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Where is my mind? How sponges and placozoans may have lost neural cell types

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Recent phylogenomic evidence suggests that ctenophores may be the sister group to the rest of animals. This phylogenetic arrangement opens the possibility that sponges and placozoans could have lost neural cell types or that the ctenophore nervous system evolved independently. We critically review evidence to date that has been put forth in support of independent evolution of neural cell types in ctenophores. We observe a reluctance in the literature to consider a lost nervous system in sponges and placozoans and suggest that this may be due to historical bias and the commonly misconstrued concept of animal complexity. In support of the idea of loss (or modification beyond recognition), we provide hypothetical scenarios to show how sponges and placozoans may have benefitted from the loss and/or modification of their neural cell types.

1. Introduction

Animals consist of five major lineages: Ctenophora (comb jellies), Porifera (sponges), Placozoa (represented by a single described species, *Trichoplax adhaerens*), Cnidaria (e.g. corals, sea anemones and jellyfish) and Bilateria (all other animals, 99% of all species). Understanding the phylogenetic relationship of these lineages is critical for understanding the early evolution of animals. Historically, sponges were thought to be the sister group to the rest of animals, a hypothesis that was consistent with the assumption that neural cell types arose after sponges (and perhaps *Trichoplax*) diverged from the rest of animals [1]. Recent findings from phylogenomics [2–4] support the position of Ctenophora as the sister group to all other animals (figure 1) [5–7], challenging these long-held assumptions.

Under this new phylogeny, two possibilities exist, either (i) neural cell types evolved once in the stem leading to the most recent common ancestor of all animals and were subsequently lost (or modified beyond recognition) in Porifera and Placozoa, or (ii) neural cell types arose independently twice, once in the stem of Ctenophora and once in the stem leading to Cnidaria and Bilateria [8]. To avoid problems associated with defining neurons [9,10], we will consider that the definition excludes cells present in extant Porifera or Placozoa.

Much of the discussion thus far has centred around the hypothesis that neural cell types evolved independently in ctenophores and the stem lineage leading to cnidarians and bilaterians [5,11–14].

Arguments have largely focused on the absence of bilaterian nervous system components in ctenophores (e.g. neurogenic and neurotransmission genes). However, these claims are far from conclusive and in many cases have satisfactory explanations outside of parallel evolution (table 1). Other authors have rejected a scenario invoking independent evolution of neural cell types in ctenophores, but express major doubt about the evolutionary loss of neurons in sponges [16,23]. Indeed, some authors find it highly unlikely, based on comparisons of sponges and ctenophores, that sponges lost neurons regardless of the phylogenetic position of ctenophores [24]. In this review, we argue that sponges and placozoans had recognizable neurons at some point in history that were lost or modified
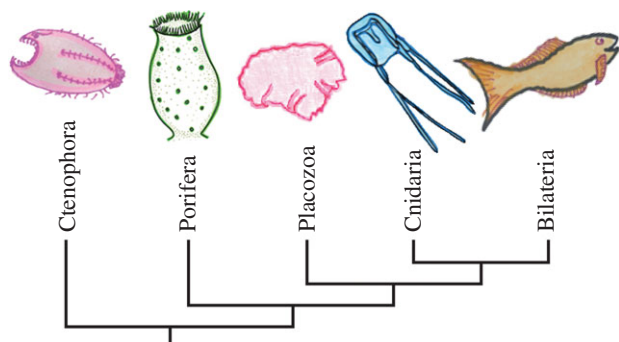
Figure 1. Evolutionary relationships of the five main branches of animals based on recent phylogenetic analyses [2–6]. (Online version in colour.)

beyond recognition. We discuss why this view might not be looked upon favourably and provide hypothetical scenarios as to how this transition may have occurred.

2. Historical bias impedes acceptance of neural cell-type loss

Surprisingly, the increasing support for ctenophores as the sister to the rest of animals has led many to either rally behind the independent evolution of neurons [13] or to assume that the phylogeny is an artefact (e.g. [25]). Few authors have acknowledged the possibility that sponges and *Trichoplax* may have lost a nervous system (though see [26]). We contend that this may be due to historical bias.

