COMMENTARY

Do jellyfish have central nervous systems?

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Summary

The traditional view of the cnidarian nervous system is of a diffuse nerve net that functions as both a conducting and an integrating system; this is considered an indicator of a primitive condition. Yet, in medusoid members, varying degrees of nerve net compression and neuronal condensation into ganglion-like structures represent more centralized integrating centers. In some jellyfish, this relegates nerve nets to motor distribution systems. The neuronal condensation follows a precept of neuronal organization of higher animals with a relatively close association with the development and elaboration of sensory structures. Nerve nets still represent an efficient system for diffuse, non-directional activation of broad, two-dimensional effector sheets, as required by the radial, non-cephalized body construction. However, in most jellyfish, an argument can be made for the presence of centralized nervous systems that interact with the more diffuse nerve nets.

Key words: central nervous system, Cnidaria, jellyfish, nerve net.

Introduction

In cnidarian life cycles with medusoid stages, free-swimming jellyfish represent dispersal and reproductive forms that are capable of directional swimming and that can have elaborate feeding, protective and escape responses. The richness of the behavioral repertoires of many jellyfish is at odds with the common perception of nervous system organization in this group (e.g. Mackie, 2004a; Lewis et al., 2005; Garm et al., 2007a). This perception, usually with suggestions of simplicity and primitiveness, is presented in both introductory and higher-level undergraduate textbooks with comments similar to the following (paraphrased from actual texts):

(1) Cnidarians have nerve cells that are organized in a nerve net – a diffuse network where neurons interact wherever they cross one another.

(2) There are two contrasting types of body symmetry: radial and bilateral. Nerve nets are associated with the former and central nervous systems with the latter.

(3) The simplest and most primitive of multicellular nervous systems is the nerve net, in which neurons are not grouped into nerves or a central nervous system, but rather are diffusely dispersed, seemingly at random.

(4) In the nerve nets of cnidarians and ctenophores, conduction is unpolarized – it can run in any direction through the network of neurons.

(5) A central nervous system coordinates the various parts of the body of bilateral animals but not of sponges or radially symmetrical animals.

A few of the texts point out that some jellyfish have ganglion-like structures, but they stop short of suggesting a centralized nervous organization. The intent of this commentary is to show that the nervous organization of jellyfish from all three cnidarian classes with medusoid stages (Cubozoa, Hydrozoa and Scyphozoa) is largely misrepresented by these textbook treatments, and also to present evidence that supports the suggestion of Mackie (Mackie,

2004a) that the organizational features of jellyfish nervous systems are favorably comparable to the central nervous systems of bilateral animals.

Before anyone suggests that a straightjacket may be in order for those of us who hold this view, the semantic difficulty in claiming that a nervous system that rings the margin and subumbrella of a bell-shaped organism has a 'centralized' nature is acknowledged. But consider that one of the most basic features of a centralized nervous system is found in these radially organized animals. Whether centralization of bilateral animals is considered a driving force or a consequence, a general organizational trend stands out. Integrative centers tend to be more closely aligned with sensory specializations than with effector elaborations, such that these integrative centers provide the opportunity to weigh a variety of sensory inputs to produce appropriate variability in motor output and thus behavior. This idea is not consonant with the depictions of the nerve net listed above. Two goals of this commentary are to show that what we may be teaching our students is a gross oversimplification bordering on dead-wrong (depending on the specific comments), and to suggest that many of the medusoid members of the phylum have what can be considered a centralized nervous system based on this key organizational feature of the nervous systems of bilateral animals. Even the polypoid forms of some cnidarians have ring-like condensations of neural tissue in the oral disc region that connect with tentacular networks, suggesting a degree of centralization (see Grimmelikhuijzen et al., 1996; Grimmelikhuijzen et al., 2002).

Constraints on nervous system organization of cnidarians

In discussing cnidarian nervous systems, we have to acknowledge some rather severe constraints on their organization. First and foremost is the radial symmetry. There can be no cephalization because all regions of the circumference of the bell- or plate-shaped organism are more or less equally responsive to environmental

perturbations. This requires that sensory integrative capabilities are distributed around the bell, in proximity to the sensory structures. And from this we have a secondary constraint – it is necessary to design conducting and/or integrating systems that are capable of transmitting information in more than one direction around the bell from any number of stimulation sites.

An additional secondary constraint comes from the organization of the locomotory machinery of jellyfish. If we take an imaginary anemone-like polyp and free it from the substrate, with an expansion of the oral disc to form the bell- or disc-shaped medusa, we see that the mouth takes a superior, internal position (at the apex of the subumbrellar cavity) and the margin of the bell retains the ring of tentacles. An internal (subumbrellar) lining of circular muscle serves to decrease the diameter of the subumbrella and eject water, forming a biological fluid pump (e.g. Costello et al., 2008). If biological fluid pumps are attached within an organism, their contractions will move the fluid (as in hearts). If the fluid pump is unattached, contractions will move the pump through the fluid. This requires the circular muscle of jellyfish to take the form of a broad and flat two-dimensional sheet that lines the subumbrellar cavity. The sheet may be restricted to ring-like muscular regions in some species, but they still are relatively broad and two-dimensional.

This gives rise to a tertiary constraint, which results from a combination of the primary and secondary constraints. A distributive conducting system has to be designed to activate the broad two-dimensional muscle sheet such that impulses can be conducted from virtually any point of origin around the umbrella. Even though we can demonstrate this unpolarized conduction within the swimming system experimentally, the 'degrees of freedom' for activation sites are restricted primarily to the margin of most intact meduase, barring injury or unusual stimulation, both of which do happen in nature. Still, our challenge is to design a conducting system that can efficiently activate this two-dimensional sheet of muscle in a diffuse, multi-directional manner.

