from circuits they innervate

also coupled, but through inhibitory dendro-dendritic synapses within their nuclei and by inhibitory afferents between the nuclei. Last, a basic difference exists in the regulation of appetite. These contrasts contribute to the body of discussion below.

5-HT in neural circuits

5-HT receptors span a range of functional mechanisms mediating synaptic effects that endure variously over ranges of tens of milliseconds to minutes and longer. Multiple types include ionotropic non-specific cation channels, G protein-coupled receptors that either inhibit or stimulate adenylyl cyclase in various allelic and alternatively spliced forms, and G protein receptors coupled to phospholipase-C to produce second messengers IP_3 and DAG, with marked and various effects on metabotropic ion channel responses and biochemical pathways mediating neural plasticity. The qualities they bring to the computational abilities of 5-HT target neurons are similarly broad. Although less information is available for invertebrates, a similar heterogeneity of these receptors in vertebrates and molluscs indicates that the serotonergic systems of the 2 lines retain a similar toolkit after 600 million years of independent evolution (cf. Barbas and others 1998).

Serotonergic neurons are well conserved among gastropods as groups or individuals, although clusters vary across species in number of elements (cf. Sudlow and others 1998). Unlike mammals, where serotonergic cells concentrated in the raphe nuclei innervate the rest of the CNS, those of gastropods and leeches, so far as is known, are dispersed around the CNS where they are embedded as intrinsic elements in the different motor networks. These include the feeding, escape swim/turn, and locomotor networks, where they generate the intrinsic excitation to express stereotypic motor patterns and where they can function in mechanisms for switching among metastable states of motor coordination (Gillette and Jing 2001; Popescu and Frost 2002; Jing and Gillette 2000, 2003).

Independent labs have shown that the serotonergic neurons of these 3 networks are coupled through excitatory chemical and electrical connections, so that they appear to form a "compartmental serotonergic system" (Norekian and Satterlie 1996; Satterlie and Norekian 1996) or a "distributed arousal network" (Jing and Gillette 1995, 1999, 2000, 2003; Gillette and Jing 2001) that may underlie the general behavioral arousal of the hungry animal, which can transit rapidly between orienting and avoiding, locomotion, feeding, and escape. The distributed network in *Aplysia* was found to be persistently activated by sensitizing stimuli, thereby regulating the learned sensitization of avoidance behavior (Marinesco and others 2004). Similarly, serotonergic neurons of leech are electrically coupled, also receive synaptic feedback from the swim network they modulate, and are synaptically regulated by sensory afferents (Kristan and Nusbaum 1983; Gilchrist and Mesce 1997; review, Kristan and others 2005).

For premotor networks of locomotion, escape swimming, and turning in *Tritonia* and *Pleurobranchaea*, a small bilateral population of multifunctional neurons provides the motive force that permits those networks to self-organize. The firing frequency and laterality of activity in these cells determine the animal's decision to

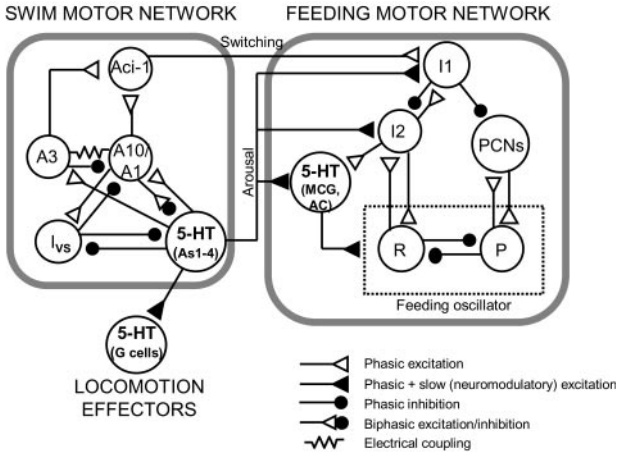

Fig. 1 Serotonergic neurons as elements of motor networks and their inter-connectivity. A summary diagram of the motor networks for escape swimming, feeding behavior, and locomotion in *Pleurobranchaea* shows how serotonergic neurons are embedded in the motor networks as intrinsic elements. The As1–4 cells are multifunctional, with roles in motor control and general arousal. They provide excitation to the escape swim network for swim pattern generation, acting as elements of an orienting/avoidance turn network (data not shown; Jing and Gillette 2003) and driving serotonergic locomotion neurons of the pedal ganglion. They also excite serotonergic and other cells of the feeding motor network (MCG and its coupled neighbors). Modified from Jing and Gillette (2000).