For as long as sponges have been firmly recognized as animals, they have been considered the earliest offshoot of the animal tree [27]. As such, complex characteristics such as neural cell types that are seemingly absent from sponges and present in most other animals were thought to have arisen after sponges diverged from other animals [28]. It has been acknowledged that sponges have the machinery necessary for a nervous system, but that for whatever reason neurons did not evolve in this lineage [9]. Consequently, sponge research has operated from the perspective that these animals are an important model for understanding the transition from an aneural to neural condition [29]. The view is summarized in this quote from a recent perspective piece in the journal *Science*: 'Many scientists think these marine and freshwater filter feeders are the living creatures most similar to the common ancestor of all animals. And to many researchers, sponges look like animals on the verge of a nervous breakthrough.' [30]

Because multicellularity is considered more complex than unicellularity, it is assumed that a certain amount of early animal evolution had to fit into the mould of 'simple to complex'. Consideration of the sponge lineage as an 'Almost Group' [9] is compatible with this notion and puts the last common ancestor of all animals within this upswing of complexity. We argue that the age and perceived elegance of this view has led to bias against the possibility that the absence of neural cell types in sponges, and to a lesser extent placozoans, can be explained by secondary loss.

However, there are flaws in the view even at the level of its elegance, as complexity is difficult to measure and more often based on anthropocentric criteria. If sponges, instead of humans, were writing the textbooks they would dismiss human complexity criteria (e.g. number of distinct cell types, the presence of brains, appreciation of art, etc.) as these criteria do not enhance sponge lifestyle. Rather, they might measure complexity by an organism's ability to filter water in terms of volume compared with body size, efficiency in distinguishing between very small (less than 1 μm) organic and inorganic particles, or its level of production of bioactive metabolites. Under these criteria, sponges would be at the apex of the complexity ladder. Similarly, placozoans might rank complexity by an organism's ability to quickly adjust body shape to the current environment, or to split itself in two. From this perspective, it is clear that 'complexity' should be left out of the equation as to the phylogenetic position of sponges and placozoans, as well as to the question of whether they lost neural cell types or any other feature found in other animals. All animals are complex in their own way and as such they gain and lose components, which contributes to their ability to adapt to a particular lifestyle.

3. Porifera: 'neural' genes, cells and behaviour

Sponges are said to lack true neural cell types [31], yet these animals have genes associated with neurons, behaviours indicating sensing of external stimuli, and secretory cells involved in sensory behaviour that express neural genes. Sequencing of the demosponge genome *Amphimedon queenslandica* [32] and multiple transcriptomes from all major sponge lineages [33] have revealed a remarkable number of genes present in sponges that are associated with neural cell types in bilaterians and cnidarians. These include (i) genes associated with fast synaptic transmission (e.g. voltage-gated ion channels [34], and the neurosecretory SNARE proteins syntaxin1, SNAP25 and synaptobrevin [35,36]), (ii) primary specification of neurogenic fields (e.g. components of the Notch-Delta signalling system and a pro-neural basic helix loop helix gene [37], (iii) enzymes involved in the synthesis of neurotransmitters (e.g. DOPA decarboxylase and tryptophan hydroxylase [33]) and (iv) the vast majority of genes associated with the postsynaptic scaffold (e.g. NOS, Citron and ionotropic glutamate receptors [33,38]). It should be noted that many of these genes date back prior to animal multicellularity and have roles outside of the nervous system [19]. These other roles may be why these genes have not been lost, despite the loss of a nervous system.

Much work has been performed to characterize the larval cell types of the demosponge *A. queenslandica*. There are three cell types of the sponge larvae that are thought to be sensory-related. The posterior end of the larvae contains specialized photoreceptor cells (including a ring of pigmented cells with photosensory membranes and a long cilium) that regulate phototaxis [39]. Another larval cell type, globular cells, is packed with vesicles and migrates from the subepithelial (middle) layer (throughout the larva) during development to intercalate with columnar epithelial cells [37]. These subepithelial cells express notch, delta and a bHLH gene related to atonal and neurogenin. The globular cells express delta, as well as a subset of the postsynaptic genes including DLG, GKAP, GRIP, HOMER and CRIPT. At the anterior end of the larvae a group of monociliated epithelial cells, called flask cells, receive metamorphic cues [40]. These cells have putative intracellular junctions connecting them to surrounding epithelial cells, though no chemical synapses have been found [40].

