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REVIEW

The search for ancestral nervous systems: an integrative and comparative approach

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ABSTRACT

Even the most basal multicellular nervous systems are capable of producing complex behavioral acts that involve the integration and combination of simple responses, and decision-making when presented with conflicting stimuli. This requires an understanding beyond that available from genomic investigations, and calls for a integrative and comparative approach, where the power of genomic/transcriptomic techniques is coupled with morphological, physiological and developmental experimentation to identify common and species-specific nervous system properties for the development and elaboration of phylogenomic reconstructions. With careful selection of genes and gene products, we can continue to make significant progress in our search for ancestral nervous system organizations.

KEY WORDS: Cnidaria, Ctenophores, Nervous system evolution

Introduction

The description of basal nervous systems, and the search for evidence of the evolutionary history of nervous system development, has seen a major advance through the use of genomic (and related molecular) techniques (e.g. Moroz, 2009; Moroz et al., 2014). A major advantage of this new approach is the generation of huge data sets of useful genetic and molecular information. It also provides a wealth of candidate genes for phylogenetic analysis. This is where an integrative approach, extending from genomes to neuroethology, can provide a mutually beneficial dialogue that can help focus genomic analyses on critical components of nervous systems at the same time as it points the way for morphological examination, and physiological and developmental experimentation. Furthermore, combining the integrative approach with comparative analyses should allow improved description of between-group and within-group evolutionary relationships.

Genomic and transcriptomic information tells us what is there, where it is expressed and when it is expressed. It does not provide information on how the gene products are used, particularly in the time course of behavioral events. For example, we can have a thorough understanding of the ion channel and synaptic protein components of specific neurons or classes of neurons within an organism. Yet, this does not tell us how these proteins act in concert, and through various forms of modulation, to impact behavioral variability. Through comparative analyses, we can search for common themes in neuronal organization and function, and note species-specific properties that are superimposed on these common themes. This type of analysis of behavioral variability on extant animals can be used to focus on common nervous system

arrangements within and between phylogenetic groups as a stepwise strategy to look backward toward ancestral neuronal organizations, and thus provide new foci for phylogenetic reconstructions at the genomic level.

When dealing with nervous systems, genomic and anatomical knowledge is not enough, particularly when an understanding of the capabilities of basal nervous systems is a major goal. The behavioral repertoires of some of the least centralized cnidarians, for example the anemones, underline this point. *Stomphia*, when stimulated by a predator, releases the basal disk from the substrate and produces a repetitive series of alternate-side column contractions to 'swim' away from the predator (Ross and Sutton, 1964a; Ellis et al., 1969). The swimming response is inhibited in the presence of food on the oral disk or following nematocyst discharge in response to food stimuli (Ross and Sutton, 1964b; Ross, 1965). Together, the generation of this integrated behavioral pattern coupled with decision making when swimming and feeding stimuli are both present belies the apparent anatomical simplicity of the anemone nervous system.

When the anemone *Calliactis* encounters a shell of the snail *Buccinum*, the anemone will attach with its tentacles, release contact with the substratum, bend its body around and attach the basal disk to the snail shell (and release the tentacles) (Ross and Sutton, 1961a; Ross and Sutton, 1961b; Ross, 1965). If a hermit crab does not inhabit the shell, the anemone will move to another shell.

These specific and integrated behaviors encourage a second look at a concept raised by Horridge (Horridge, 1968): 'What constitutes a … central nervous system? How many neurons must be brought together before patterns of interaction can evolve between them that confer on the group some recognizably new features, which are not those of single neurons?' These questions suggest that an additional organizational complexity exists in a centralized nervous system that justifies the concept of 'central properties'. Certainly, the most basal extant animals that possess multicellular nervous systems exhibit integrative capabilities and sufficiently complex behaviors to suggest the existence of such 'central properties'.

In the following sections are a few examples where an integrative and comparative approach to the understanding of nervous system functional morphology can be used to provide useful contributions to the understanding of nervous system function in basal metazoans, and in the search for likely properties of ancestral nervous systems.

