Evolution of the first nervous systems – what can we surmise?

The success of the Metazoa can be attributed, in large part at least, to the presence of a nervous system. This provides them with the means to integrate multiple sensory inputs and produce appropriate and directed responses that typically require rapid intercellular communication over large distances. Nervous systems are exceedingly complex: it has been determined that as much as 70% of an animal's genome is expressed in a single nerve cell (Moroz et al., 2006; Citarella et al., 2011). Thus, harnessing the multiple tool boxes that enable what we would define as neuronal function – the ability to produce and propagate action potentials, and to produce and release neurotransmitters, and to receive synaptic input from other neurons, and to produce and maintain long processes and transport molecules and organelles along the length of those processes – was clearly not a trivial evolutionary step. Nevertheless, it evolved at least once and perhaps multiple times (Moroz, 2009; Moroz et al., 2014).

Our understanding of neurobiology, as a whole, has advanced enormously over the past 50 or so years, but the evolutionary origins of nervous systems, and questions such as 'when did nervous systems first appear?', 'what was the selective pressure that drove what is an exceedingly complex and energetically expensive trait?' and 'did that indeed happen multiple times?' have barely been addressed. These questions, amongst others, were discussed at a meeting held in Florida in May 2014 – entitled 'The Evolution of the First Nervous Systems II' – from which the articles in this special issue were commissioned. This Inside JEB article summarizes the many ideas put forward by participants during organised discussion sessions at the meeting, together with the consensus views of the participants, which have shaped the articles in this issue.

When did neurons evolve?

The recently completed genomes of the ctenophores *Mnemiopsis leidyi* (shown here) and *Pleurobrachia bachei* have radically altered our understanding of early metazoan phylogeny and, most notably, the origin of nervous systems. Photo credit: Peter Anderson.

A natural starting point for any discussion about the origin of the first nervous systems is to look at which taxa actually have neurons. Porifera and Placozoa do not have clearly defined neuronal cell types and for a variety of reasons have been held to be basal to the rest of the metazoans. Therefore, it was most parsimonious to assume that the ancestral metazoan did not possess neurons, but that nervous systems evolved in the lineage towards the last common ancestor of Cnidarians, Ctenophores and Bilateria. This view is challenged, however, by two ctenophore genomes that have recently been completed (Ryan et al., 2013; Moroz et al., 2014). Both publications argue for a phylogenetic position of Ctenophores as the sister group of all other metazoans, and for the possibility that either Ctenophore neurons evolved independently from neurons in Cnidaria and Bilateria, or that Porifera and Placozoa have lost neuronal cell types,

possibilities that are discussed in this special issue by Leonid Moroz (Moroz, 2015).

In this context it is interesting to note that some synapses in ctenophores (Hernandez-Nicaise, 1973) and cnidarians (Anderson and Grunert, 1988) share a novel presynaptic morphology, consisting of a single layer of a few large synaptic vesicles that is sandwiched between the cell membrane and a large cisterna that has, in turn, one or several large mitochondria positioned against its cytoplasmic surface. The presence of this 'presynaptic triad' (Hernandez-Nicaise, 1973) in representatives of two phyla (Ctenophora and Cnidaria) but its absence in any other organism with a nervous system, might argue for a closer relationship between the two phyla than the latest phylogenies would suggest.

One caveat recognized by scientists working in this field is that the latest metazoan phylogeny, which has the Ctenophora as the most basal group, is based on the genome of a single genus of Porifera, making it imperative that other sponge genomes be fully sequenced. Only then will it be clear that the ctenophores are the last common ancestor to all metazoans and that the sponges did indeed lose their nervous system. The same can be said for the Placozoa, but as this phylum contains only a single species, *Trichoplax adhaerens*, no additional genomic information can be obtained to confirm this phylogenetic position. The status of the Placozoa in nervous system phylogeny is further complicated by a measure of uncertainty with respect to the question of whether the Placozoa have indeed lost their nervous system. Earlier work (Grell and Ruthmann, 1991) identified only four cell types in *Trichoplax*, none of which were described as neurons. More recent work (Smith et al., 2014), however, has identified six somatic cell types, one of which contains proteins typical of neurosecretory cells, including a FMRFamide-like peptide, opening the door, at least, to the possibility that neurons or neuronal-like cells exist in this phylum.

What is a neuron?

The motor nerve net of the scyphozoan jellyfish *Cyanea capillata*, shown here in an exposed cell preparation, is composed of long bipolar neurons that are functionally non-polarized and connected by bidirectional chemical synapses. Photo credit: Peter Anderson.

