Evolving Concepts of Arousal: Insights from Simple Model Systems

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SYNOPSIS
Arousal states strongly influence behavioral decisions. In general, arousal promotes activity and enhances responsiveness to sensory stimuli. Earlier work has emphasized general, or non-specific, effects of arousal on multiple classes of behaviors. However, contemporary work indicates that arousal has quite specific effects on behavior. Here we review studies of arousal-related circuitry in molluscan model systems. Neural substrates for both general and specific effects of arousal have been identified. Based on the scope of their actions, we can distinguish two major classes of arousal elements: localized versus general. Actions of localized arousal elements are often limited to one class of behavior, and may thereby mediate specific effects of arousal. In contrast, general arousal elements may influence multiple classes of behaviors, and mediate both specific and nonspecific effects of arousal. One common way in which general arousal elements influence multiple behaviors is by acting on localized arousal elements of distinct networks. Often, effects on distinct networks have different time courses that may facilitate formation of specific behavioral sequences. This review highlights prominent roles of serotonergic systems in arousal that are conserved in gastropod molluscs despite extreme diversification of body forms, diet and ecological niches. The studies also indicate that the serotonergic elements can act as either localized or general arousal elements. We discuss the implications of these findings across animals.

KEYWORDS
arousal, molluscs, neural network, modulators, serotonin, feeding, defense, behavioral sequences

INTRODUCTION
Arousal phenomena are present in vertebrates and invertebrates alike, and are essential to animals’ survival and well-being. Previous reviews of arousal in a variety of contexts are available /30, 33,62,84,101,125,126,133,141,161,163,168/ (see also Ann NY Acad Sci, 2008, Vol. 1129, edited by Pfaff and Kieffer). We review arousal-related work in simple model systems, with a focus on circuitry findings in gastropod molluscan model systems. Work in both vertebrates and invertebrates underscores the fact that actions of modulatory systems are central in establishing the arousal state (e.g., /81,126,168/). However, as will become apparent later in this review, circuitry studies in molluscan model systems can provide essential experimental evidence that may distinguish between alternative neural models of arousal. Furthermore, studies of possible roles of arousal elements in multiple behavioral networks suggest the need to classify neuronal elements involved in arousal as localized versus general arousal elements. We believe that such a distinction may be useful in understanding common outstanding issues in both vertebrates and invertebrates. In classifying modulatory elements, we take into account their roles in multiple neural circuits. This classification scheme differs from the...
previous distinction of intrinsic versus extrinsic neuromodulators in that the latter /69/ focused primarily on modulators’ role in a single circuit. Thus, this scheme may offer a novel conceptual framework for understanding the functions of various neuromodulators.

In this review, arousal is used to refer to the internal factors that energize and potentiate behaviors. In particular, these factors are manifested as an internal state of the animal that may account for readiness to initiate behavior, as well as for the persistence of behavioral expression, in the presence of relative constant sensory inputs /40,41/. With heightened arousal, animals are more active, and more responsive to sensory stimuli. These features of arousal may facilitate goal seeking and interaction. In this sense, arousal organizes and optimizes behavioral expression. Thus, in addition to sensory inputs, the arousal state is a major determinant of behavioral decisions (e.g., /23,56/).

GENERAL VERSUS SPECIFIC AROUSAL?

From its early formulation, the arousal concept usually implied some general or nonspecific effects on multiple behaviors /1,40,41/. Modern studies of arousal began with work identifying the brain systems underlying arousal. Early work in vertebrates suggested that primitive brainstem systems (ascending reticular activating system) exerted powerful, monolithic controls over activation of the forebrain /109/ (Fig. 1A). Later work, however, suggested differentiation in anatomy, physiology and neurochemistry in the brainstem arousal system (see the last section). Based on these data and relevant behavioral findings, Robbins and Everitt /133/ discounted the general arousal theory and asserted that there could not be a unitary, general arousal function.

There is growing realization that arousal can be more specific, and may exist in multiple forms (Fig. 1B). In particular, different forms of arousal are often associated with particular physiological drives, including sex, fear, hunger motivation, thirst, pain, etc. (Fig. 2). These particular physiological drives propel generation of a variety of motivated behaviors (goal-directed behaviors) and exploratory/exploratory behaviors. Widely recognized motivated behaviors are ingestive, thermoregulatory, defensive, and reproductive behaviors /163/. Foraging/exploratory behaviors, such as locomotion and orienting behaviors, allow the animal to seek and interact with a goal object. Indeed, these forms of arousal may be released by different external or internal stimuli that often signal specific goal objects, such as food or a sexual partner. Along these lines, there are a number of experimental studies that identified neural mechanisms underlying these forms of arousal, such as sexual arousal and feeding arousal (e.g., /125,126,168/).

Some effects of arousal can be general or nonspecific. Pfaff and colleagues advocated the concept of general or generalized arousal /125-127/ without positing that a single centralized arousal system underlies all aspects of arousal (Fig. 1A), as was previously suggested /109/. Thus, there may be some arousal mechanisms that mediate a generalized arousal accounting for a portion of several or all forms of arousal (Fig. 2A). For instance, knocking out the gene for estrogen receptor-a reduced every measure of arousal in female mice, including mice’s behavioral responses to vestibular, tactile, olfactory and auditory sensory stimuli /28/.

Although multiple alternative mechanisms could account for this phenomenon (see Fig. 1C), Pfaff et al. proposed that the generalized arousal mechanisms may be mediated, at least in part, by the brainstem arousal systems that have broad projections to diverse regions of the CNS, including the cortex.