Before reading on, it would be a good exercise to think about possible ways to deal with these three levels of constraint, to produce a coordinated contraction of the subumbrellar circular muscle and eject water from the subumbrellar cavity to move the animal in the water column. This system also must be responsive to environmental and biological perturbations. In considering possible designs, a few factors will ease the burden. In those jellyfish from which convincing intracellular recordings have been made from swim muscle cells, the properties of action potentials are similar to those of other fluid pumps (including hearts). Notably, the action potentials (and resulting contractions) are of extremely long duration (Spencer, 1978; Satterlie and Spencer, 1983; Satterlie, 1985b; Mackie, 2004a) (Fig. 1). These long, squeezing contractions are necessary to

efficiently move the viscous fluid from the pump cavity. This property also has a smoothing function on the coordination of individual muscle cell contractions where there may be significant conduction delays from cells near the activation site to cells farthest from that site (Spencer, 1981). With long-duration contractions, the near-stimulus muscle cells may still be in the contraction phase when the most distant cells are activated. Keep in mind that, barring any conduction specializations, a point stimulation at the margin will produce diverging waves that will run in both directions away from the stimulation site, yet those waves only have to run halfway around the subumbrella before they meet and cancel in mutual refractoriness (under normal conditions).

A nerve net is a nerve net is a nerve net?

In addressing the challenge of designing the motor distribution side of our jellyfish nervous system, we can cheat and borrow a trick from the fluid pumps known as vertebrate hearts. What if we forego neurons altogether and hook the muscles together with electrically conducting gap junctions? Does such a conducting pathway exist in cnidarians? The answer is an emphatic yes. All hydromedusae (jellyfish of the Class Hydrozoa) that have been investigated show either electrical or dye coupling (or both) between swim muscle cells, and/or morphological evidence for intercellular gap junctions (e.g. Spencer, 1979; Satterlie and Spencer, 1983; Satterlie, 1985b; Mackie, 2004a). In *Polyorchis penicillatus*, the muscle sheet quadrants are aneural with excitation for each swim contraction originating in the marginal inner nerve ring and spreading up the radii and across the oral edge of the muscle sheet so the neuronal motor network encircles and activates each quadrant *via* chemical synapses (Anderson and Mackie, 1977; Spencer, 1978; Spencer and Satterlie, 1980; Spencer, 1981; Lin et al., 2001). Conduction of action potentials through the muscle sheets is *via* electrical connections (gap junctions) between muscle cells, which show both electrical and dye coupling (Spencer, 1978). In *Aequorea victoria*, muscle cells are similarly electrically and dye coupled (Fig. 2A), but a subumbrellar nerve net also innervates the muscle sheet (Satterlie, 1985b; Satterlie, 2008). Thus, different hydromedusan species show the basic plan of electrical coupling throughout the swim muscle sheets, but with variability in the presence or absence and organization of subumbrellar nerve nets or neuronal projections, as in *Aglantha* (Mackie, 2004a).

We might envision intercellular electrical coupling as an efficient way to spread excitation through a two-dimensional muscle sheet because gap junctions can pass current in either direction (provided they do not rectify) and they are metabolically cheap to operate compared with chemical synapses. Despite these perceived advantages, and the retention or independent appearance of a similar mechanism of conduction in the fluid pumps of higher
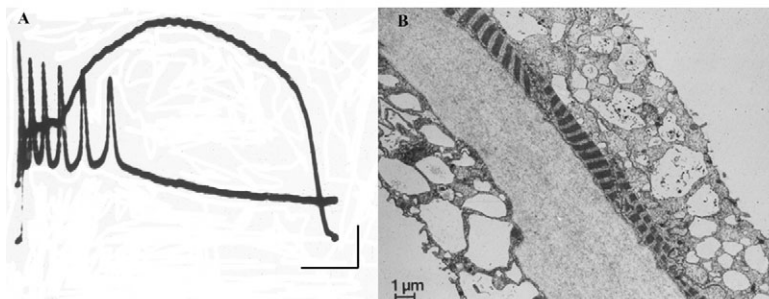

Fig. 1. (A) Dual intracellular recording from a neuron from the motor network of the inner nerve ring (burst of action potentials) and a post-synaptic circular muscle cell (long-duration action potential) of the hydromedusa *Aequorea victoria* (from Satterlie, 1985a). The muscle action potential corresponded to a single contraction of the swim musculature. Scale bar, 100 ms (horizontal), 30 mV (vertical). (B) Electron micrograph of subumbrellar epitheliomuscular cells of the hydromedusa *Polyorchis penicillatus*. The striated feet of the circular muscle cells abut the mesoglea (amorphous band of connective tissue between the two epithelial layers).

Fig. 3. Diffuse nerve net of the scyphomedusa *Cyanea* sp. labeled with an antibody to the bioactive peptide FMRFamide. The photo was taken in the region of the swim musculature, and the circular muscle cells had their long axes in the horizontal plane of the figure.
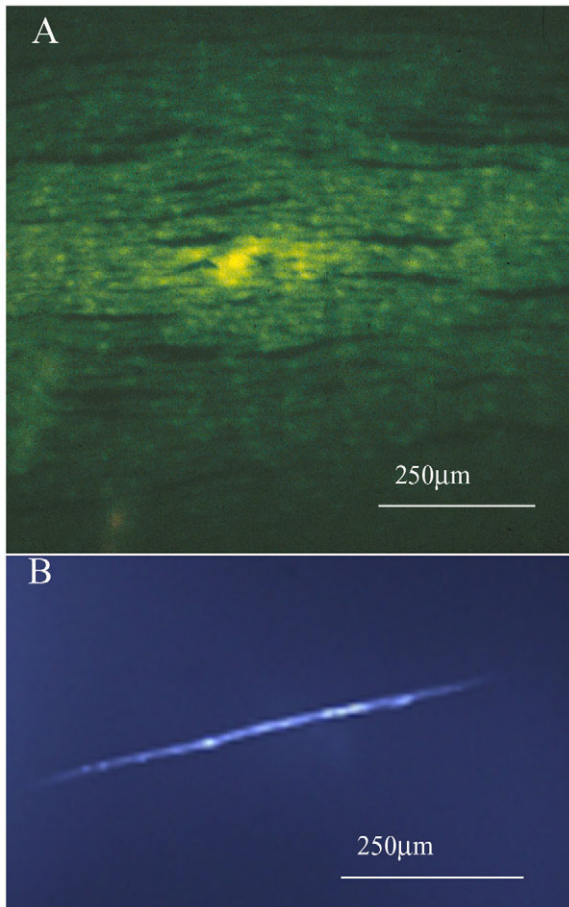
Fig. 2. Injection of a single circular muscle cell of the subumbrella (responsible for swim contractions) of the hydromedusa *Aequorea victoria* (A) and the cubomedusa *Tamoya haplonema* (B). In both animals, the fluorescent dye carboxyfluorescein was intracellularly injected through a recording microelectrode. Extensive dye coupling is seen in this and other hydromedusae (verified in five species). No dye coupling has been observed in cubomedusae (verified in three species); only the injected muscle cell contains the dye.