Table 1. Review of evidence supporting independent evolution of nervous systems.

evidence put forth to support independent evolution of neurons	reason evidence is not conclusive
ctenophores lack the majority of components essential for canonical neuronal signalling (acetylcholine, serotonin, dopamine, noradrenaline, adrenaline, octopamine, histamine or glycine) [2–6,13,14]	acetylcholinesterase is absent from *P. bachei*, but present in *M. leidyi* [4], and acetylcholine (along with adrenaline) have been shown to play a role in *M. leidyi* bioluminescence [15]
ctenophores use a different subset of small secretory peptides for inter-neuronal signalling [5,13,14]	lineage-specific alteration of neuropeptides beyond the limits of recognition of homology has been observed in bilaterians, e.g. in flatworms [16]
ctenophores are missing many genes that control neuronal fate and patterning in bilaterians (e.g. neurogenin, NeuroD, Achaete–Scute, REST and HOX/otx) [13]	many of the genes that control neuronal fate are present (e.g. Sox, Lhx, bHLH, Hes) [4] and several Sox, Lhx, and bHLH genes show expression patterns consistent with neural expression in ctenophores [17,18]
genes critical for pre- and postsynaptic genes functions in cnidarians and bilaterians have a reduced representation in ctenophores [13,14]	the vast majority (approx. 25/32) of the postsynaptic scaffolding components of cnidarians and bilaterians are clearly present in *Mnemiopsis leidyi* [4]. There has yet to be a comprehensive analysis of presynaptic genes in ctenophores but many of the vesicle exocytosis and presynaptic active zone proteins (e.g. Erc/Cast, Mint and Liprin-α) are present in non-metazoan eukaryotes [19]
ctenophore synapses have a unique organization forming a 'presynaptic triad' [20]	this morphology is not unique to ctenophores. The cnidarian *Cyanea capillata* has tripartite synapses similar to ctenophore synapses [21,22]

Sponge larvae are able to rapidly respond to environmental stimuli using a range of orienting behaviours including geotaxis, phototaxis and rheotaxis (reviewed in [41]). They are able to select substrates for settlement [42]. Adults from all four classes are able to expel sediment obstructions via contraction [24,43]. Similarly, adult sponges are capable of locomotion (discussed in [44]). All adult movements are very slow compared with other adult animals. In cnidarians and bilaterians, these behaviours are often associated with rapid neurotransmitter-based signalling, but this does not seem to be the case in sponges [43,45,46].

Sponges have genes, cells and behaviours associated with nervous systems. Historically, these data have been interpreted from the perspective that sponges never possessed neural cell types. However, the data are compatible with a nervous system being present in the stem ancestor of sponges.

4. Placozoa: 'neural' genes, cells and behaviour

Placozoans are flattened discoidal creatures surrounded by flat epithelial cells that enclose multinucleate cells. *Trichoplax* is thought to consist of six different cell types, none of which present an obvious similarity to a neural cell type [47]. Despite the apparent lack of neural cells, a surprisingly high number of genes orthologous to animal neural genes have been described from the *Trichoplax* genome. These include: (i) transcription factors with known pro-neural activity in cnidarians and bilaterians (e.g. Sox, LIM, bHLH, Six-HD and Pou-HD families), (ii) components of neurotransmitter biosynthesis, (iii) pre- and postsynaptic components (e.g. syntaxin, synaptobrevin, SNAP25, and subunits of potassium, sodium and calcium channels), (iv) several members of the GPCR family, which are typically involved in signal transduction, and (v) genes associated with

axon guidance and neural migration (NCAM, netrin and slit, among others) [48]. Likewise, at least five predicted regulatory peptide precursors have been identified in *Trichoplax* [49].

While *Trichoplax* has no obvious neurons, it does have neurosecretory cells concentrated in the periphery of the ventral epithelium [50]. These cells express typical neural proteins including syntaxin, synapsin, synaptobrevin and a FMRFamide-like neuropeptide [47]. These cells are currently viewed as evolutionary precursors to true neural cell types, but might just as easily be viewed as remnants of a more traditional nervous system.

Placozoans have complex behaviours. They have distinct dorsal and ventral sides and when they are flipped ventral side up, these animals will 'right' themselves (i.e. ventral side down) [51]. Unpublished observations of both positive and negative phototaxis are reported in the *Trichoplax* genome paper [48]. *Trichoplax* show preference towards slides that have been conditioned in natural seawater (with food) versus sterile seawater (without food) [52]. Lastly, *Trichoplax* has distinct feeding behaviours including flattening at higher food concentrations, and increased locomotion in the presence of food deprivation [53].

Placozoans have genes, cells and behaviours associated with nervous systems. These have been viewed as precursors of neural components and innovations of an ancestor that never had neural cell types. However, an equally compatible idea is that these genes, cells and behaviours are vestiges of an ancestral nervous system.