While CNS mechanisms underlying the generalized arousal proposed by Pfaff et al. are presently difficult to study experimentally, other related phenomena may be more amenable to experimental investigation. Specifically, pertinent to the general arousal concept, there is evidence that different forms of arousal may interact with each other, i.e., stimuli that elicit arousal may have broad effects on multiple classes of behaviors /56,126/. Most prominently, moderately noxious or threatening stimuli can promote both defensive behaviors (e.g., locomotion) and competing behaviors such as feeding /2,64/, grooming /138/, and sexual responses /6/. In principle, there may be at least two possible mechanisms that may mediate the nonspecific effects of noxious stimuli (Fig. 1C).
Fig. 1: Simplified neural models for various concepts of arousal. A. General arousal theory, in which arousal is proposed to be mediated by a single centralized ‘general arousal system’ (G arousal). B. An extreme version of the notion of multiple forms of arousal, in which it is believed that arousal for different classes of behaviors is mediated by distinct ‘arousal systems’ embedded in individual networks. C. Two alternative neural models that may account for the ability of alerting/noxious stimulus to promote two classes of behaviors. C1. Alerting/noxious stimulus has direct access to the dedicated ‘arousal systems’ for two motor networks, thereby promoting two classes of behaviors. C2. Alerting/noxious stimulus has access to a ‘general arousal system’ that in turn acts on the ‘arousal systems’ embedded in the two motor networks, thereby promoting two classes of behaviors.

Fig. 2: Conceptual models of arousal underlying behavior. A. Arousal is hypothesized to have two components in vertebrates /127/: a generalized arousal (A_gen) and particular forms of arousal for each class of behavior, e.g., sex (A_s), thirst (A_t), hunger motivation (A_h), and fear (A_f). Redrawn from /127/ with permission. B. A conceptual model of arousal mechanisms in gastropod molluscs based, in part, on the work reviewed in this paper. General arousal elements composed of serotonergic interneurons (A serotonin) may act in concert with localized arousal elements for locomotion (A locomotion), defense (A defense), feeding (A feeding) and cardiovascular (A cardiovascular) systems to promote behavioral responses of locomotion, withdrawal, feeding and heart beat. Note that locomotion may be expressed as a component of exploratory/foraging behaviors, or as a component of defensive behaviors (escape locomotion). Presently, it is not known whether serotonergic interneurons may play a role in sex behaviors, but in some species, there are extensive serotonergic innervations of sexual organs /108/.
As illustrated in Fig. 1C, although it is possible that noxious stimuli may act directly on the specific arousal systems for various behaviors (Fig. 1C1), the more attractive hypothesis would be that noxious stimuli have access to a hypothesized general arousal system, which in turn acts on different behavioral networks to promote expression of multiple behaviors (Fig. 1C2). If we can obtain experimental support for the second hypothesis, it will suggest that some kind of general arousal mechanism does exist. In the next section, we summarize behavioral and circuitry findings that appear to support the second hypothesis in experimentally advantageous molluscan model systems.

MOLLUSCAN MODEL SYSTEMS: MULTIPLE FORMS OF AROUSAL

Most animals exhibit basic manifestations of arousal, organized on the basis of diurnal and seasonal rhythms, as shown by the presence of multiple forms of arousal, e.g., sex behavior, feeding, defensive responses, etc. (Fig. 2). Among them, molluscs (Fig. 2B) provide model systems that are highly accessible to analysis in terms of the simplicities of both behavior and central nervous systems. Here we focus on the two forms of arousal in molluscs: feeding arousal usually elicited by food, and defensive arousal usually elicited by noxious stimuli. In each case, the aroused state has both specific and nonspecific effects on behaviors. Specific effects of arousal refer to enhancing effects on behaviors that are directly relevant to feeding or defensive (e.g., consummatory feeding) or escape locomotion, withdrawal. Nonspecific effects of arousal refer to effects on behaviors not directly related to feeding or defensive, but which are important components of the overall state.

Circuitry studies indicate that the arousal states are established by persistent actions of modulatory neuronal elements or intrinsic circuitry neuronal elements that contain modulatory transmitters, such as biogenic amines and neuropeptides. Moreover, nonspecific and specific effects of arousal have neural substrates in the nervous systems. In work to date, arousal elements may be divided into two general classes: localized versus general. Localized arousal elements have actions that are limited to the class of behaviors directly relevant to feeding or defense, and thus primarily mediate specific effects of arousal. In contrast, general arousal elements act on more than one motor network, and therefore may mediate both specific and nonspecific effects of arousal. This classification is made possible by one significant advantage of molluscan circuitry studies, i.e., the ability to identify the inputs and outputs of neuronal elements, and their physiological actions in multiple behavioral networks.

Feeding arousal:
From appetitive to consummatory phase

Like other motivated behaviors, feeding behavior in molluscs involves sequential stages. The first involves appetitive behaviors by which animals detect and locate food, and approach and orient to it. The second stage entails consummatory behaviors by which animals ingest the food. To organize these behaviors efficiently, food-seeking animals establish an internal state, during which behavioral responses are facilitated after pre-exposure to food. This behavioral state has been called ‘food-induced feeding arousal’, and is akin to incentive motivation.