animals, convincing evidence for electrical conduction or dye coupling in the muscle sheets of jellyfish from the other two classes (Cubozoa and Scyphozoa) has not been forwarded (Mackie et al., 1984; Satterlie, 2002) (Fig. 2B). In fact, three out of the four cnidarian classes (including the Anthozoa) do not appear to have functional gap junctions (but see Germain and Anctil, 1996).

Without the possibility of electrical conduction, we have to shift our thinking on the most efficient way to distribute motor commands through our two-dimensional muscle sheet, and it is hard to come up with anything better than a nerve net. So, for the cubomedusae and scyphomedusae, the subumbrellar motor nerve nets have persisted as an efficient means of spreading motor commands in extant jellyfish (assuming this was an ancestral trait) (Satterlie, 1979; Anderson and Schwab, 1981; Anderson and Schwab, 1983; Anderson, 1985; Satterlie, 2002).

Many aspects of the classical view of nerve net function are then accurate. Neurons show an apparently random orientation within the network (Fig. 3), although some preferred directionality is sometimes seen. Where neurons cross one another, chemical synapses allow inter-neuronal communication. Symmetrical synapses have been described and physiologically characterized in
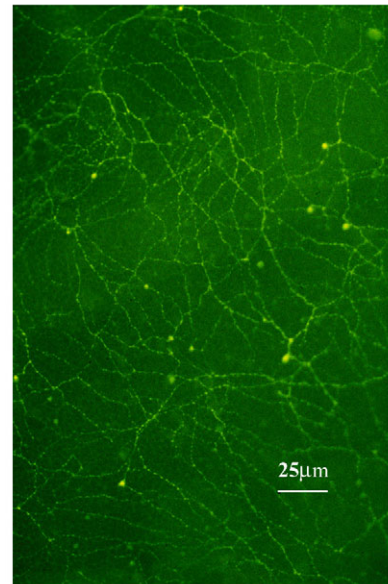
the motor nerve net of *Cyanea* (Anderson, 1985), and have been demonstrated on morphological grounds for several other cnidarians (see Westfall, 1996). This provides a mechanism of unpolarized conduction through the nerve net for activation of effectors from various points of marginal origin. These nerve nets are also considered through-conducting because impulses or contraction waves will invade the entire area of innervation without decrement. Some nerve nets in cnidarians, in particular in colonial anthozoans, do show decremental or incremental conduction (Shelton, 1975; Anderson, 1976); however, this has yet to be demonstrated in the motor nerve nets of jellyfish.

Peripheral integration and the scyphozoan swim system

If we stick with the classical nerve net organization for effector control, as seen in scyphomedusae, behavioral richness comes from two primary mechanisms. First is a characteristic of the neuromuscular junctions, which take the form of unidirectional chemical synapses. Neuromuscular facilitation represents a primary integrative property of the swim system, and shows a frequency dependence such that the strength of muscle contractions is related to the frequency of impulse traffic in the motor nerve net (Bullock, 1943; Horridge, 1956; Passano, 1973). Similar peripheral facilitation profiles are seen in the cubomedusan locomotory system (Satterlie, 1979) (Fig. 4). Additional variability in swim contractility is achieved in scyphomedusae through the activity of a second nerve net with a distribution that at least partially overlaps that of the motor nerve net (Horridge, 1956; Passano, 1973). This 'diffuse nerve net' represents a key characteristic of cnidarian nervous systems: the use of parallel conducting systems. In the case of the diffuse nerve net of scyphomedusae, the function is twofold, including conduction of sensory information to the rhopalia (location of swim pacemakers) for modification of the basic swim rhythm, and a peripheral innervation of the swim musculature, which is believed to serve a modulatory function for an additional source of variability in contraction strength. This 'double
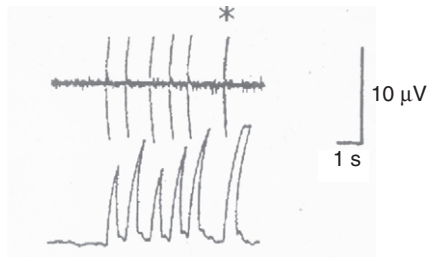
Fig. 4. Extracellular electrophysiological recording (top trace) and a force transducer recording (bottom trace) from the cubomedusa *Carybdea marsupialis*. The recording suction electrode was placed on the pacemaker region of a rhopalium while the force transducer was placed to register the contraction of the subumbrellar swim muscle (circular musculature). The successive swim contractions have amplitudes show a frequency-dependent facilitation with a consistent relationship between the amplitude of contractions and the intervals between contractions (shorter intervals=larger contractions; verified with a large data set of recordings of this type). The asterisk indicates a rapid 'double pulse' in the pacemaker recording, which produced a large contraction that exceeded the travel of the chart recorder.

Fig. 5. Dual subumbrellar nerve nets of the scyphomedusa *Aurelia aurita*, double stained with antibodies against α-tubulin (A; FITC label) and FMRFamide (B; TRITC label). The two photos represent a single location in the subumbrella (area of swim musculature) and were taken by switching the filter cubes (on a slide-changer) for the two antibody stains. Tubulin stains the motor nerve net whereas FMRFamide stains the diffuse nerve net.

innervation' of the swim musculature thus provides an additional level of peripheral integration.