A complex behavior: feeding in the ctenophore *Pleurobrachia*

The comb rows that produce locomotory movements are controlled by an apical organ, including a statocyst that directly influences animal orientation in the water column through reflex adjustment of comb plate beating in the eight comb rows (Horridge, 1966). In addition, comb plate beating frequency and intensity can be up or down modulated by conducting systems that are presumably represented by anatomically defined nerve nets (Horridge, 1966;

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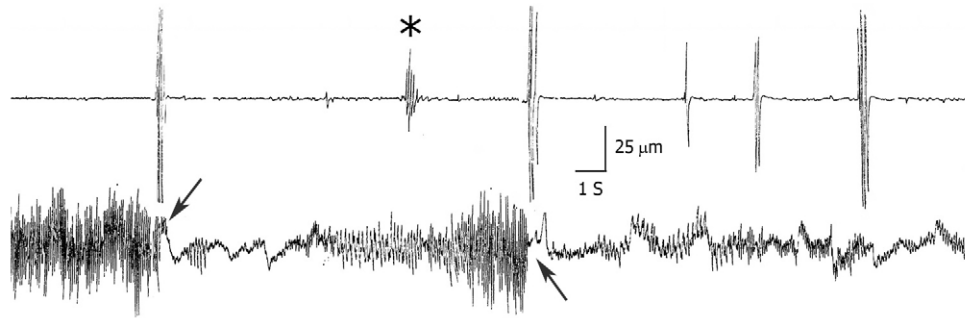*Author for correspondence (satterlier@uncw.edu)

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Fig. 1. Extracellular recording immediately adjacent to a comb plate in the ctenophore *Pleurobrachia pileus*. The lower trace is a record of comb plate movements, produced by placing a photoresistor above a comb row with substage illumination. Large impulse bursts are associated with comb plate inhibition (arrows), while bursts of smaller impulses trigger an acceleration in comb plate beating (asterisk). Original recording by the author with polyethylene suction electrodes and standard AC amplification and display.

Moss, 2004). Distinct electrical impulses can be recorded with suction electrodes that correspond, respectively, to excitation and inhibition of comb plate activity (Fig. 1).

In the 'fishing' mode, *Pleurobrachia* are oriented mouth-uppermost with tentacles extended downward (aborally). When prey become attached to a tentacle (via colloblast activity), a series of coordinated actions are initiated (Fig. 2) (Tamm and Moss, 1985). The tentacle shortens, pulling the prey closer to the body. Comb plate rows on opposite sides of the body undergo opposite reactions – ciliary excitation on one side and ciliary reversal on the other side. These reactions produce a spinning of the body in the plane of the tentacle sheaths so both tentacles become wrapped around the body in contact with the mouth. The tentacle holding the prey is then contracted until the prey is brought to the mouth, where is it consumed. This integrated behavior, which is species specific, involves several coordinated responses: ciliary excitation on one side of the body, ciliary reversal on the other side, shortening of the stimulated tentacle, an override of the normal statocyst geotactic response, and coordination of tentacle and mouth activity. While each individual response may be explained by activity in a body or tentacular nerve net, the coordination of the component responses suggests an organism-wide level of integration. Each nerve net can produce animal-wide changes in comb plate activity and, as seen in feeding reactions, very specific regional changes. One important concept in the integration of multiple conducting systems is the coordinated and behaviorally relevant interaction between these systems. This level of integration, of coordinated cross-talk between independent conducting systems, forms a neuronal hierarchy that suggests the use of a form of Horridge's 'central properties'.

Behavioral convergence in cnidarian medusae

The swimming systems in all three cnidarian groups with medusoid members (Hydrozoa, Scyphozoa, Cubozoa) are similar in that each has a layer of subumbrellar, circular muscle that decreases the volume of the subumbrellar cavity, and thus moves water to provide motive force for forward locomotion. Biomechanical analyses of hydromedusae show that prolate forms (bell taller than it is wide) use a form of jet propulsion, while oblate forms (bell wider than it is tall) produce marginal rowing movements (Colin and Costello, 2002). Scyphomedusae, which are nearly all of the oblate type, similarly use marginal rowing (Costello and Colin, 1995). Cubomedusae are interesting as their bell is typically as wide as it is tall, and they show an intermediate condition – jet propulsion in smaller medusae and marginal rowing in larger forms (Colin et al., 2013).
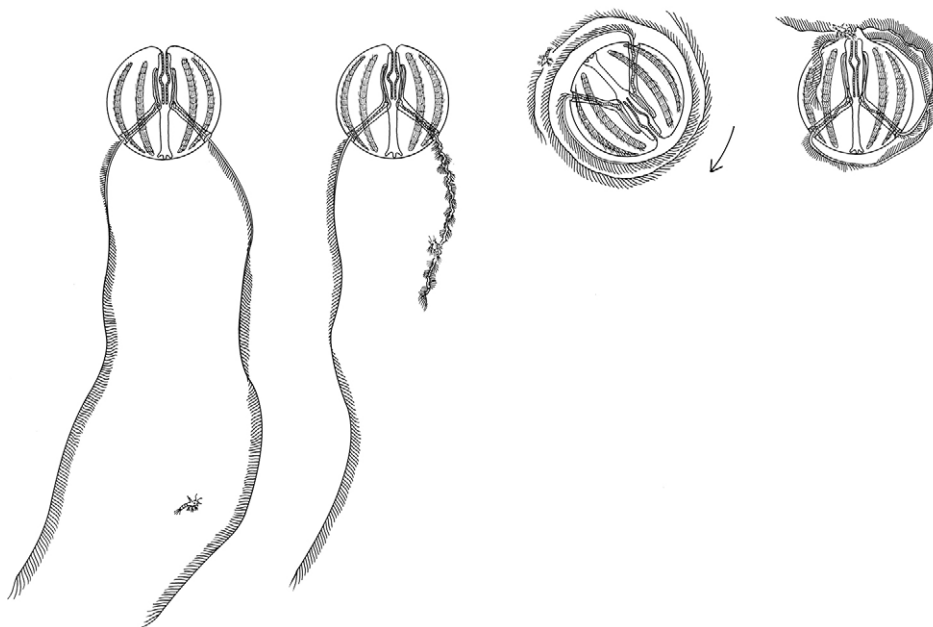