locomote, turn, or escape swim (Popescu and Frost 2002; Jing and Gillette 2003). In the feeding motor network a coupled population in the cerebral ganglion lobes, including the giant "metacerebral neurons" identifiable across opisthobranchs and pulmonates (Pentreath and others 1982), provides slow excitation to the network (Gillette and Davis 1977; Kupfermann and Weiss 1981; Straub and Benjamin 2001). Figure 1 illustrates the position of 3 sets of identified clusters of serotonergic neurons in motor networks and how they are connected through direct serotonergic and nonserotonergic synaptic paths. These neurons promote excitation in their respective networks through fast phasic EPSPs, slow modulatory EPSPs, and electrical coupling. In leech similar neurons innervate the neural network for swimming in each segmental ganglion (reviewed in Kristan and others 2005).

The 5-HT neuron somas often lie near other neurons of networks that they modulate. The excitatory roles of these invertebrate 5-HT neurons, mediated both ionotropically and metabotropically, are played out in a distinct context where they receive synaptic feedback from the pattern generating network that they drive, as well as external inputs from both other 5-HT neurons and pathways carrying arousing sensory stimuli (Gillette and Davis 1977; Katz and Frost 1995; Satterlie and Norekian 1996; Jing and Gillette 2000; Straub and Benjamin 2001; Marinesco and others 2004; Kristan and others 2005). Thus, their activity accompanies motor activity and frequently precedes motor onset. When motor activity is rhythmic, 5-HT neuron spiking is often phase-locked to the output. The positive feedback relationships between 5-HT neurons and the central pattern generators (CPGs) they innervate may embody a control principle where the gain of motor network excitation would follow a sigmoid function regulated by activity in either of the reciprocal paths.

A chief function of 5-HT in both vertebrates and invertebrates is to facilitate motor output. Similar roles for 5-HT in potentiating actions of both reflex activity and CPGs are well established in molluscs, leech, and vertebrates. In mammals, 5-HT produces a long-lasting facilitation of spinal reflexes dependent on neuromodulatory actions (Shay and others 2005), analogous to effects on reflexes well-studied in *Aplysia* (Mackey and others 1989) and possibly involving 5-HT-induced synaptic facilitation (Marek and Aghajanian 1998). Spinal pattern generator circuits for locomotion in lamprey and mammal depend on descending serotonergic inputs from raphe nuclei to enhance their excitation state for motor expression (Schmidt and Jordan 2000; Grillner 2003), similar to the actions of those serotonergic neurons embedded in the CPGs for locomotion, turning, and feeding in lophotrochozoans.

In contrast to the broad dispersal of the 5-HT neuron somas across the nervous systems of lophotrozoans, vertebrate 5-HT neuron cell bodies are concentrated in discrete nuclei of the midbrain, called the raphe nuclei (Fig. 2). While first look suggests marked dissimilarity, the difference is functionally blurred upon examination of the neurophysiology and anatomy; the scattered invertebrate serotonin neurons and small clusters appear quite analogous to the raphe nuclei in their specificity of target innervation and feedback from their targets. The different raphe nuclei innervate different areas of the CNS; moreover, the raphe nuclei themselves are differentiated into distinct domains with specific targets and inputs (Peyron and others 1997). As in the invertebrates, serotonergic cells of the raphe are heterogeneous in firing rates, patterns, and basic electrophysiological characters (Kocsis and others 2006).