Immunohistochemical staining of nervous structures in scyphomedusae provides a convenient means of selective staining of the two nerve nets (Fig. 5). The diffuse nerve net is believed to be RFamidergic, or at least to use an RFamide as a co-transmitter, because a peripheral nerve net that shows the expected anatomical distribution based on physiological evidence stains with a commercial antibody against FMRFamide or custom-made RFamide antibodies (see Anderson et al., 1992; Grimmelikhuijzen et al., 1996; Grimmelikhuijzen et al., 2002; Satterlie, 2002). This nerve net is separate from a more anatomically robust nerve net that fits the physiological distribution of the motor nerve net. This latter network stains with antibodies against tubulin (either α- or β-tubulin) (Satterlie, 2002). Double staining shows that both nerve nets stain with the tubulin antibodies; however, the RFamide-immunoreactive network is so lightly stained it can only been seen at high magnification and it fades in the time needed for photographic exposure in most preparations. The tubulin staining of the motor nerve net is similar to RFamide staining of the diffuse nerve net: staining is intense with little fading under the illumination necessary for visualization and photography.

If we stop here, we can still use several of the textbook statements concerning the organization of cnidarian nervous systems. But even for the scyphozoan swim system, this ignores the primary site of interaction between the two nerve nets – the rhopalia. Most scyphozoans have eight or more rhopalia, typically with species-specific numbers that are multiples of four. These modified tentacles represent accumulations of nervous and sensory structures that include the pacemakers for swim contraction initiation (Horridge, 1959; Passano, 1973). In most scyphomedusae, removal of the rhopalia paralyzes the swim system, although it is still responsive to imposed stimulation (Romanes, 1885; Horridge, 1959; Lerner et al., 1971; Passano, 1973; Murray, 1977). These rhopalia represent integration sites for the interaction between sensory inputs and motor outputs. This includes input from the diffuse nerve net (Fig. 6) as well as from rhopalial sensory structures, including ocelli, and some form of
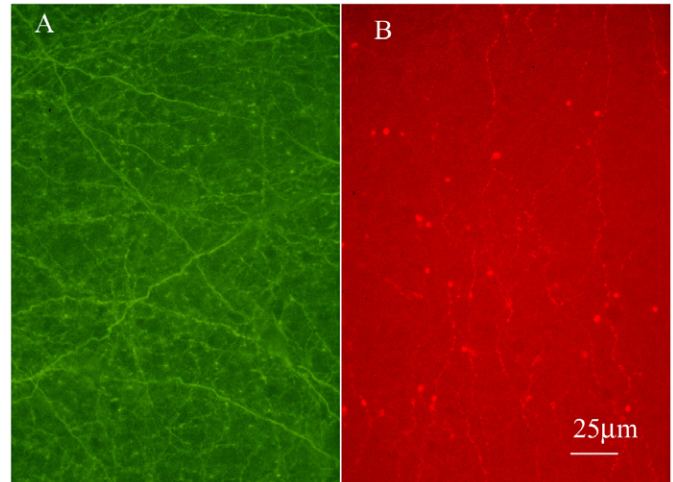
statocyst-like activity. These rhopalia thus represent integrative centers that could be considered rudimentary ganglia. They also represent a close association between sensory structures and integrative neuronal centers for the sorting of environmental information that, ultimately, will be passed to effectors. Scyphozoans lack any nerve-like interconnections between rhopalia, so inter-rhopalial coordination occurs *via* the nerve nets.

A zenith of neuronal and sensory co-condensation: the cubomedusan rhopalium

Until the mid-1900s, the cubomedusae (box jellyfish) were included within the Class Scyphozoa. They are now recognized as members of a fourth cnidarian class, the Class Cubozoa (Werner, 1973). In agreement with the previous phylogenetic grouping, the basic features of neuromuscular organization of cubomedusae are essentially identical to those of scyphomedusae (Satterlie, 2002). Swim pacemakers of cubomedusae are restricted to four rhopalia and the subumbrellar swim musculature is innervated by a motor nerve net that stains with antibodies to tubulins (Fig. 7A). This nerve net is anatomically diffuse and extends throughout the muscle sheets, including the subumbrella and velarium. A lack of dye coupling between neurons of this nerve net (Fig. 7B, Fig. 8), the sensitivity of conduction in the nerve net to high-magnesium seawater, and anatomical evidence for inter-neuronal and neuromuscular synapses (Satterlie, 1979) suggest that neuronal and neuromuscular communication occurs *via* chemical synapses. Furthermore, intracellular injection or microelectrode electroporation of small molecular weight fluorescent dyes show a lack of dye coupling between swim muscle cells as well as between radial smooth muscle cells (R.A.S., in preparation) (Fig. 2B). Similar dye injections show extensive inter-neuronal and inter-muscular dye coupling in the swim systems of hydromedusae (Fig. 2A).

Cubomedusae are best known for two dramatic characteristics: their potent venoms, which in some species can be lethal to humans, and the lensed camera-type eyes of their rhopalia. An additional
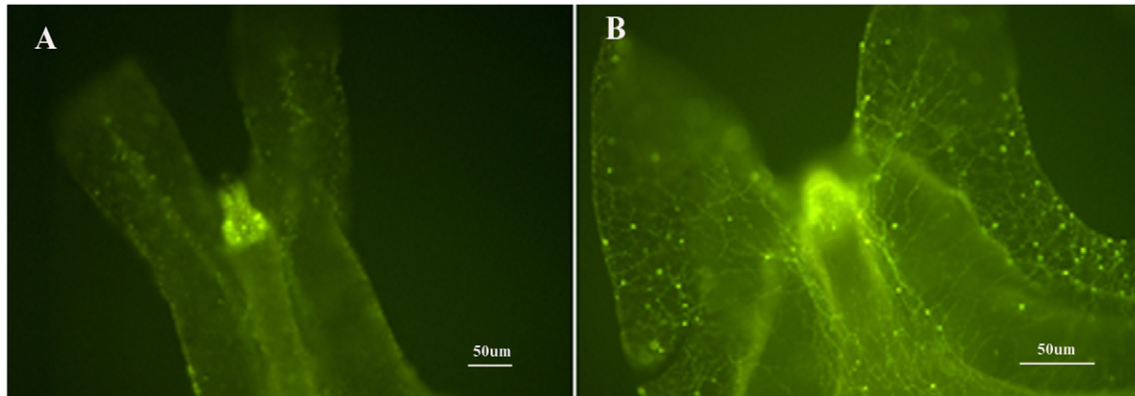
Fig. 6. Rhopalia and lappets of the ephyra of *Aurelia aurita* stained with an antibody against FMRFamide. Two different preparations are shown. (A) Immunoreactive somata of the rhopalia, which are found around the outside of the structure and surround a central neuropil (neuropil not visible at this magnification). (B) Connections between the rhopalium and the diffuse nerve net of the lappet.