Fig. 2. Summary of feeding behavior in *Pleurobrachia pileus*. From left to right: first panel – normal 'fishing' behavior. The mouth is at the top. Second panel – contact of prey with a tentacle results in contraction of that tentacle. Third panel – continued stimulation (struggling of prey) triggers a spinning response, in the tentacular plane, which wraps the tentacles around the body to contact the mouth. Fourth panel – the tentacle bearing the prey is then contracted to bring the segment bearing the prey into contact with the mouth.
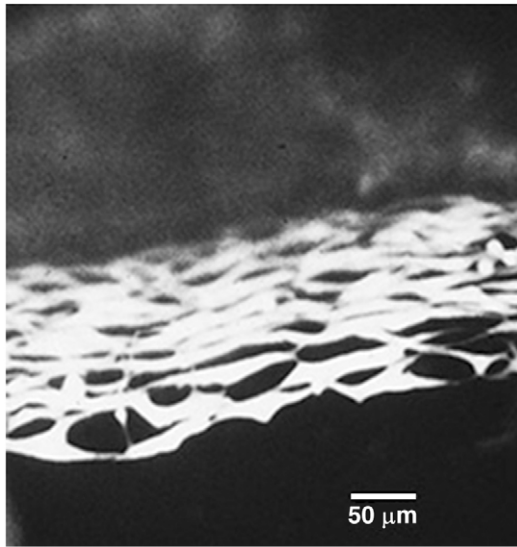
Fig. 3. Lucifer Yellow injection of a single neuron of the pacemaker/ motor neuron network in the inner nerve ring of *Polyorchis penicillatus*. The dye was iontophoretically injected from a tip-filled recording microelectrode. The entire network is dye- and electrically coupled. Dye-coupling is found within electrically coupled networks of hydromedusae and within sheets of striated swim musculature. Gap junctions have been identified via electron microscopy in these same tissues. Original image of the author (see Spencer and Satterlie, 1980).

One aspect of this apparent behavioral convergence centers on a marginal ring of muscular tissue that serves to narrow the bell opening in hydromedusae and cubomedusae – called the velum in the former and the velarium in the latter. These structures both serve to increase the rate of flow of ejected water from the subumbrellar cavity, but they also play a significant role in turning behavior through asymmetrical contractions and directional nozzle formation (Gladfelter, 1973; Petie et al., 2011). The two structures are considered to be analogous rather than homologous, as in cubomedusae they are direct extensions of the subumbrella, and in hydromedusae they are separated from the subumbrella by the nerve rings.

Despite the similarities in the behavioral/biomechanical aspects of swimming, a distinct dichotomy exists in the mechanisms of neural control of swimming (Satterlie, 2002). In hydromedusae, the neurons that initiate each swim contraction, and serve as motor neurons for activation of the musculature, are organized as an electrically coupled network, via gap junctions, that circles the bell margin in the inner nerve ring (Fig. 3). Any neuron in the network can initiate a swim contraction. These neurons activate the swim

musculature (directly and/or indirectly) via chemical synapses. The circular swim muscle cells are interconnected by gap junctions so that in some forms, the muscle sheets are aneural, only innervated at the margins of a quadrant (Spencer, 1978; Spencer and Satterlie, 1980; Lin et al., 2001). Subumbrellar nerve nets are present in some forms (primarily oblate forms), so the activation of muscle is due to both chemical synaptic inputs and intercellular current flow through gap junctions (Satterlie, 1985; Satterlie, 2008).