Specific effects of feeding arousal include those on consummatory feeding, during which animals will perform biting behavior to grasp the food, and if successful, animals will swallow the food. In molluscs, such as Pleurobranchaea, Lymnaea, Limax, Helisoma, Clione, and Aplysia, consummatory feeding behaviors are organized by a feeding central pattern generator (CPG) in the buccal ganglion that receives initiating and modulatory inputs from cerebral feeding interneurons. More specifically, as in Aplysia, both biting and swallowing are mediated by a multifunctional feeding CPG (Fig. 3A1). The feeding CPG can be activated by activity in the cerebral higher-order neurons (HONs), such as cerebral-buccal interneuron-2 (CBI-2), which is in turn activated by food stimuli.
Fig. 3: Simplified diagrams of behavioral circuitry in *Aplysia*. **A.** Transition from appetitive to consummatory feeding. 
**A1.** Consummatory feeding is mediated by a feeding central pattern generator (CPG) in the buccal ganglion, that drives motoneurons for feeding. The feeding CPG receives inputs from higher-order neurons (HONs), e.g., CBI-2, that can activate the CPG, and a modulatory (M) neuron, the serotonergic MCC, that modulates the feeding CPG, motoneurons and muscles, but does not directly activate the CPG. CBI-2 also receives feedback from the feeding CPG, and therefore is part of the feeding CPG. Notably, the MCC can be activated by food-related input, the sensory neuron (SN) C2, which is active during feeding. **A2.** Appetitive aspect of the feeding is mediated in part by the cerebral-pedal regulator (CPR) interneuron, which provides input to posture related neurons to establish a feeding posture so that the animals can perform consummatory feeding. CPR also excites the MCC, thereby facilitating the transition from appetitive to consummatory feeding. **B.** Interaction between locomotor and feeding networks. 
**B1.** Locomotion is controlled by a locomotor CPG, which is in turn activated by HONs, e.g., serotonergic CC9-10. Pedal serotonergic (PS) neurons modulate the motoneurons-elicited contraction of foot muscle. **B2.** CC9-10 enhance the excitability of the MCC of the feeding network, thereby promoting food-induced feeding in a latent fashion.
The specific effect of feeding arousal on consummatory feeding is the enhancement of consummatory feeding behavior by prior exposure to food /153,168/. This effect may be mediated at least in part by a localized arousal element, the serotonergic metacerebral cell (MCC) in the cerebral ganglion that acts on the consummatory feeding network (Fig. 3A1). Evidence supporting this claim includes that the activity of MCC speeds up feeding behavior /166/ and fictive feeding motor programs /107/. In addition, MCC modulates motoneuron-elicited muscle contraction /167/. MCC also modulates the synaptic outputs of CBI-2 onto feeding motoneurons /131/. Conversely, a lesion of MCCs slows down biting responses /137/. Additional elements that may contribute to feeding arousal include intrinsic elements of the feeding network, such as HONs (e.g., CBI-2) and motoneurons that contain peptide modulators (Fig. 3A1). Peptides in motoneurons /8,83,165/ modify the neuromuscular transform. Neuropeptides in CBI-2 may modulate feeding response during repeated activation of feeding motor programs /107,129/, and mediate homosynaptic facilitation of CBI-2 synapses /75,76/. Thus, specific effects of arousal on consummatory feeding are mediated by the localized arousal element MCC and intrinsic peptidergic elements of the consummatory feeding network.

In terms of appetitive aspects of feeding arousal, one critical element in *Aplysia* is an interneuron named cerebral-pedal regulator (CPR) (Fig. 3A2) /156,157/ in the cerebral ganglion, which projects its axon to the pedal ganglion. Distinct from the MCC, CPR can be classified as a general arousal element because it appears to mediate not only specific effects but also nonspecific effects of feeding arousal. One specific effect mediated by CPR is the feeding posture established during the appetitive phase of feeding during which the posterior foot is attached to the substrate and the head is lifted, ready to eat. Evidence in support of this includes that the CPR is persistently activated by food stimuli and provides inputs to posture-related motoneurons and modulatory neurons that modulate contractions of posture muscles /111,112/ (Fig. 3A2). Another specific effect of food-induced arousal mediated by CPR is its excitatory effects on the MCC (Fig. 3A2) /156,159/. This finding is of interest because CPR mediates appetitive aspects of feeding arousal, and its actions on the MCC constitute a circuitry linkage from appetitive to consummatory networks.

Furthermore, CPR also mediates nonspecific effects of feeding arousal on a number of non-feeding behaviors. Specifically, CPR affects a number of elements in the cardiovascular and defensive systems, all of which may underlie nonspecific aspects of feeding arousal /156/. First, during the appetitive phase, initial contact with food induces head withdrawal. However, during subsequent food contact, withdrawal quickly decreases and animals assume a feeding posture and show orienting responses to food. The inhibition of withdrawal may be mediated at least in part by CPR because CPR inhibits head withdrawal neurons /156,159/. Second, during feeding, the heart rate is faster /74/, and this may also be mediated in part by CPR because it excites the serotonergic heart exciter (RBhe) located in the abdominal ganglion.

**Defensive arousal:**

**From escape locomotion and avoidance to feeding?**

Defensive behaviors in *Aplysia* include escape locomotion and withdrawal behaviors. Thus, specific effects of defensive arousal induced by noxious stimuli, such as tail shock, include enhancement of both escape locomotion and withdrawal. Notably, spontaneously-occurring locomotion is also part of exploratory behavior. In terms of the network organization, locomotion and feeding share a number of features (Fig. 3B1) /51/. Locomotor behavior is controlled by a locomotor CPG located in the pedal ganglion /42,49,50/. On the other hand, the HONs are located in the cerebral ganglion /26/, and are persistently activated by noxious stimuli that normally elicit locomotor behavior. In turn, when directly activated, these HONs can activate the locomotor CPG (Fig. 3B1). A recent study indicates that these HONs may include serotonergic CC9 and CC10 /56/. Although interneurons may modulate withdrawal behaviors /14/, withdrawal behaviors are primarily mediated by direct interactions between sensory and motor...
elements, rather than through actions of inter-neurons of a CPG. Furthermore, withdrawal circuitry for different parts of the body is distributed in distinct ganglions. For example, head withdrawal circuitry is primarily localized in the cerebral ganglion, tail withdrawal in the pleural-pedal ganglion, and siphon withdrawal in the abdominal ganglion.

Similar to feeding arousal, defensive arousal is also mediated by localized and general arousal elements. The localized arousal elements include serotonergic modulatory neurons in the pedal ganglion (PS neurons) (Fig. 3B1). These neurons modulate the foot muscles and thereby may enhance locomotor behaviors /105/. Although less extensively studied, the motoneurons that control locomotion, like the motoneurons in the feeding network, also contain peptides /123/, and may modulate the foot muscle contraction underlying locomotion /38/. Thus, the specific effects of defensive arousal on locomotion are also mediated by localized arousal elements and intrinsic peptidergic elements of the locomotor network.