structural specialization represents a neuronal condensation not seen in scyphomedusae. A complex nerve ring connects the four rhopalia and the four tentacle bases and contains multiple conducting systems for the swim system, the visual system and for coordination of tentacle responses (Garm et al., 2006) (R.A.S., in preparation). The nerve ring contains oversized (giant) neurons as well as tracts of smaller neurites (Satterlie, 1979; Garm et al., 2007b) (Fig. 8). It thus includes multiple specialized conducting pathways in a nerve-like structure that interconnects the rhopalia and the tentacle bases.

As a further indicator of this neuronal condensation, immunoreactivity to an FMRFamide antibody is present in cubomedusae (Parkefelt and Ekstrom, 2009) (R.A.S. and J. Eichinger, in preparation) and, as in scyphomedusae, it includes sensory cells of the tentacles and rhopalia, suggesting a similar function. Recall that the FMRFamide-immunoreactive system in scyphomedusae includes a subumbrellar nerve net (the diffuse nerve net) that directly innervates the swim musculature. In cubomedusae, FMRFamide immunoreactivity is not found in a diffuse network associated with the subumbrellar swim musculature. Rather, it includes a compressed, ring-like network in the pacemaker region of each rhopalium, a similar cluster of neurons in the pedalial base of each tentacle and a few strands of neurites making up small tracts in the nerve ring that extend into the rhopalia and tentacle bases (Fig. 9). The FMRFamide-immunoreactive system of cubomedusae is primarily contained in the centralized nervous system that includes the rhopalia, nerve ring and tentacle bases. This has a significant implication for the physiological organization of the nervous systems of scyphomedusae and cubomedusae and highlights the more centralized nature of the latter group. In scyphomedusae, effector innervation by the diffuse nerve net as well as projection of the network to the rhopalia suggests a combination of RFamidergic peripheral and central inputs. In contrast, the cubomedusan organization suggests a compression of this system to where the peripheral inputs are missing, but with an elaboration of the system within a more centralized system of ganglion-like rhopalia and an interconnecting nerve ring. This places the integrative capabilities of the subsystem within the more condensed nervous system. This analysis assumes a similarity of function of FMRFamide-
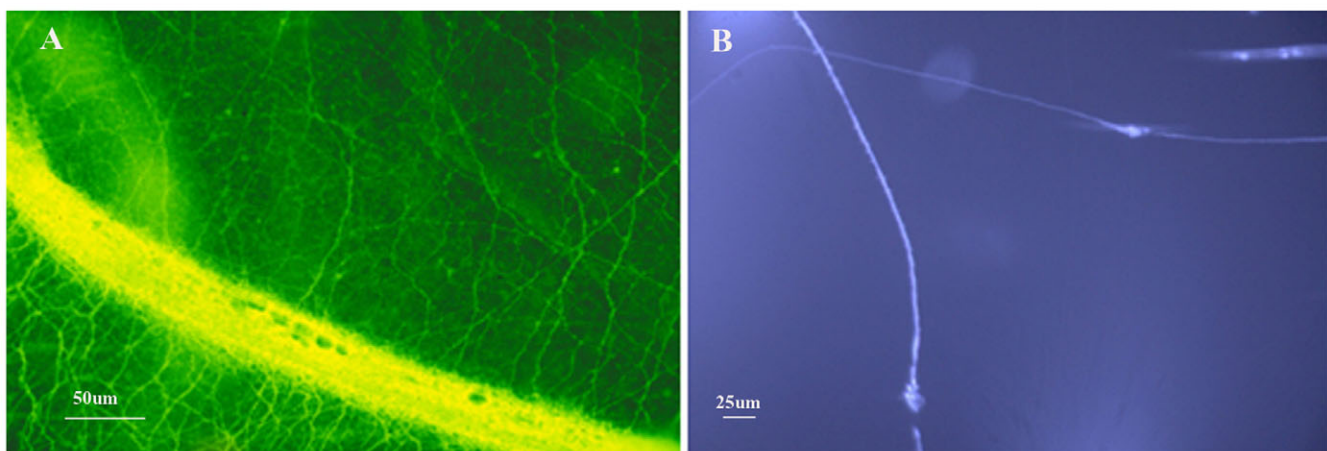