In scyphomedusae and cubomedusae, gap junctions have not been found (Fig. 4) (Mackie et al., 1984), nor have they been found in anthozoans. Swim pacemakers in scyphomedusae and cubomedusae are restricted to sensory/ganglion-like structures called rhopalia, and activation of the subumbrellar swim musculature is entirely through chemical synaptic inputs (Satterlie, 2002). The organization of the neuronal distribution system for excitation of swim musculature fits the classical description of a non-polarized, diffuse nerve net.

Within this motor control dichotomy is an interesting situation that could be useful for further analyses of evolutionary relationships within the cnidaria, and between cnidaria and other basal groups. It also could contribute to our search for ancestral nervous systems of basal metazoans. The important observation is that three of the cnidarian classes (Anthozoa, Cubozoa and Scyphozoa) do not appear to have gap junctions, while gap junctions are found between neurons, between muscle cells and between epithelial cells in both polyploid and medusoid forms of the Hydrozoa (Mackie et al., 1984). This is contrary to the suggestion that all multicellular animals develop gap junctions (Fushiki et al., 2010). Ctenophores do have gap junctions (Satterlie and Case, 1978). Twelve innexins/pannexins have been found in the ctenophore *Pleurobrachia*, while none have been found in the anthozoan *Nematostella* (Moroz et al., 2014). A representative hydrozoan, *Hydra*, has 18 innexins/pannexins (L. L. Moroz, personal communication), while data for scyphozoans and cubozoans are lacking.

This raises interesting evolutionary questions about these basal forms. Foremost is the need for genomic and transcriptomic investigations of innexins/pannexins in scyphozoans and cubozoans. Two possibilities can account for the lack of gap junctions: innexins and pannexins are missing in these groups, as in anthozoans, or they could be present but unable to form functional gap junctions. The former should be straightforward to test, and this paper represents a call for such a search. If innexins and pannexins are missing from the three cnidarian classes, an immediate question is whether innexins/pannexins were secondarily lost or if an ancestral form also lacked these gap junction proteins. From a logical standpoint, it would seem unlikely that an entire class of innexins/pannexins (12 in a ctenophore and 18 in a hydrozoan) would be lost entirely.
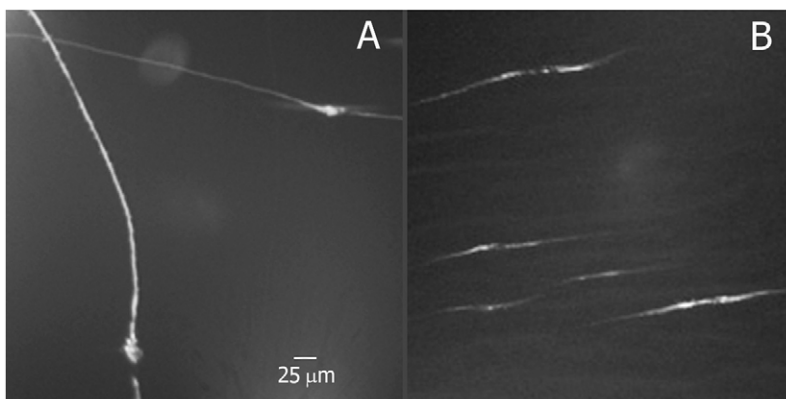

Fig. 4. Lack of dye-coupling in the motor nerve net and in the subumbrellar muscle sheet of cubomedusae. Carboxyfluorescein injection of two neurons of the subumbrellar motor nerve net (A) and five swim muscle cells (B) of the cubomedusa *Tamoya haplonema*. In these, and 54 additional injections, dye-coupling was never observed. The dye was iontophoretically injected from a tip-filled microelectrode with techniques identical to those used to obtain dye-coupling in hydromedusae (as shown in Fig. 3). Original data of the author.