The general arousal elements of defensive arousal may include serotonergic HONs CC9-10 /56/ (Fig. 3B) and perhaps CC3(CB1) /91,94,172/. Interestingly, although, similar to CBI-2 of the feeding network, CC9-10 of the locomotor network also act as intrinsic modulators of locomotor activity, the functional role of CC9-10 decidedly differs from CBI-2 in that CC9-10 function as general arousal elements in defensive arousal, as they act not only on locomotor and other defensive networks, but also on other networks unrelated to defense, e.g., feeding, as described below. In this sense, CC9-10 and CC3(CB1) are similar to CPR. Importantly, CC9-10 and CC3(CB1) are activated by noxious stimuli applied to either the head or tail, thus their sensory responses are not site-specific. Upon presentation of brief alerting/noxious sensory stimuli, these neurons show persistent activity for minutes following stimuli. Thus, they function to transform brief external events critical for animals’ survival into persistent CNS activity.

In terms of their outputs, CC9-10 and CC3(CB1) have specific effects on locomotion and withdrawal. For locomotion, activity of CC9-10 initiates locomotor activity that persists even after their activity is terminated. CC9-10 also excite the pedal serotonergic (PS) neurons that are themselves modulator of foot muscles /105/. Furthermore, we have shown that CC9-10 can modulate locomotor circuitry because prior stimulation of CC9-10 can enhance nerve-evoked locomotor activity /56/. We have not directly shown that this modulatory action is mediated by serotonin, but it is likely that this is the case because serotonin and its precursor have been shown to promote locomotion /90,96,121/.

Although effects of CC9-10 on withdrawal have not been examined, CC3(CB1) appears to modulate withdrawal. It is well known that tail or siphon withdrawal is modulated by noxious stimuli, such as tail shock, that induces a defensive arousal. In fact, a noxious stimulus has been used as a reinforcement for sensitization paradigms of tail and siphon withdrawal (e.g., /91,162/) (see reviews in /10,12,14,149/). In part, the modulatory actions of noxious stimuli on defensive withdrawals may be mediated by serotonin /36,95/ (see reviews in /5,11/). Furthermore, CC3(CB1) /91/ has been shown to modulate the siphon withdrawal circuit. CC3(CB1) sends its axon to the abdominal ganglion, where the sensory and motor neurons for siphon withdrawal are located.

CC9-10 also mediate nonspecific effects of defensive arousal. Specifically, alerting/noxious stimuli, in addition to promoting escape locomotion and withdrawal, may promote feeding /85/. This effect can be considered a nonspecific effect of defensive arousal, because feeding is obviously not part of defensive behaviors. (In fact, active feeding suppresses withdrawal responses /78,156/, and may interrupt locomotion /32,80/.) Recent work /56/ suggests that CC9-10 may be one of the critical elements underlying this phenomenon. Inspired by earlier findings in another mollusc that serotonergic neurons embedded in diverse motor networks may interact /54/, we have now provided evidence that CC9-10 excite the MCC of the feeding circuitry. Their primary actions on the feeding network are to promote excitability of the MCC, without directly activating it. This conditional activation allows CC9-10 to promote MCC activation in the presence of food-related inputs, e.g., sensory/modulatory neuron C2 /166/ (Fig. 3B2). Moreover, serotonin, for which CC9-10 are immunoreactive, appears to
enhance MCC excitability, possibly through cAMP activation /47/. Thus, direct activation of the locomotor CPG and conditional activation of the feeding network by CC9-10 provide an elegant mechanism for enhancing the generation of multiple behaviors without creating a conflict between them.

Comparison of feeding and defensive arousal: Emergence of behavioral sequences?

The findings on the neural basis of feeding and defensive arousal suggest that the internal states underlying arousal are mediated by partially overlapping sets of neuronal elements. In particular, two groups of arousal elements, localized and general, can be distinguished based on their functional roles. Localized arousal elements include neurons that act exclusively as neuromodulators (e.g., MCC, PS neurons), and peptidergic neurons that are intrinsic elements of the motor network. These neurons act to enhance the class of behaviors that are directed to a specific goal object, and therefore mediate specific effects of arousal. Because of their specificity, localized arousal elements are often targets of general arousal elements in order to promote a specific behavior.

In contrast, general arousal elements include multifunctional neurons that have diverse effects on multiple motor networks. In this role, general arousal elements may mediate both specific and nonspecific effects of arousal, and may potentially enable generation of behavior sequences. In the case of feeding arousal, due to its actions on the posture system and the localized arousal element MCC, CPR both promotes appetitive behaviors (e.g., the feeding posture that enable contact with the food) and also prepares the animals for consummatory behaviors, therefore facilitating transition from appetitive to consummatory phase (Fig. 3A2). Similarly, in the case of defensive arousal, CC9-10 may drive locomotor response immediately following an alerting/noxious stimulus. In addition, through its actions on the MCC, CC9-10 promote feeding when the animal encounters food in the aftermath of the stimulus, thereby promoting behavioral transition from locomotion to feeding (Fig. 3B2). In this sense, both CPR and CC9-10 contribute significantly to the formation of behavioral sequences.