Fig. 7. (A) Nerve ring, subumbrellar nerve net (above nerve ring) and velarial nerve net (below nerve ring), stained with an α-tubulin antibody in the cubomedusa *Tripedalia cystophora*. The nerve nets represent the motor nerve net (verified electrophysiologically in other species). (B) Two individual neurons of the motor nerve net of the cubomedusa *Tamoya haplonema* injected with carboxyfluorescein *via* intracellular recording electrodes. Two independent injections were used. Note the lack of dye coupling in the nerve net. The swellings in the cells are the cell bodies, which were the sites of recordings. In the upper right corner, the out-of-focus cell is an injected circular muscle cell (swim muscle cell).
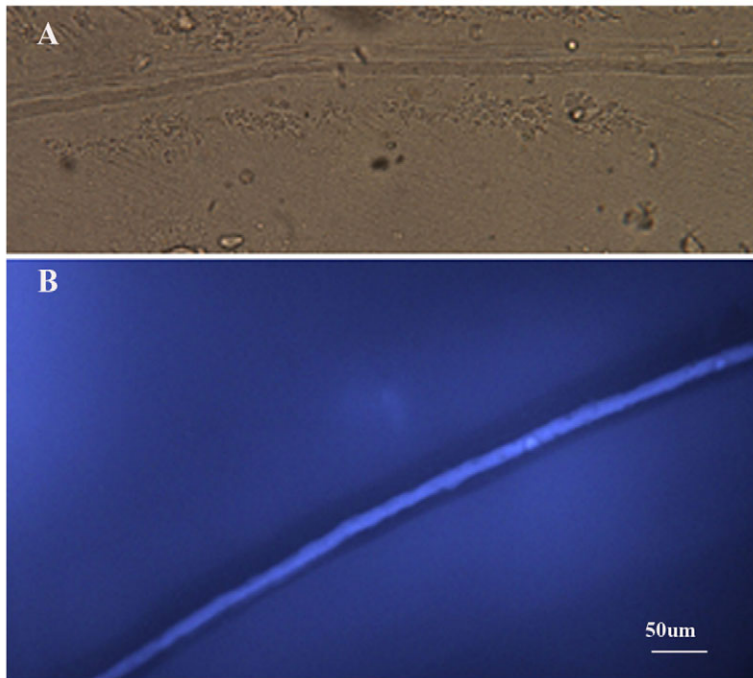
Fig. 8. (A) Nerve ring of the cubomedusa *Tamoya haplonema* in a live preparation viewed with oblique substage illumination. What appears to be a large-diameter axon is seen in the center of the nerve ring. (B) Injection of this large neuron with carboxyfluorescein (injected *via* an intracellular recording electrode). The nerve ring includes the 'clear' regions on either side of the injected neuron.

immunoreactive systems in cubomedusae and scyphomedusae. Such anatomical similarities cannot be used as proof of homology. Yet, these similarities do illustrate a difference in nervous system organization that is also seen in other jellyfish – a compression of nerve nets resulting in a closer association between integrative systems and sensory structures, as well as a compression forming bundles of neurons from multiple conducting systems into nerve-like, directional connectives.

The elaborate structure of cubomedusan rhopalia is demonstrated by more than the photoreceptor complexities. Each of the four rhopalia contains two lensed, complex eyes, two pit ocelli, two slit ocelli, a statolith-like concretion, sensory epithelia, a 'central' neuropil of neuronal processes and synapses, and an outer layer of neuronal and non-neuronal somata (Berger, 1898;

Laska and Hundgen, 1982; Nilsson et al., 2005; O'Connor et al., 2009). In all anatomical respects, these latter structures give the rhopalia the look of ganglia found in bilateral animals (Fig. 10). As a testament to the integrative capabilities of the rhopalia, the various types of eyes have bilateral, directional neuronal tracts that run between the eyes and the pacemaker neuropil, and commissures connecting the tracts (Parkefelt et al., 2005; Garm et al., 2006; Skogh et al., 2006). Both bidirectional and unidirectional synapses have been found in the retinal and pacemaker regions (Gray et al., 2009). Furthermore, the different types of eyes in each rhopalium have been shown to produce different types of modification of pacemaker output when selectively activated (Garm and Mori, 2009). If we then include the possibility of a gravitational sense as well as input from
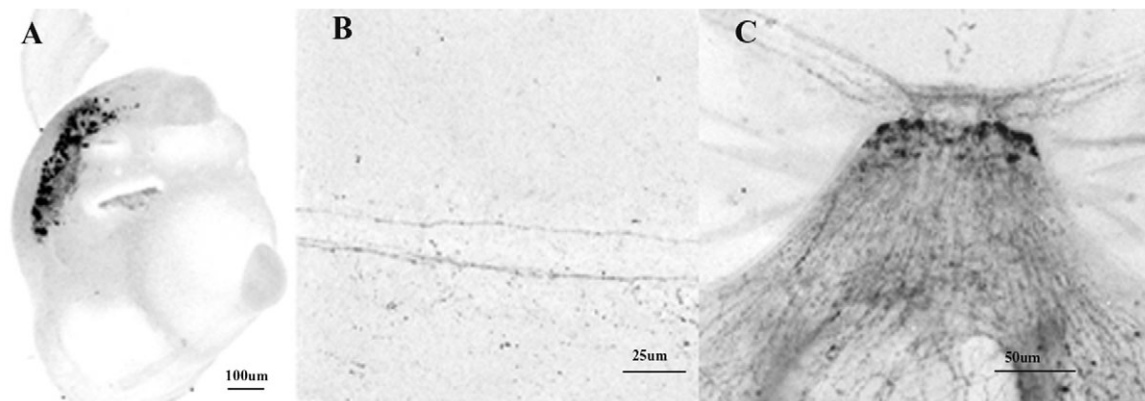

Fig. 9. FMRFamide immunoreactivity of the cubomedusa *Tripedalia cystophora*. The fluorescent photos were converted to grayscale and then 'inverted' because the black-on-white showed greater detail of the fine processes. (A) The immunoreactive cells form a compressed network in the pacemaker region of the rhopalium (in a horseshoe-shaped band partially surrounding the rhopalial stalk; top of photo). (B) The nerve ring has two or three immunoreactive tracts of small fibers, and a very sparse network limited to the vicinity of the nerve ring. This network does not extend throughout the subumbrellar or velarial muscle sheets. (C) A network of immunoreactive cells, somewhat similar to the rhopalial network, is found in the pedalial bases of the tentacles. Note the strands of immunoreactive fibers that run from the pedalial base in each direction in the nerve ring. The network of pedalial neurons gives rise to dense immunoreactive nerve nets that run (downward) through the pedalia and into the tentacles.
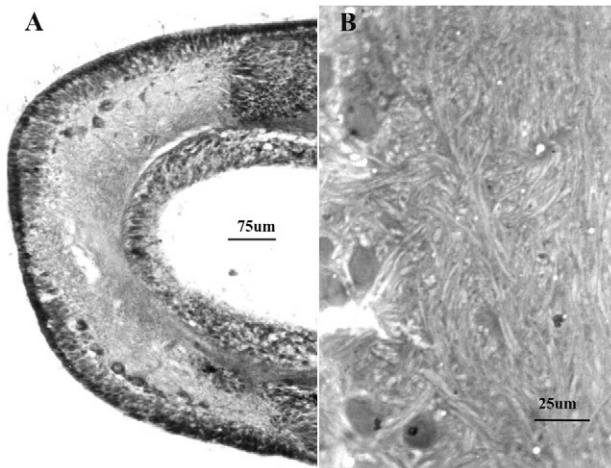
Fig. 10. Silver-stained histological tissue sections from the rhopalium of the cubomedusa *Carybdea marsupialis*. (A) The pacemaker region near the emergence of the rhopalial stalk partially surrounds the central gastrovascular cavity of the rhopalium, and is made up of an outer layer of cell bodies and a central neuropil. A few large somata are found in the neuropillar region. (B) Higher magnification of the neuropillar region, showing the tangle of fibers and a few somata.