However, innexins could have been lost before the full radiation of innexin/pannexin types. There is a close relationship between invertebrate innexins and vertebrate pannexins, and current evidence suggests the divergence of the innexin subfamilies occurred after the evolutionary appearance of insects and nematodes (Fushiki et al., 2010). This study used only one innexin sequence from *Hydra* as a representative of the cnidarians. Additionally, ctenophores were not included. As gap junctions are central players in the nervous system organizational dichotomy in cnidarian medusae (see below), and innexins are present in ctenophores, the innexin/pannexin relationships in basal metazoans should hold significant value in the search for common ancestors, particularly as organized nervous systems appear to have multiple origins (Moroz, 2009; Moroz et al., 2014) and ctenophores are likely the earliest lineage in the Metazoa (Moroz et al., 2014).

A search for an ancestral nervous system within a metazoan subgroup

One strategy for the search for nervous system origins within a phyletic subgroup is illustrated by examining the swim system of hydromedusae. The method of activation of subumbrellar swim muscle is variable and species specific, although some generalized properties can be seen [e.g. electrical conduction within the muscle sheets (Satterlie, 2002)]. Despite the variation in muscle activation, a comparative examination of 13 species from four different suborders revealed a common component of the swim system – the presence of a network of electrically coupled neurons within the inner nerve ring, frequently oversized compared with the surrounding neurons, that function as the pacemaker network and primary motor network for activation of the swim musculature (Satterlie and Spencer, 1983). This suggests that a common medusoid ancestor may have had a similar method for initiating swim muscle contractions. As such, it represents a target for genomic/transcriptomic analyses of protein profiles specific for this network, possibly including ion channel types and properties that produce endogenous pacemaker activity.

A similar common feature can be found in the swim systems of scyphomedusae and cubomedusae, in the form of the sensory ganglia called rhopalia, which contain the pacemakers for swim contractions (Satterlie, 2002). The development of marginal rhopalia could yield interesting features that allow molecular comparisons between the rhopalial pacemakers and the pacemaker networks of hydromedusae to strengthen evidence for the origins of nervous system development within the cnidaria and between the cnidaria and other basal metazoans.

In contrast, the feeding behavior described above for the cydippid ctenophore *Pleurobrachia* is certainly a species-specific activity. However, it enlists individual responses that are more common throughout the phylum, such as comb plate excitation (acceleration) and comb plate inhibition/reversal. In this way, very specific, complex behaviors can be constructed through the integration and combination of independent responses, some of which may be common to the larger phylogenetic group.

Nervous system origins

The anatomical organization and function of neural circuits and nervous systems are shaped by four types of variables: adaptive influences, developmental constraints, historical influences from ancestral forms, and architectural features based on the design of the organism (Dumont and Robertson, 1986). This suggests that parsimony does not always reflect a best approximation of within- and between-group relationships.

One common characteristic of basal metazoans that is considered a possible ancestral feature is the basiepidermal diffuse nerve net (typically stated in the singular). Furthermore, recent evidence suggests that a cnidarian ancestor was bilaterian (Finnerty et al., 2004; Martindale, 2005), so if such a nerve net organization was present, it was not a specific adaptation to a radial body plan. However, the nervous system organization of extant ctenophores and cnidarians goes well beyond that of a simple nerve net. Two features of the nervous systems of these basal organisms are important. First, multiple nerve nets, some parallel and some coordinating different body parts, will have areas of interaction where we would expect to find either an increased density of neurons or areas of physiological integration, or both. In polypoid cnidarians, such densifications are sometimes found in the oral region, where body wall, mesenteric, oral disk and tentacular nerve nets interact (e.g. Grimmelikhuijzen, 1985; Koizumi et al., 1992). Similar integrative interactions occur between the largely parallel conducting systems of the inner and outer nerve rings of hydromedusae. Here, the number of distinct conducting systems can be considerable, as shown in the hydromedusa *Aglantha digitale* (Mackie, 2004).

Second, the accumulation of neurons, sometimes into ganglion-like structures, is frequently associated with a similar accumulation of sensory cells. This is best seen in the rhopalia of scyphomedusae and cubomedusae, and in the nerve rings of hydromedusae (see Satterlie, 2002; Satterlie, 2011). But even in the planula larva of the hydrozoan *Clava multicornis*, the sensory networks (as revealed by FMRamide and GLWamide immunoreactivity) show a concentration in the anterior end, which corresponds with the sensory cell-rich forward-directed region for these benthic, crawling larvae (Piraino et al., 2011).