One potential difference between feeding and defensive arousal may be the relative degree of persistence of the underlying internal states. Specifically, the persistence of the internal state underlying a form of arousal depends on at least two aspects. One is how persistent is the activity of the modulators activated by the particular stimulus. For example, both activity of CPR evoked by food /156,157/ and activity of CC9-10 and CC3(CB1) elicited by noxious stimuli /56,91,94/ are persistent, but between the two, the latter may last longer (tens of seconds vs minutes). Second is how persistent are the actions of modulators on their targets in specific motor circuitry. Actions of CPR on its targets may have a relatively short time course. However, actions of CC9-10 on their targets, e.g., MCC, are persistent, i.e., CC9-10 enhancement of MCC excitability persists for minutes after termination of CC9-10 activity (Fig. 4A). Furthermore, the modulatory actions of the MCC on motoneuron-elicited muscle contraction also outlast the stimulation of MCC for tens of seconds (Fig. 4B). These data suggest that persistence may be a prominent feature of serotonergic neurons, and therefore serotonergic neurons may be especially important in molluscan arousal states. Other modulatory systems, such as peptidergic neurons, may exert persistent actions as well /57,59,75,106,154/. More generally, these studies suggest that in studying the role of specific neurons in arousal, it is important to document the temporal aspects (i.e., dynamics) of neuronal actions.

In the previous section we hypothesized two possible mechanisms to account for nonspecific effects of defensive arousal (Fig. 1C). The evidence in Aplysia appears to support a scheme that is similar to the second hypothesis (Fig. 1C2). More specifically, CC9-10 may act as general arousal elements to modulate activity of circuitry elements of several motor networks to promote expression of multiple behaviors. It is notable, however, that CC9-10 also belong to the locomotor circuitry by initiating locomotor activity, while their actions on the MCC are conditional, thus their actions on different motor networks differ.
The previous section emphasized a critical role of serotonergic systems in behavioral expression of molluscs (Figs. 2B, 3). It is useful here to examine this issue from an evolutionary perspective, since the serotonergic system of gastropods appears to be highly conserved in both structure and function across species (Table 1). Why should this be, in the face of the remarkable variation in body plans and life-styles in the gastropods? The answer likely lies in a marked evolutionary conservation of structure and function in the CNS, whose consideration lends dimension to the roles played by the arousal system.

Innovations in evolution that led to the spectacular radiations of the greater animal clades often had to do with the morphology of the feeding apparatuses, enabling pronounced efficiency and versatility in food-handling. For vertebrates, acquisition of the jaws and associated buccal and
## TABLE 1

Probable homologs and well studied functions of serotonergic neurons across several gastropod molluscan species

<table>
<thead>
<tr>
<th>Cerebral posterior 5-HT cluster</th>
<th>Aplysia</th>
<th>Pleurobranchaea</th>
<th>Tritonia</th>
<th>Clone</th>
<th>Lymnaea</th>
</tr>
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<tbody>
<tr>
<td>*</td>
<td>As1</td>
<td>Slow EPSPs to MCC</td>
<td>DSI1</td>
<td>Cr-SP (CPB1)</td>
<td>Extrinsic modulators (locomotor speed), excite (slow) Pd-SW</td>
</tr>
<tr>
<td>CC9-10</td>
<td>As2-3</td>
<td>Strong EPSPs (both fast and slow) to MCC</td>
<td>DSI2-3</td>
<td>*</td>
<td></td>
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<tr>
<td>Output to MCC and PS, initiate and modulate locomotion</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CC5(CB1)</td>
<td>As4</td>
<td>Strong outputs to G neurons</td>
<td>*</td>
<td>Cr-SV</td>
<td>Excite (weak) Pd-SW; HE; inhibit withdrawal</td>
</tr>
<tr>
<td>Output to PS and RBhe</td>
<td>*</td>
<td>MCC</td>
<td>CGC</td>
<td>Gating role to feeding</td>
<td></td>
</tr>
<tr>
<td>Cerebral anterior 5-HT cluster</td>
<td>MCC</td>
<td>Modulate CPG, MN, muscles</td>
<td>MCG</td>
<td>MCG neighbor</td>
<td>Some are coupled to MCG</td>
</tr>
<tr>
<td>* (cells adjacent to the MCC)</td>
<td>MCG neighbor</td>
<td></td>
<td>*</td>
<td>Cr-SA (CPB2)</td>
<td>Similar to Cr-SP, but with fast EPSPs, and also excite HE</td>
</tr>
<tr>
<td>Pedal 5-HT cluster</td>
<td>PS-californica; POP-brasiliana</td>
<td>PS; foot/body wall muscles; POP; parapodial muscles</td>
<td>G neurons</td>
<td>Pd-SW</td>
<td>Modulate wing muscle</td>
</tr>
<tr>
<td>Heart 5-HT exciter S</td>
<td>RBhe</td>
<td>Enhance heart rate * (pedal)</td>
<td>* (pedal)</td>
<td>HE (pedal)</td>
<td>Ventricle activation</td>
</tr>
<tr>
<td>* Likely present based on serotonin immunoistochemistry, but currently remain unidentified physiologically.</td>
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<tr>
<td>$ Depending on the species, serotonergic heart exciter appears to be distributed in either abdominal/visceral ganglions or the pedal ganglion.</td>
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| # Cr-SA neurons in Clone have projections to the pedal ganglion, which may be different from cells at the similar location in other species, e.g., MCG neighbors in Pleurobranchaea, which may project their axons to the periphery through cerebral nerves.
hyoid musculature, and for the arthropods, the adaptation of jointed appendages to handling food, represented novel introductions of basic machinery that could be modified through simple changes in topology and material chemistry to handle wide ranges of foodstuffs and foraging strategies. The amazing success of the molluscs appears to be largely based on the radula and its associated musculature.

Gastropods, among all the molluscs, show the widest range of prey exploitation, ranging from scraping and grazing on plants and sessile animals, like *Aplysia* and *Tritonia*, to wide-ranging active predation in *Pleurobranchaea* and *Conus* spp /13,65/. They also show extremes in morphological variation, but extremes in which the radula/buccal mass remains adapted for the rhythmic actions of biting or rasping and swallowing.