5. Hypothetical scenarios

It is difficult to imagine how losing a nervous system could benefit a lineage of animals when considering how reliant
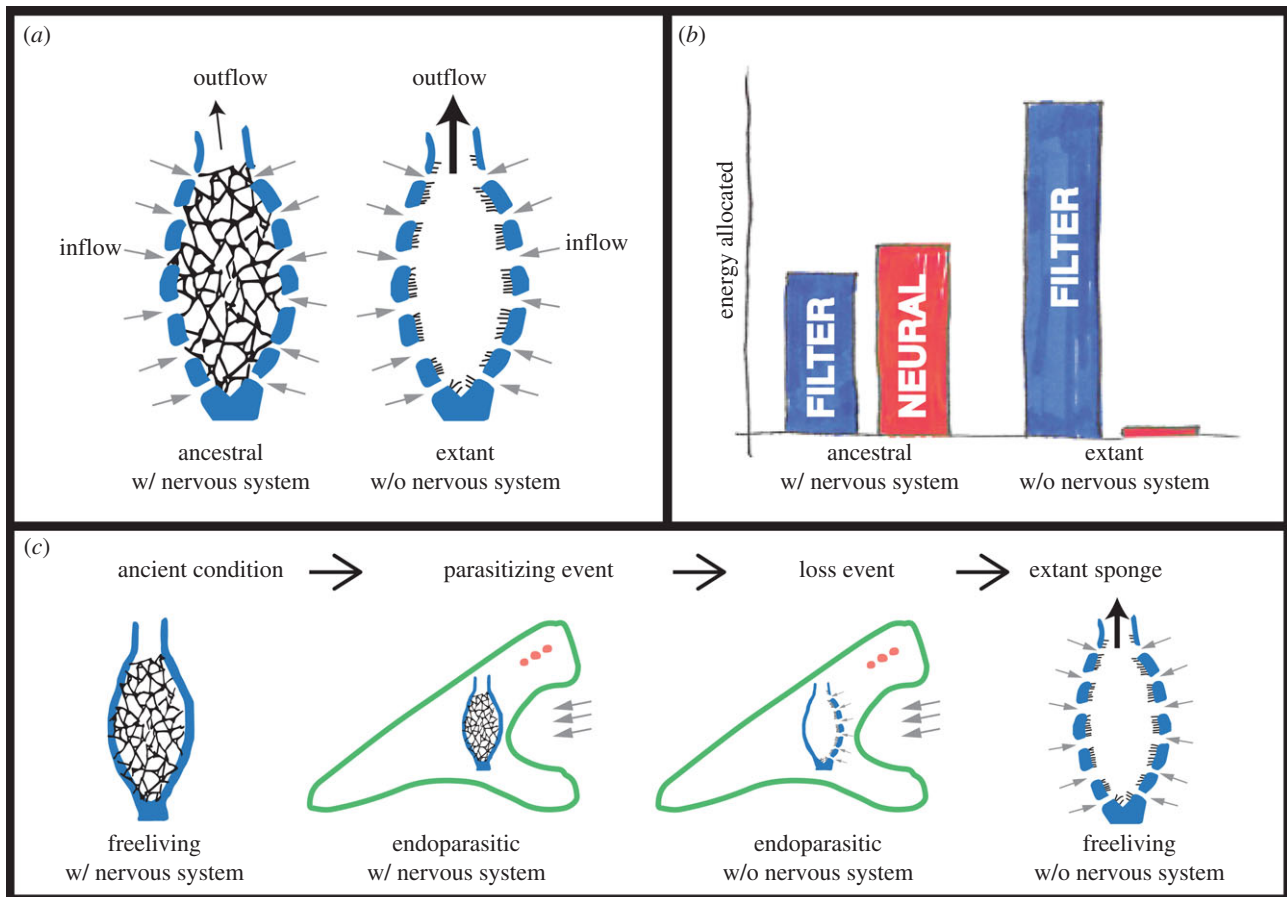
Figure 2. Hypothetical scenarios for the loss of neural cell types in sponges. (*a*) A nerve-net type nervous system in ancestral sponge may have provided a physical obstruction to maximal filtering efficiency. Losing this nerve net may have been selectively advantageous. (*b*) The metabolic cost of neural mechanisms is high. The loss of neural cell types may have provided a selective advantage by allowing for the reallocation of resources to filtering activities. (*c*) Ancient endoparasitic lifestyle of Porifera and/or Placozoa. It is possible that the stem ancestors of either poriferans or placozoans lived as endoparasites, and that some event led to them reverting to a free-living lifestyle. As parasitism can be associated with loss of many cell types including neurons [59], this hypothetical scenario might explain why it is not possible to represent neural cell types in poriferans and placozoans. (Online version in colour.)

so many animals are on sensing the environment with neurons. It is helpful then to consider hypothetical scenarios as to how neural cell types could have been lost or altered beyond recognition.