For the vertebrate 5-HT system there is reciprocal connectivity between raphe nuclei and the networks they innervate (Peyron and others 1997; Jacobs and Fornal 1999), similar to connectivity observed in the invertebrates where 5-HT neuron neuromodulatory

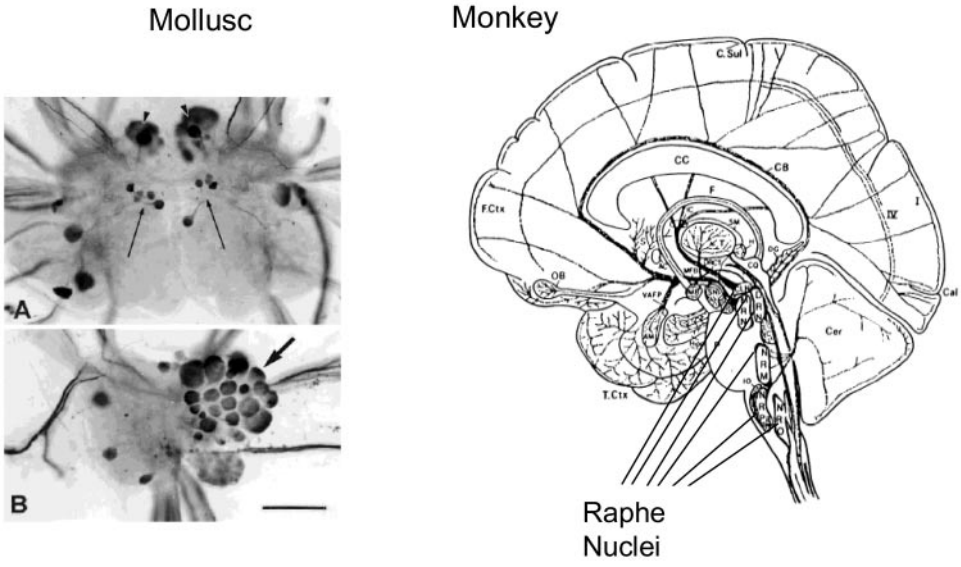

Fig. 2 Localizations of serotonergic neurons in CNS of lophotrochozoan (*Pleurobranchaea californica)* and mammal. *Left:* 5-HT neurons of the mollusc, labeled immunocytochemically, are dispersed as clusters and individuals among cerebropleural and pedal ganglia. Modified from Sudlow and others (1998). *Right:* 5-HT neurons in the monkey are concentrated in the several discrete raphe nuclei of the midbrain. Modified from Jacobs and Azmitia (1992).

output is governed both by activity in the network it innervates and by external afferents. For instance, a population of dorsal and median raphe neurons discharge in time-locked fashion to the theta rhythm of the hippocampus (Kocsis and others 2006). Neurons of these nuclei also innervate the hippocampus and may be the same individuals receiving feedback. Neurons of the *raphe nuclei obscurus* and *raphe pallidus* project to brainstem motor nuclei and spinal motorneurons, where they may function as gain-setters of motor activity; increases in putative serotonergic neuron activity were observed during treadmill-driven locomotion, active feeding, and increased ventilatory response to hypercapnia (Veasey and others 1995). Positive feedback paths can account for increases in dorsal raphe neuron activity caused by stimulation and overexpression of 5-HT_4 receptors in medial prefrontal cortex (Lucas and others 2005).

Still, a contrast remains, since the coupling between 5-HT neurons within and between the raphe nuclei is through widespread inhibitory connections, instead of the excitatory interconnections characteristic of the lophotrochozoans. This situation suggests existence of lateral inhibition, which conceivably could sharpen configurations of activity in the raphe population when specific neurons are recruited into CPG and other motor circuits. What might be the advantage of the connectivity for the raphe neurons over the invertebrate organization of the 5-HT system? It may be related to the condition, elaborated below, that the 5-HT neurons are not the mainstay of the arousal system in vertebrates, but rather they are subsidiary to a peptidergic network that is the core controller of both appetite and arousal. In this case, positive feedback and feed-forward relations within and among 5-HT clusters, as seen in lophotrozoans, could result in marked loss of precision of control by the peptidergic network.

5-HT—a promotor of lophotrochozoan appetitive state

Aside from the many similarities in the 5-HT network in lophotrochozoans and vertebrates, there are distinctive and basic differences in serotonergic regulation of appetite and overall arousal functions. A comparative perspective suggests that those differences could have evolved in parallel with differing strategies for handling of nutrient stores.