The serotonergic arousal system of gastropods appears to be highly conserved across the class Gastropoda (cf. /30,33,54,56,114,146/) (see below), and, indeed, may be recognizable in both form and function in other molluscs like the cephalopods. A basic reason, we argue, is that evolutionary changes in the nervous system are basically more conservative than those that occur in the periphery, and that natural selection tends to operate more drastically on the periphery than on the basic structure of the CNS. For snails, the basic circuitry scheme of the gastropod CNS appears to show fewer changes between species of quite different morphology and ecological niche than does the peripheral morphology, physiology and sensory apparatus. The many homologous individual neurons and neuron groups identified across species in pulmonates and opisthobranchs are consistent with this interpretation.

A notable example of CNS conservation with radical peripheral evolution comes from the work of Dickinson, who identified gill withdrawal neurons in the sea-slug *Pleurobranchaea*, which has a true gill /22/. Moreover, she identified homologous neurons in doridacean nudibranchs (*Triopha* spp) possessing true gills as well as secondary gills derived from the mantle, and found that those same neurons innervated both the old true gill and the newcomers /21/. Finally, in nudibranchs lacking true gills (*Tritonia*) she found that those homologous neurons were still present and had come to solely innervate the new, derivative gills. Thus, CNS neurons were highly conserved in basic CNS circuits and function, while adapting to major changes in the periphery.

The similarities of serotonergic systems between gastropod molluscs are two-fold. First, the general distributions of serotonin immunoreactive neurons are similar. The serotonergic systems in gastropod molluscs are largely localized in the central nervous systems. Indeed, in some species, such as *Pleurobranchaea* and *Tritonia*, they appear to be exclusively localized in the CNS (cf. /25,108,151/). In the CNS, serotonergic neuron somata are mostly distributed in distinct clusters in the cerebral and pedal ganglions with apparently no serotonin immunoreactive somata in the buccal ganglion, and usually absent or few in the pleural ganglion /15,25, 71,89,113,116,119,122,147,151,160/. In molluscs with widely divergent ganglion fusions and partitioning in CNS development, such as *Aplysia* /13,65/, with its abdominal ganglion formed of asymmetric fusions of pleural and visceral ganglia, 5-HT neurons innervating the visceral organs and their motor networks are likely to be of origins traceable to homologous cells in other species’ CNS (see the serotonergic heart exciter in Table 1).

Second, the chief functions of serotonergic neurons, where data are available (Table 1), appear to be modulatory, i.e., to facilitate motor outputs and to regulate sensory inputs. This modulatory function is conserved despite the fact that some of these neurons may serve species-specific behavioral functions. Congruent with the central theme of this review, the serotonergic neurons can be grouped into two major classes: localized arousal elements that are embedded in individual motor networks, and general arousal elements that act upon multiple motor networks through their modulatory roles. Figure 5 shows a proposed basic organization of the serotonergic system and its network actions underlying behavioral expression in gastropods, drawing from studies of *Tritonia, Pleurobranchaea, Clione, Lymnaea*, and *Aplysia*.
Localized arousal elements

Cerebral: MCC – The feeding network

Perhaps due to a lack of the radula, jaws and buccal mass complex in a few nudibranch species, a clear homolog of the MCC may not be present in these animals /113/. However, in most gastropods, a homolog of the MCC in the cerebral ganglion is easily identifiable as a serotonergic giant cell /31,124,139,169/. MCC homologs, where present, modulate the feeding network so as to promote feeding activity /31,39,137,177,178/. Although some early work suggested that MCC, if activated at high frequency, may be capable of initiating feeding motor programs in Pleurobranchaea /31/,
Helisoma /37/, and Lymnaea /102/, later work with a more physiologically-relevant level of activity of MCC showed that MCC is primarily modulatory /178,179/. It is of interest that recent work in Lymnaea suggests a role for MCC in appetitive learning /73,115/.

**Pedal: PS – Body movement and locomotion**

The pedal serotonergic (PS) neurons in Aplysia brasiliana /104/ and Aplysia californica /105/ do not directly elicit muscle contraction, but modulate motoneuron-elicited contraction. Similarly, the homologous neurons in Clione /144/ modulate contractility of wing muscles underlying parapodial flapping.

**Heart exciter**

Although cardiovascular systems of gastropod molluscs may be composed of several groups of excitatory and inhibitory neuronal elements (cf. /92,97,148,174/), at least a subset of heart exciters may be serotonergic. In Aplysia, serotonergic heart exciters are located in the abdominal ganglion /88,94/. Similarly, some heart exciters located in the visceral ganglion of Lymnaea are also likely serotonergic /9/ based on pharmacological evidence. In contrast, although there are heart exciters in the intestinal ganglion in Clione /92/, the serotonergic heart exciter /3,93,145/ is located in the pedal ganglion rather than the intestinal ganglion.

**General arousal elements**

**Cerebral serotonergic interneurons, posterior clusters**

Homologous cerebral serotonergic interneurons have been identified in a number of species (Table 1, Fig. 5), where they play a specific role in locomotor-related behaviors that are critical components of the animals’ defensive and exploratory/foraging behaviors. In Aplysia californica (CC9-10) /56/, Tritonia (DSI-3) /128/, and likely in Pleurobranchaea (As1-4) /54/, they initiate locomotor activity despite the fact that the locomotor mechanisms in these animals are drastically different. Aplysia locomote by pedal-waves through muscular contraction, while Tritonia and Pleurobranchaea locomote through pedal cilia movement. In addition, type CN2 and/or CN4 command-like neurons that drive yet another form of locomotion, i.e., parapodial swimming in Aplysia brasiliana /27/, may also be serotonergic, although this has not yet been shown. The conservation of specific serotonergic neuron populations to drive locomotor behaviors mediated by markedly different peripheral mechanisms across these species is another prominent instance of conservation of CNS structure and function in the face of broad peripheral changes.