sensory epithelia of as yet unknown function, we have a ganglion-like structure that is capable of integrating multimodal sensory inputs for distribution of information to the other integrating centers as well as for distribution of motor output.

Do hydromedusae have ganglion-like structures?

Hydromedusae do not have rhopalia, although some species have small accumulations of neurons in the region of sensory elaborations. Presumably coincident with the prevalence of inter-neuronal and inter-muscular electrical coupling in this group, the 'centralization' of the hydrozoan nervous system takes a different form than in cubomedusae, although it is based on a common organizational feature – the compression of nerve nets into multiple, parallel conduction systems. Some species of hydromedusae have subumbrellar nerve nets that are diffusely organized, but the primary components of the hydromedusan nervous system include inner and outer nerve rings that run around the margin of the bell at the junction of the subumbrella and velum (see Mackie, 2004a). Both nerve rings include multiple conducting pathways which have interconnections within and between nerve rings. In those conducting systems that have been studied at the intracellular level, the individual pathways are each made up of a compressed network of electrically coupled neurons (Anderson and Mackie, 1977; Spencer and Satterlie, 1980; Satterlie and Spencer, 1983; Satterlie, 1985a; Arkett and Spencer, 1986a; Arkett and Spencer, 1986b). As an example, a motor network of large neurons is found in the inner nerve ring that serves as both the pacemaker and motor network for swim contractions (Fig. 11A,B). Similar electrically coupled networks are found in the outer nerve ring and are associated with sensory systems (Arkett and Spencer, 1986a; Arkett and Spencer, 1986b). Within and between nerve rings, the networks interact *via* chemical synapses (Spencer and Arkett, 1984; Mackie, 2004a). The integrative functions of the nerve rings center on properties of the inter-network connections as well as on intrinsic properties of the networks themselves. The nerve rings thus serve a dual role; sorting of various sensory information with intrinsic activity related to rhythmic and directed activities, and radial conduction and coordination of outputs of this centralized system. In other words, the nerve rings serve as the ganglia and connectives of the hydromedusan nervous system, as noted by Mackie (Mackie, 2004a).

In addition to the marginal nerve rings, varying degrees of network compression give rise to radial pathways that can be diffuse or nerve-like, and can include directional conducting routes (Fig. 11C,D) [see Mackie (Mackie, 2004a) for a thoroughly studied example]. Giant neurons are found in some species in both nerve rings and radial nerves. As an indication of the complexity of the hydromedusan nervous system, 14 distinct conducting systems have been described electrophysiologically in one hydromedusan species, *Aglantha digitale* (Mackie, 2004a).
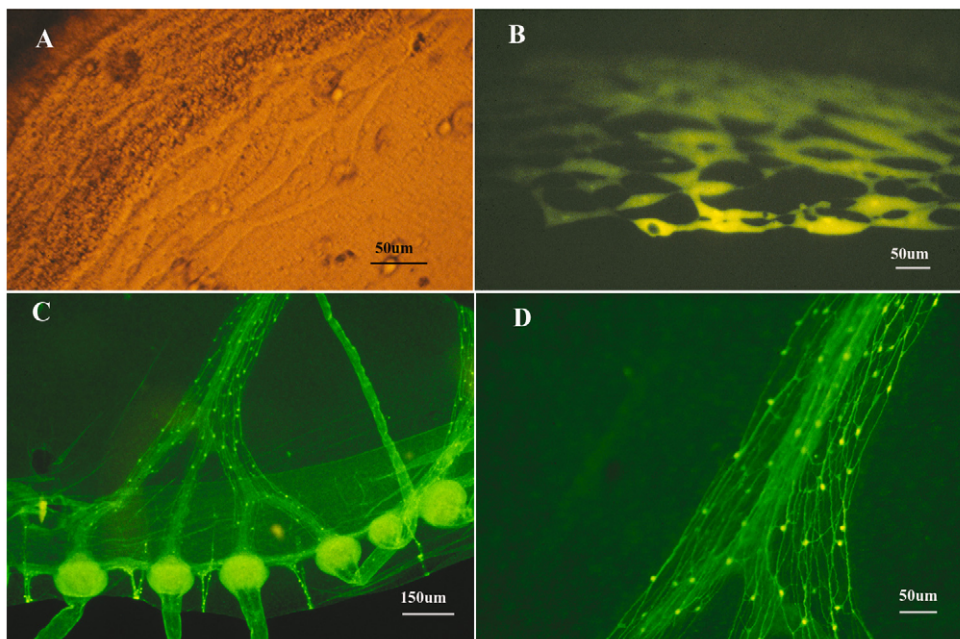

Fig. 11. (A) Inner nerve ring region of a live preparation of the hydromedusa *Polyorchis penicillatus* viewed with oblique substage illumination. The large banana-shaped cells are neurons of the swim motor network. (B) Intracellular injection of Lucifer Yellow from a recording electrode into a single neuron of the swim motor network of *Polyorchis* indicates that the neurons of this compressed nerve net are dye coupled (electrical coupling was also verified with paired intracellular recordings). (C) FMRFamide-immunohistochemistry of the hydromedusa *Proboscidactyla* sp. shows a compressed nerve net that follows the branching pattern of the radial canals. The margin of the animal (with marginal tentacles) is at the bottom. (D) A higher magnification view of the same animal shows the directional nature of the compressed net.