Nutrient handling and regulation of appetitive state in sea slugs, snails, and leech are markedly simpler than in mammals. These animals may grow in size throughout their lives, which rarely last more than a single reproductive season. Their foraging behaviors are consistent with strategies for eating opportunistically and investing nutrient directly in growth and production of gametes. Adult animals tend to lay large numbers of eggs and thereby lose appreciable mass. Satiation in the gastropods and leeches arises primarily from bulk stretch of the gut (Susswein and Kupferman 1975; Croll and others 1987; Elliot and Benjamin 1989; Groom and others 1993; Horn and

others 2001). Those inputs reconfigure the feeding motor network for inhibition of feeding command neurons, biasing the pattern generation in the retraction phase of the retraction/protraction feeding cycle (Davis and others 1983; London and Gillette 1984, 1986; Elliot and Benjamin 1989), and thereby raising sensory thresholds for feeding. *Pleurobranchaea* does not respond to glucose injections with changes in feeding thresholds, nor is *Pleurobranchaea*'s appetitive state robustly altered by transfer of hemolymph between hungry and satiated animals (unpublished data). The appetitive feeding behavior of these animals apparently is not affected by the states of their nutrient storage depots (for example, glycogen stores of hepatopancreas and mantle), as it is in mammals. Thus, gut stretch may well be the principle peripheral factor that translates nutritional status into appetitive state, as manifested in readiness-to-feed.

The reproductive system, not the CNS, may be the main accountant of nutrient stores outside of the gut and normally responsible for signals initiating release of reproductive activity. Aside from a full gut, learned food-avoidance, and simple ill health, only reproductive activity appears to affect feeding thresholds. At reproductive maturity, nutrient stores are invested in gametes, particularly in masses of eggs. The signal for reproductive readiness is not known, but egg-laying is triggered by release of a well-characterized egg-laying hormone into the blood from the CNS (Kupfermann 1967; Ram and others 1977). The hormone raises feeding thresholds and induces egg-laying behavior (Davis and others 1974; Stuart and Strumwasser 1980). Reproductive hormonal regulation of feeding threshold is presently the only known peptidergic regulation of feeding behavior in these molluscs.

The prominent actions of exogenous 5-HT, and injections of the precursor 5-hydroxytryptophan, in feeding behavior of sea slug and leech are to lower feeding thresholds (Palovcik and others 1982; Lent and others 1989), indicative of the actions of serotonergic neurons of the feeding motor networks that stimulate the excitability and reactivity of those networks to appetitive sensory inputs. Thus, 5-HT acts here similarly as it does in most instances, by directly augmenting excitation state of the neural networks involved. A significant role for 5-HT in the regulation of satiation state is suggested by observations that the serotonergic metacerebral neurons of the feeding network of *Aplysia* are less active in satiated animals (Kupfermann and Weiss 1982). Moreover, 5-HT content of the metacerebral neurons is markedly higher in active, hungry *Pleurobranchaea* than in satiated animals (manuscript in preparation), perhaps due to metabotropic effects of gut afferents or to enforced inactivity. Both observations are consistent with 5-HT being a major central factor in the regulation of appetite, through modulating excitation state of the feeding motor network. This simple scheme for regulating goal-directed behavior differs markedly from that of mammals.

A simple neural network model was previously proposed for decision-making in foraging/feeding behavior in *Pleurobranchaea* based on behavioral observations and partial knowledge of underlying neural circuitry (Gillette and others 2000). In it, satiation state regulated excitation state of the feeding motor network, a neural manifestation of appetite, which in turn tipped the balance between orienting and avoidance behavioral responses to appetitive stimuli. Here, we present a modified minimal model incorporating plausible actions of 5-HT and gut stretch in regulating arousal state of the feeding motor network. Corollary outputs from the feeding network, whose activity encodes appetite, regulate decision between orienting and avoiding appetitive responses (Fig. 3). 5-HT function in this simple model can be compared with another minimalist model for organization of mammalian feeding and arousal presented later.