Clione /146/ locomotes (swims) by parapodial flapping, which occurs spontaneously and practically continuously throughout the animals’ life. In these cases, cerebral serotonergic interneurons can speed up the swimming. They can also initiate swimming activity in non-swimming preparations. In Melibe, which swim by alternating lateral body flexion, homologous neurons may play similar modulatory roles /114/. Thus, these neurons may be considered as extrinsic modulators /69/ in Clione and Melibe.

In Pleurobranchaea, the serotonergic As1-4 ensemble promotes avoidance turning /55/. These neurons also function as CPG elements for escape swimming (alternating dorsal and ventral body flexion) in Tritonia /29/ and Pleurobranchaea /53/. In their roles as members of the CPG for escape swimming, they function as intrinsic modulators in Tritonia (e.g., /68-70,100,140/) and likely in Pleurobranchaea too /53/.

More commonly, cerebral serotonergic interneurons may organize the loosely coupled serotonergic arousal networks embedded in individual motor networks by providing excitatory inputs to them (Fig. 5). These neurons excite pedal serotonergic neurons in Clione /118,145/, Pleurobranchaea /54/, Tritonia /128/ and Aplysia /56,94/. They also provide excitatory input to the serotonergic heart exciter in Clione /118,145/ and Aplysia /94,172/. Finally, they provide persistent excitation to the MCC homologue in Pleurobranchaea /54/ and possibly in Tritonia /67/, as well as to the MCC in Aplysia /56/. In Lymnaea, even though homologous cerebral serotonergic neurons have not yet been identified physiologically, MCC
does show elevated firing during locomotion compared with during quiescence /179/, which is potentially mediated by these serotonergic interneurons.

Although overall functions of serotonergic neurons within a neuronal cluster may be similar, they do show some differential actions, indicative of functional differentiation. For example, cerebral posterior cluster interneurons provide excitation to serotonergic neurons embedded in different motor networks, but their outputs may have distinctive targets (Table 1). In Aplysia /56,94,172/, CC9-10 act on MCC and PS, but do not act directly on RBhe because they do not project to the abdominal ganglion where RBhe are located. In contrast, CC3(CB1) act on PS and RBhe. Functional differentiation is also observed in Pleurobranchaea /54/ and Clione /118,145/ (Table 1).

Taken together, serotonergic modulatory elements that act as localized or general arousal elements are present across many species of gastropod molluscs. In particular, although there are differences in their roles in species-specific behaviors, overall the arousal functions of cerebral serotonergic interneurons are very much conserved, reinforcing the central role of these interneurons in organizing molluscan animals’ behaviors.

**IMPLICATIONS: WHAT CAN WE LEARN FROM GASTROPOD MOLLUSCS?**

**Basic similarities**

Converging evidence from contemporary work in both invertebrates and vertebrates suggests that arousal is not a unitary construct, but exists in multiple forms /125,126,133,161,168/. Multiple forms of arousal are evident not only behaviorally, but also mechanistically. First, different forms of arousal are defined by the class of behaviors serving a specific behavioral goal, e.g. feeding, defense, or sex. Second, these forms of arousal are in turn implemented by actions of partially overlapping sets of arousal elements that establish multiple, persistent internal states in the CNS. Specifically, these internal states are manifested as specific and persistent physiological changes in circuitry elements of behavioral networks exerted by persistent actions of arousal elements.

In particular, we can distinguish specific and nonspecific effects of arousal that are implemented preferentially by two major groups of arousal elements: localized versus general arousal elements. Specific effects are largely implemented by actions of localized arousal elements embedded in individual motor networks. These arousal elements include neuronal elements that act exclusively as modulators, and neuronal elements that contain peptides and are intrinsic to the motor network. Although various sites of the specific circuitry may be affected, actions of these localized arousal elements are largely confined to specific circuitry. For example, feeding arousal in Aplysia is dependent, in part, on the actions of localized arousal elements /168/ (see the last two sections). Sexual arousal in the female rat may also depend, in part, on the actions of sexual hormones on specific elements of the circuitry mediating lordosis behavior /125-127/. In addition, general arousal elements act on multiple motor networks and therefore they may mediate both specific and nonspecific effects of arousal.

The distinction of localized versus general arousal elements may be applicable to the vertebrates. For example, among other neurons, many projection neurons in specific neural circuitry in the cortex, basal ganglia and brainstem are known to contain peptide modulators (e.g., /66,98/), which may potentially function as localized arousal elements.

In contrast, the general modulatory systems in vertebrates (see Fig. 6) /126/, including the ascending reticular activating system, are likely to function as general arousal elements. The general arousal elements in molluscan (Fig. 5, Table 1) and vertebrate modulatory systems may share a prominent anatomical feature: the broad projections in the CNS. Thus, many components of the ascending reticular activating system (e.g., serotonergic, noradrenergic and dopaminergic) project to and modulate diverse cortical and subcortical structures responsible for sensory, motor and cognitive functions (e.g., /66/) (cf. Fig. 6). Results from diverse molluscan systems indicate that, functionally, although actions of general arousal elements may be broad, their actions in different
Motor networks may differ. Thus, they may exert direct activating actions versus conditional activating actions on two different motor networks, allowing them to promote two classes of behaviors without creating a conflict between them /56/, and to contribute to the formation of behavioral sequences. It is possible that similar mechanisms may also operate in vertebrates.