Scenario no. 1 involves a modification of neural cell morphology to enhance filtering efficiency (figure 2*a*). Sponges are capable of filtering nanoscaled particles from large quantities of water using a sophisticated pipeline of different cell types [31]. It is possible that neuronal projections may have negatively affected filtration. An adaptive advantage may have been achieved by the lineage that lost these projections perhaps through the shutdown of axon guidance machinery.

Scenario no. 2 involves the loss of neural cell types for reallocation of resources to filtering (figure 2*b*). Adult sponges are able to filter water to a near-sterile state with great efficiency [54]. The metabolic cost of this process is probably one of the greatest expenses to a sponge. Maintaining a nervous system would certainly shift metabolic resources away from filtering processes. Individual sponges that had developed alternative low cost solutions to larval taxis, adult contractions and other responsibilities served by neural cell types may have been able to invest more resources in filtering and developed a selective advantage.

Scenario no. 3 involves *Trichoplax* and/or Porifera having had an endoparasitic lifestyle at some point in history, which led to the loss or modification beyond recognition of neural cell types (figure 2*c*). Several endoparasites including orthonectids,

dicyemids and myxozoans appear to have lost nervous systems [55,56]. Interestingly, myxozoans, like poriferans and placozoans, exhibit complex behaviours (helical swimming and stationary coiling [57]), and appear to have neural genes like syntaxin, synaptotagmin, DLG, CRIPT, Erbin and CASK (we recovered reciprocal best BLAST hits from the recently published transcriptome of the myxozoan *Polypodium hydriforme* [58]) [55,56]. It is possible that, like myxozoans, ancient stem ancestors of *Trichoplax* and/or Porifera lived as endoparasites and lost their nervous system, but then later reestablished a free-living lifestyle. The feeding modes of both Porifera and *Trichoplax* (i.e. filtering and external digestion, respectively) are perhaps not far off from what one might expect from a former endoparasite.

6. Proving loss of nervous systems?

Directly proving that sponges and placozoans lost neural cell types will be extremely difficult if not impossible. One can think of transgenic experiments involving 'neural' genes from a sponge, *Trichoplax*, or even a non-metazoan eukaryote, but as we do not know the genetic background of the ancestors where neural cell types were lost, these experiments are problematic [60]. Instead, proof will depend on two avenues of research involving ctenophores. First, it will be important to establish the homology of neural cell types in ctenophores

and cnidarians/bilaterians. This will involve functional experiments in ctenophores showing that the majority of the 'neural' genes in ctenophores are involved in ctenophore neural cell types [8]. Secondly, despite the mounting evidence supporting ctenophores as the sister group to the rest of animals and the lack of strong data supporting sponges in this position, it remains necessary for claims of long-branch attraction to continue to be addressed and for more data to be applied to the question. If both of these requirements are met, it will be necessary to infer that loss or severe modification are responsible for the apparent absence of neural cell types in sponges and placozoans.

ancestors of these lineages, and these cell types were lost completely. Our functional understanding of cell-type make-up and specification are at an early stage. As more animal genomes are characterized and more model systems are functionally analysed at the scale of individual cells, we will have much more resolution from which to understand the history of animal cell types. In the meantime, it is useful to throw a wide net and in the case of sponges and *Trichoplax* entertain the possibility that they lost neurons or that cells with sensory characteristics in these animals might represent vestiges of neural cell types rather than ancestors of cells that almost made it to neuronville.

7. Conclusion

It is quite possible that the ancestor of all animals had neural cell types similar to the neurons of modern-day Ctenophora, Cnidaria and Bilateria. The make-up and specification of these cell types would have been continuously modified by natural selection and random processes for hundreds of millions of years. In the case of poriferans and placozoans, it is possible that these modifications have left these cell types unrecognizable. Alternatively, the specification of neurons may have been actively suppressed in one or both stem

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