5-HT as an appetite suppressant in vertebrates

Mammals share with lophotrochozoans common functions of 5-HT in motor arousal. However, augmenting 5-HT activity (for example, with uptake blockers) does not promote appetite, but rather has the effect of suppressing it. Appetite and major aspects of appetitive behavioral arousal in mammals are organized around peptidergic hormones and transmitters, instead of 5-HT. The satiety mechanisms in mammals go far beyond the simple gut stretch of lophotrochozoan model systems. Indeed, the mammalian brain keeps an account of its nutrient resources in terms of blood glucose, glycogen stores, fat stores, and digestive activity. Glucose-sensor neurons abound in hypothalamic areas regulating appetite (Oomura and Kita 1981; Yamanaka and others 2003). The hormones leptin, secreted from adipose cells proportionate to mass, and ghrelin and other peptides secreted from the stomach act at the hypothalamus on the peptidergic neuron circuits driving appetitive arousal and behavior (Willie and others 2001; Yamanaka and others 2003). Neurons in the hypothalamus also produce leptin and ghrelin, among other potent peptide neuromodulators, and exert effects on neurons producing orexin and NPY peptides that promote appetite and cortical arousal. Orexins are key peptides both in stimulation of appetitive behavior and of the arousal side of sleep and wakefulness. Receiving inputs from all nutrient

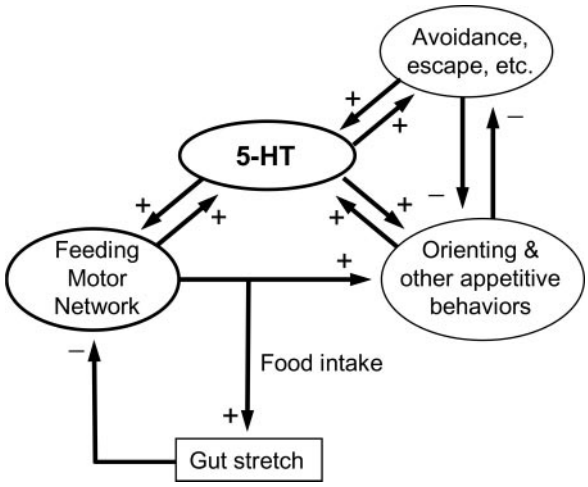

Fig. 3 Roles of 5-HT neurons in appetite and arousal in lophotrochozoans. The serotonergic system, abstracted here to a single entity, lies in positive feedback relationship to motor networks, including the feeding motor network. The model elaborates an earlier one for cost-benefit decision in behavior (Gillette and others 2000). It indicates a role of 5-HT in promoting appetitive state, as manifested in the excitation state of the feeding motor network. Similarly, 5-HT augments excitation state in most motor networks. Reciprocal inhibitory connections between networks ensure singularity of behavior expression and with specific excitation of appetitive behavior by the excitation state of the feeding network promotes appetitive behavior. Satiation, through inhibitory feedback from the gut stretch receptors, regulates appetite and feeding thresholds through the excitation state of the feeding motor network.

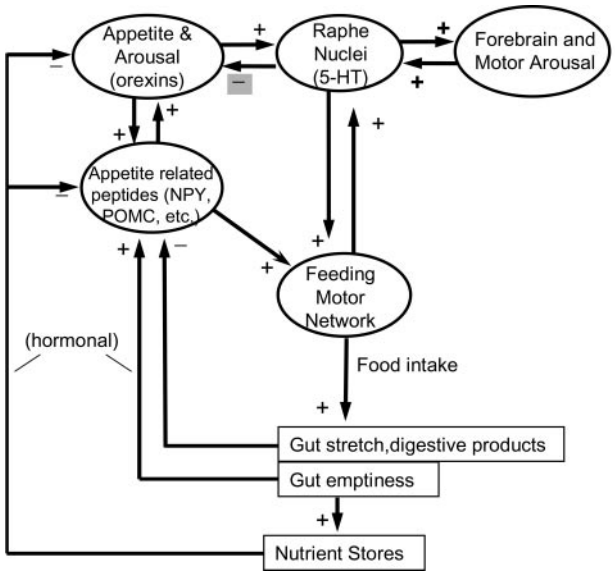

Fig. 4 A simplified model of the role of the 5-HT system in vertebrates. It emphasizes both the conservation of 5-HT function in promoting excitation state of motor and other neural circuitry and the subordinate role of 5-HT to the peptidergic network that is the mainstay of appetite and arousal in these animals. The orexin neurons at the core of the appetite/arousal network influence cortical arousal and motor activity through their connections to 5-HT neurons of the raphe nuclei. Negative feedback from the 5-HT neurons to the orexin neurons is a feature that may have significance for precision in control (see text). Regulation of appetite is through feedback pathways from nutrient stores (for example, leptin) and gut, and contrasts with the simple regulation of the feeding motor network by gut stretch in lophotrozoans.

stores, orexin neurons couple behavioral arousal to energy balance and appetite. 5-HT has important, but now adjunctive, roles in mammalian appetite.