Fig. 6: Interacting modulatory systems underlying arousal and wake-sleep cycle in vertebrates /62,141,163/. The central regulator of wakefulness is the orexinergic (OR) neurons in the lateral hypothalamic area (LHA), which also promote feeding. In addition, OR neurons provide excitatory inputs to cholinergic (ACh), noradrenergic (NA), and serotonergic (5-HT) neurons in hindbrain nuclei, and histaminergic (Hist) neurons in the hypothalamus, all of which also promote wakefulness. Sleep is divided into two major types, slow wave sleep (SWS) and paradoxical sleep (PS) or REM sleep (REMS), which are promoted by VLPO and extended VLPO (eVLPO, not shown), respectively /141/. Both types of sleep are characterized by the absence of muscle tone, but differ in the activation of forebrain. During SWS, there is no forebrain activation, while during PS, forebrain is activated. Among other things, GABAergic neurons in the basal forebrain may contribute to SWS, while cholinergic systems in the basal forebrain may contribute to cortical activation during PS and wakefulness. The figure illustrates intricate modulatory networks contributing to the wake-sleep cycle, and suggests that the serotonergic system may contribute to wakefulness, but only serves a secondary role. Connections between components of the arousal network are in gray. Connections are excitatory unless indicated with (-), when they are inhibitory. For clarity, some connections are not shown, e.g., inhibitory connections from VLPO to LC, and from LC, RN and TM to VLPO. Thus, VLPO vs LC, RN and TM (all shown in bold) are mutually inhibitory. LDT = laterodorsal tegmental nucleus; PPT = peduncular pontine nucleus; LC = locus coeruleus; RN = raphe nuclei; TM = tuberomammillary nucleus; VLPO = ventrolateral preoptic nucleus; GAL = galanin.

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The literature also highlights the idiosyncrasy of different stimuli, either external or internal, that can elicit an aroused state. Although some stimuli, such as food or a sexual partner, tend to be more specific, effects of other stimuli, like noxious stimuli, tend to be broader, and, as we explained earlier, this difference in scope of actions can be largely accounted for by the set of general arousal elements that is being activated by different stimuli (e.g., compare CPR vs CC9-10 in Aplysia). Perhaps this is not accidental because the biological importance of noxious stimuli may demand the broad attention of animals and therefore readiness of multiple motor networks. Obviously, reaction to this type of stimulus is essential for animals’ survival not only in molluscs but also in vertebrates. It is therefore not surprising that noxious stimuli can induce widespread changes in the CNS. Although it is debatable whether molluscs might have emotions /82,134/, noxious stimuli are emotionally arousing in higher animals. Thus, emotionally arousing events, such as an encounter with a noxious stimulus, are readily encoded in the CNS, which may underlie different forms of learning and memory /94,96/. Indeed, tail shock has been used extensively to sensitize defensive reflexes in Aplysia, which may be mediated in part by serotonergic interneurons /91/. In vertebrates, the amygdala, which is involved in processing emotional stimuli, is also important for memory consolidation (/18,43,86,103/). Nonetheless, it may be that these other modulatory systems serve only secondary roles in arousal in molluscs /30/.

In contrast, the modulator systems of vertebrates are necessarily more complex because of their apparently larger behavioral repertory. The complexity of the modulatory systems in vertebrates is well illustrated by systems controlling the sleep-wake cycle (e.g., /62,101,141,163/) that is adaptively coupled with diverse behavioral activities, including feeding /141/ (Fig. 6). There are a number of modulators, including ACh, GABA, NA, 5-HT, Hist, orexin, to name a few. Neurons that contain these modulators are distributed in nuclei of multiple brain regions, such as basal forebrain, hindbrain, and hypothalamus. The network interactions between them are complex, and many interactions likely play specific roles in various aspects of the sleep-wake cycle. Specifically, each state, e.g., wakefulness, can be promoted by several different modulatory systems, i.e., the ACh, NA, Hist, and 5-HT systems.

In part, this situation may be due to the fact that these modulatory systems promote different aspects of wakefulness, e.g., alertness or attention. For example, the cholinergic (ACh) system stimulates cortical activation during both wakefulness and REM sleep, irrespective of motor activity and muscle tone, while the NA system may promote both cortical activation and behavioral arousal during wakefulness. As is also evident from Figure 6, although serotonergic neurons in raphe nuclei do play some roles in wakefulness (e.g., motor activity /48/), their actions are not central, and likely only serve a role complementary to other modulatory systems, e.g., peptidergic (orexin) systems. A recent review noted that although serotonergic systems play a major role as excitatory modulators of appetite and feeding in lophotrochozoa (molluscs and leeches) systems, they do not play such a role in vertebrates. Instead, peptidergic systems, many of them like orexin linked to monitoring of long-term nutrient stores, are more important regulators of appetite, allowing appetitive regulation by the long-term needs of vertebrate animals /30/.

Differences

The general similarities between arousal networks of simple model systems and vertebrates stand out, but there are notable differences as well. Specifically, the modulator systems underlying arousal in gastropod molluscs are relatively simple. In terms of arousal functions, serotonergic systems appear to play a central role in molluscs (Figs. 2B and 5). Although it is presently unclear whether noradrenalin (NA) or orexin systems are present, clearly, other modulator systems that contain transmitters, such as dopamine, histamine (Hist), and peptides are present in the nervous systems of gastropod molluscs /20,57,63,99,135,154,164,165/.
CONCLUSIONS

Arousal is one of the internal states of animals that endow animals with the ability to generate goal-directed behaviors. By integrating sensory inputs with the internal states, animals can make adaptive behavioral decisions. Differences between simple model systems and higher animals notwithstanding, circuitry studies in simple model systems can help us obtain a mechanistic understanding of arousal. More specifically, circuitry studies are valuable in distinguishing specific neuronal models of arousal (Fig. 1C) and are advantageous in characterizing specific roles of arousal elements in multiple behavioral networks. Thus, localized arousal elements mediate specific effects of arousal and are associated with dynamic aspects of internal states that are more confined in nature, and may optimize expression of a class of behaviors interacting with a specific goal object. General arousal elements, such as the cerebral serotonergic interneurons in molluscs, may mediate both specific and nonspecific effects of arousal and are associated with dynamic aspects of internal states that are more global in nature, i.e., affecting multiple motor networks, possibly in different ways. Thereby they may facilitate goal seeking and help organize adaptive behavioral sequences. Further experimental and comparative work in simple model systems and more complex animals will provide better understanding on why and how we do what we do.

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