Nervous systems are assemblies of nerve cells or neurons. Inherent in any discussion on the evolution of the first nervous systems, therefore, is the question of what is a neuron. In most cases, anatomists and physiologists find it straightforward to identify cells as neurons ('we know them when we see them'), in a way that is reminiscent of the pluralistic biological species definitions (Mishler and Donoghue, 1982). However, this approach is less useful when there is little knowledge about cellular function or when cells lack the long processes (the axons and dendrites) that, for many, are the defining feature of a neuron.

Why is it so difficult to develop a set of criteria to define what we mean by a neuron? First, there is no universal genetic marker for neurons. As noted above, the transcriptome of a single nerve cell can be as much as 70% of an animal's genome (Moroz et al., 2006; Citarella et al., 2011). Furthermore, a large percentage of neuronal genes are also expressed in non-neural cell types and no specific genes are expressed in all neurons. Second, even the functional components that are used diagnostically to identify a cell as a neuron are not exclusive to neurons or are not present in all neurons. The genes underlying components generally associated with what we would call a neuron have evolved in different contexts and are mostly older than metazoans. For example, six transmembrane domain voltage-gated ion channels certainly play a prominent role in neuronal excitability. However, they are present in bacteria (Liebeskind et al., 2011; Zakon, 2012;

Moran et al, 2015), and are also present in non-neural cell types like muscle cells, epithelial cells and osteocytes. Interestingly, whole signaling complexes seem to have been recruited as gene expression networks into neuronal function; for example, the presynaptic machinery for vesicle release at chemical synapses, and the postsynaptic receptor and scaffold complexes contain interacting proteins that are already present in secretory and receptor structures in fungi, choanoflagellates and Porifera (Conaco et al., 2012), as discussed in this issue by Pawel Buckhardt (Burkhardt, 2015).

Structural criteria are also inadequate. Some glial cells and osteocytes bear long processes reminiscent of dendrites, while some interneurons have a fairly compact morphology lacking clearly defined axons and dendrites. In this respect, it is interesting that none of the authors in this special issue, or other participants at the associated meeting and beyond, are aware of any evolutionary precedent for what inevitably remains that most 'neuronal' phenotype: the permanent or semi-permanent microtubule-based process that forms the axon or dendrite. Harnessing the components of the tool box required to generate such a structure is surely far from trivial, yet may have occurred more than once. In the context of axons and dendrites, it is clear that neurons need not be functionally polarized. The motor nerve net of the jellyfish *Cyanea* is composed of bipolar neurons that make random contact with one another to create a classical, diffuse nerve net. Conduction through this nerve net and individual neurons in that nerve net is completely non-polarized (Anderson, 1985) thereby enabling activity to be conducted to the swimming muscle from any one of the eight marginal ganglia.

Recognizing that there will inevitably be exceptions, the most pragmatic definition of the neuron might be a functional one, along the lines of 'a cell dedicated to electrical communication that targets discrete, distant cells by way of synapses with both pre- and post-synaptic elements'. This definition excludes electrically excitable epithelia as they only target adjacent cells and would also exclude neuroendocrine cells as there is no post-synaptic element to the release site of such a cell.

What were the first neurons for?

Another subject worthy of much discussion is why neurons evolved. It is easy to see the advantages that nervous systems provide extant animals – the ability to integrate complex sensory information and translate the results of that integration into complex behavior – but what was the selective advantage gained by the first organisms that harnessed the complex machinery inherent in a neuron? Electrically excitable epithelia, which are prevalent in the Hydrozoa, for example, can and do function to gather sensory information (Mackie and Passano, 1968) and coordinate effectors (Mackie, 1976) but they lack specificity and directionality – mechanosensory information acquired by the exumbrellar epithelium of a hydromedusa (Mackie and Passano, 1968) or skin of a frog tadpole (Roberts, 1969) merely informs the animal that it has received a mechanical stimulus but provides no information as to the exact point of stimulation that the animal could use to direct any escape response. Similarly, effectors activated by an electrically excitable epithelium are all activated simultaneously with no specificity. It is arguable that neurons likely provided animals with, for the first time, the ability to control opposing effectors; to work against a hydrostatic skeleton, for example, to elongate or contract the body, or to open or close feeding appendages.

The need for functional studies

In terms of future research on the subject, several important issues can be identified. As noted earlier, it is imperative that additional sponge genomes are sequenced in order to validate the latest phylogenetic trees that position the Ctenophora as a sister group to all other metazoans and, as such, the first group to have evolved a nervous system. Second, while molecular biology has provided enormous insights into early metazoan phylogenies, there has been a distinct dearth of new information on the 'neurobiology and neurophysiology' of the same groups, and thus it is imperative that a significant effort be made to address this shortfall.

Only when we have far more information on how the behavior of the basal Metazoa is regulated will we be able to appreciate when that most important of metazoan attributes, the nervous system, evolved and whether that happened once or multiple times.

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