Are cnidarian nervous systems made up of nerve nets?

Getting back to the generalized view of the jellyfish nervous system, the answer to this question is yes and no. Even the compressed networks are just that – net-like groups of neurons that serve particular functions. The cubomedusan ganglia (the rhopalia) include both compressed networks and distinct neuronal tracts and commissures (Parkefelt et al., 2005; Garm et al., 2006; Skogh et al., 2006), suggesting a development of integrating centers that goes beyond the concept of nerve net compression and showing a bilateral anatomy that bucks the organization trend of radial symmetry. But regardless of how we want to label the condensation of nervous tissue in jellyfish, one common feature brings it in parallel with bilateral central nervous systems – the close association between this condensation and the elaboration of sensory structures, some of which are diffusely arranged in marginal structures including tentacles, and some of which are placed in more widely spaced structures around the bell. The best examples of the classical diffusely arranged nerve nets are used for distribution of the excitation to effectors, which, in the case of swim musculature, takes the form of broad two-dimensional sheets of muscle cells.

What should be considered 'primitive' in the cnidarian nervous system?

The phylogenetic position of cnidarians begs for comment on the most 'primitive' or 'basal' properties of multicellular nervous systems, even though we study extant animals that may not closely resemble true basal forms. Nonetheless, this where the nerve net looms large. But is it accurate to say the nerve net is a defining primitive feature? In other words, is it the hallmark of the cnidarian nervous system or merely a feature that has persisted, but with varying degrees of elaboration that include co-condensation of sensory and neural structures into more compressed central systems? More importantly, with the range of nervous system complexity seen in jellyfish (and in the phylum as a whole), an important question becomes: what is primitive within cnidarian nervous systems? In the older literature (e.g. Parker, 1919; Lentz, 1968), we read about how epitheliomuscular cells, some of which also have a sensitivity to some environmental perturbation, can form an epithelium that functions as a single tissue-type reflex arc. We read about independent effectors, and how the cnidarian nervous system does not quite fit the sensor–adjustor–effector wiring plan of higher animals. In this view, nerve nets are comprised of 'ganglion cells' that lie between the sensors and effectors but are arranged in a diffusely conducting network and thus cannot be considered true 'adjustors' (Parker, 1919). We also read about how concentrations of neurons can form ganglia, but this system is still too diffuse to be considered 'central' (Lentz, 1968).

But let's take another step back and think about what is primitive in a general sense in the cnidarians. The generalized view, which has also been questioned, is that sponges are at the cellular level of organization, cnidarians are at the tissue level of organization and higher animals are at the organ or organ-system levels of organization. If we ignore the possibility of some cnidarian structures being organ-like and focus on structures like epitheliomuscular cells, conducting epithelia and other multi-functional tissues, we can form a loose correlation between the relative specialization of cell types and what can be considered primitive, realizing that a lack of specialization can be of secondary development. For many cnidarian tissues, individual cells serve multiple roles, i.e. they are more generalized in their function.

Hence, we have epithelial cells that have muscular feet and serve the appropriate functions of a surface epithelium as well as produce appropriate movements for the animal. Some epithelia can even conduct meaningful electrical signals to or from the nervous system (Mackie, 2004b). In some cnidarians, muscle cells have lost their epithelial component and, at least functionally, may be considered more specialized.

How about the nervous systems? Individual neurons in cnidarians are highly specialized. In comparison to neurons of bilateral animals, they contain close to a complete set of signaling molecules that are involved in nervous system development, maintenance and communication (see Watanabe et al., 2009). This puts the individual neurons of cnidarians on a level of complexity similar to that of neurons of higher animals. What is left, then, is to figure out what is primitive in the way these neurons are arranged and used. The important part of this challenge goes beyond structural characteristics of the nervous system, and includes what they do for the animal. For example, in some cnidarians, a single nerve net may include cells that sense some environmental variable and convey the information directly to effectors with network and neuromuscular properties serving as the integrative source of effector variability. This organization could be considered to be of the sensory–effector type. Now consider the situation in cubomedusae, where sensory structures provide information to a ganglion-like rhopalial–nerve ring integrating system. Appropriate output from this 'centralized' system is then passed out to effectors *via* a nerve net that, anatomically, looks identical to the previously described nerve net. This latter system is definitely of the sensory–adjustor–effector design and the nerve net is specialized for innervation of effectors. Yet on anatomical grounds, a nerve net is a nerve net is a nerve net. Even if a significant degree of integration still occurs at the level of neuromuscular connections, this does not signal primitiveness unless we want to put that label on neuromuscular systems of higher invertebrates as well. A familiar caution must be extended here: it is not possible to accurately predict physiology from anatomy. In our case, it is not possible to determine primitive or basal features from anatomy alone. This may seem like a strange statement as nearly all of the evidence presented here is anatomical. However, nearly all of the work cited is from laboratories that combine neuroanatomy with neurophysiology or at least emphasize functional morphology. And from this perspective, a nerve net that is used solely for the distribution of motor information is more specialized than a nerve net that has sensory, integrative and motor distribution functions. We actually see a gradation in this regard when comparing the organization and function of scyphomedusan and cubomedusan motor systems. This caution is similar to one that must be heeded for the new genomic data – primitiveness or basal position cannot be determined by gene sequences and BLAST searches alone. It is necessary to determine what genes are expressed and when, and what they do for the animal. We need functional morphology as much as we need functional genomics. And this does more than emphasize the need for a more integrative look at processes of evolutionary change or of animal design and adaptation. It brings organismal biology fully into the loop of this integrative approach.

Back to the original question, and bring on the straightjacket if you will, but the sensor–adjustor–effector organization of cubomedusae and hydromedusae certainly argues for the existence of a centralized nervous system in cnidarians. But even in portions of a nervous system that are composed of diffuse networks of neurons that conduct information in an unpolarized manner, a nerve net is not a nerve net is not a nerve net.

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