Serotonin effects on appetite in mammals are markedly distinct, even opposite, from those of the lophotrochozoan model systems. 5-HT uptake blockers promote appetite suppression, apparently in part through inhibitory effects on orexin neurons. However, orexin neurons have strong excitatory projections to the serotonergic dorsal raphe and through them can affect arousal in cortex and amygdala (Willie and others 2001; Liu and others 2002). The serotonergic neurons of the dorsal raphe feed back to the orexin neurons, on which 5-HT has direct inhibitory effects (Muraki and others 2004). This inhibitory feedback pathway suggests that the serotonergic connections are acting as a negative governor of the orexin neurons at the same time as they promote arousal of the forebrain. Such a delicate tuning function for 5-HT in appetite and arousal in mammals is an interesting and marked contrast with its apparent direct participation as an excitatory neuromodulator of appetite in the lophotrochozoans.

While the picture is still somewhat incomplete, these observations indicate that the role of 5-HT in appetite and arousal in lophotrochozoans is superceded by a network of peptidergic neurons in mammals. Figure 4 presents an abbreviated model stressing the apparent different roles of 5-HT and peptides in regulating appetite and arousal in a vertebrate.

Evolution in the 5-HT network system and in the regulation of appetite

How did peptidergic paths take over an ancient function of 5-HT in the course of vertebrate evolution and relegate it to an adjunctive role? Or alternatively, how might 5-HT have taken over appetite regulating functions in the course of evolution of greatly simplified, model lophotrochozoan systems. These are fertile grounds for speculation, for which insights might come from broadening comparative research to other species with diverse nutrient storage strategies. Certainly, the robust peptidergic pathways of lophotrozoan nervous systems involved in neuromodulatory computations in

the feeding network and musculature (Sweedler and others 2002; Jimenez and others 2006) form potential evolutionary substrates for developing innovative regulation of appetite.

The regulation of feeding behavior by long-term nutrient stores would appear to have considerable potential for supporting evolution of complexity in brain and behavior. It is probably quite significant that the cozy connections of the vertebrate body's nutrient stores to the interwoven peptidergic pathways of appetitive behavior and arousal can confer a potential for making cost–benefit behavioral decisions on a basis of nutrient need going temporally far beyond simple gut content. Undeniably, social behaviors involving much more than copulation and cannibalism are not found in the lophotrochozoan model systems used in the laboratory. Conspecific aggression, defense, and cooperation are characters shown by other invertebrates, primarily arthropods, and by vertebrates, for all of whom accumulated nutrient stores (stores cached both bodily and externally) influence appetitive behavior (for example, Zinke and others 1999; but surprisingly little information exists on the role of 5-HT in appetitive behavior of arthropods.). Brooding of young and consequent evolution of affiliative behavior might be facilitated by suppression of hunger-induced cannibalism through nutrient depot feedback. The possibility that connection of nutrient storage depots to CNS circuitry mediating appetite and appetitive behavior can supply a critical substrate for the evolution of complexity in brain and behavior merits further exploration.

Conclusion

Relative to many arthropods and most mammals, the model lophotrochozoan systems of the laboratory are markedly simple animals in both body forms and behavior. 5-HT in snails and leeches plays major roles in regulating tone in motor pathways and is also a central factor in setting internal states of behavioral arousal and appetite. In contrast, vertebrates center states of arousal and appetite around a peptidergic hypothalamic network closely in touch with nutrient storage depots via humoral signals. The comparison immediately brings up the question of whether the lophotrochozoans represent a primitive state or are simplified from more complex ancestors? The question also remains open as to whether the CNS bookkeeping of nutrient stores in the vertebrates confers particular advantages for cost-benefit decision-making in the longer-term. If so, it could supply substrate for evolution of the marked complexity in behavior characteristic of the group. The answers may be best sought in further comparative studies of those factors regulating appetite and the roles of 5-HT in other species.

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