This means there is a distinction between the organization of motor nerve nets and nets that primarily have a sensory function. In the *Clava* planula, a tubulin-immunoreactive network did not show the anterior concentration, but rather was more evenly distributed throughout the larva (Piraino et al., 2011). In both scyphomedusae and cubomedusae, the tubulin-immunoreactive nerve nets are considered to be the motor networks, while the FMRFamide-immunoreactive networks were associated with sensory structures (Eichinger and Satterlie, 2014; Satterlie and Eichinger, 2014). This is seen in the separation of the two diffuse networks of the subumbrella of scyphomedusae – the 'giant fiber nerve net' and the 'diffuse nerve net' (see Satterlie and Eichinger, 2014). Similarly, separate motor and sensory networks are found in the nerve rings of hydromedusae (Arkett and Spencer, 1986a; Arkett and Spencer, 1986b; Mackie et al., 2003; Mackie, 2004), and in four species representing three hydrozoan orders, sensory cells were components of all FMRFamide-immunoreactive nerve nets while immunoreactivity was absent in known motor networks (Mackie et al., 1985). Further examination of the FMRFamide-immunoreactive nets of *Polyorchis* indicated that, in addition to inclusion of sensory cells, the networks were frequently associated with smooth muscle bands for fields, but not the striated musculature used in swimming (Grimmelikhuijzen and Spencer, 1984; Spencer, 1988), raising the possibility that the FMRFamide networks are mixed sensory–motor nets.

A common theme that emerges from these preparations suggests that the organization of motor nerve nets follows the distribution of their effectors (diffuse in the body wall of *Clava* planulae and in the swim musculature of medusae), and sensory networks follow the development and arrangement of sensory structures and appropriate effectors in mixed sensory–motor nets. Once again, areas of interaction between these different nerve nets will represent sites of neuronal integration representative of Horridge's 'central

properties', where emergent properties of the nervous system will go well beyond those of the individual contributing nerve nets. These interactions are carried to the extreme (for cnidarians) in those species that have more centralized nervous components, including the ganglion-like rhopalia or nerve rings composed of multiple, compressed nerve networks (Mackie, 2004; Parkefelt et al., 2005; Garm et al., 2006; Skogh et al., 2006; Satterlie, 2011.

Several organizational concepts are suggested for the evolution of nervous systems in basal organisms and their ancestors that go well beyond the singular diffuse nerve net concept. (1) Evidence exists for multiple and distinct conducting systems in extant basal forms and their larvae, particularly with a separation of motor and sensory systems. (2) With this separation, motor systems follow the distribution of their effectors, so that a broad, two-dimensional sheet of muscle (or epitheliomuscular cells), or of other distributed effectors, tends to be innervated by a nerve net or a nerve plexus, and restricted bands of muscle are associated with tract-like compressed neuronal networks (Satterlie, 2002; Satterlie, 2011). The nerve net/nerve plexus organization is seen well beyond the basal phyla (e.g. Koopowitz and Chien, 1974; Lawrenz-Miller, 1977). (3) The organization of sensory-related conducting systems will be influenced by the distribution, and particularly the aggregation, of sensory structures, frequently with an accumulation of nervous tissue in areas or regions of high density of sensory cells or sensory structures. (4) The distribution of effectors and sensory cells will be dependent on body patterning and body axis formation. This is best seen in the anatomical concentration of sensory networks in the anterior region of *Clava* planula larvae, related to its anterior–posterior body axis and its forward-directed locomotion (Piraino et al., 2011). (5) The areas of interaction between the multiple conducting systems will represent areas of neural integration and the beginnings of centralization through the emergence of Horridge's 'central properties'. These areas could also represent anatomical nucleating sites for accumulation of neurons involved in the integration of multiple sensory systems and multiple motor systems to aid in the production of complex, integrated behavioral responses.

Neurobiology and neuroethology still hold important positions in integrative and comparative approaches to the search for ancestral nervous system organizations within the Metazoa, particularly when teamed with a mutually interactive program of genomic analysis. An understanding of nervous system organization must include analyses of 'central properties' involved in complex behaviors and behavioral hierarchies, which will not show up in genomic databases. In addition, just as the wealth of genomic information is directing new physiological experimentation, physiological studies hold the potential to pinpoint specific areas for genomic/transcriptomic research concentration. A few examples are indicated here, including behavioral convergence in the swimming systems of cnidarian medusae, the presence/absence of gap junctions and innexins/pannexins in basal metazoan groups, and the description of common versus species-specific neuronal organizations within behavioral systems of basal metazoans. The evaluation of neural circuit analogy or homology can point to potential properties of common ancestors deserving of more in-depth genomic evaluation, particularly through the identification of the convergence of analogous behavioral systems and neural circuits, and the divergence of similar homologous systems (Dumont and Robertson, 1986).

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