Essays in Biochemistry (2022) **66** 781–795
https://doi.org/10.1042/EBC20220042

PORTLAND PRESS

Review Article

The premetazoan ancestry of the synaptic toolkit and appearance of first neurons

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Neurons, especially when coupled with muscles, allow animals to interact with and navigate through their environment in ways unique to life on earth. Found in all major animal lineages except sponges and placozoans, nervous systems range widely in organization and complexity, with neurons possibly representing the most diverse cell-type. This diversity has led to much debate over the evolutionary origin of neurons as well as synapses, which allow for the directed transmission of information. The broad phylogenetic distribution of neurons and presence of many of the defining components outside of animals suggests an early origin of this cell type, potentially in the time between the first animal and the last common ancestor of extant animals. Here, we highlight the occurrence and function of key aspects of neurons outside of animals as well as recent findings from non-bilaterian animals in order to make predictions about when and how the first neuron(s) arose during animal evolution and their relationship to those found in extant lineages. With advancing technologies in single cell transcriptomics and proteomics as well as expanding functional techniques in non-bilaterian animals and the close relatives of animals, it is an exciting time to begin unraveling the complex evolutionary history of this fascinating animal cell type.

Introduction

Consistent with an early evolutionary origin, many of the core characteristics and molecular protein complexes found in neurons predate animal multicellularity. Neurons are highly polarized cells and rely on polarized secretion during differentiation and neurite development [1–4]. Asymmetric distribution of proteins and cellular structures is a pan-eukaryotic characteristic and the core components of polarized secretion show deep conservation [5–13]. One of the defining characteristics of bilaterian neurons is the localized, rapid, and highly synchronous release of vesicle contents. As the core components and major trafficking of vesicles is conserved, this regulated secretion appears to be an extreme example of constitutive polarized secretion [14]. Important factors in this form of exocytosis are the accumulation of vesicles in close proximity to the membrane and the tight coupling of calcium influx to fusion events [15–18]. This probably represents a key step in the evolution of neuronal signaling. The evolutionary connection between neurons and secretory cells has been nicely reviewed in [19], and we postulate that the assembly of pre-animal sensory, excretory, and signaling complexes paved the way for the first true neurons to arise from sensory-secretory cells following the occurrence of constitutive multicellularity.

Deep conservation of the components of neurons in unicellular eukaryotes

Unlike constitutive exocytosis, regulated secretion relies on maintaining vesicle pools near the plasma membrane while actively preventing fusion from occurring. This is achieved through the activity of various proteins including RIM, Unc13, complexin, and synaptotagmin 1, some of which can be found in the close unicellular relatives to animals [18,20–23] (Figure 1). The clustering of voltage-gated calcium

Received: 22 July 2022
Revised: 31 August 2022
Accepted: 13 September 2022

Version of Record published:
08 December 2022

Essays in Biochemistry (2022) **66** 781–795
https://doi.org/10.1042/EBC20220042

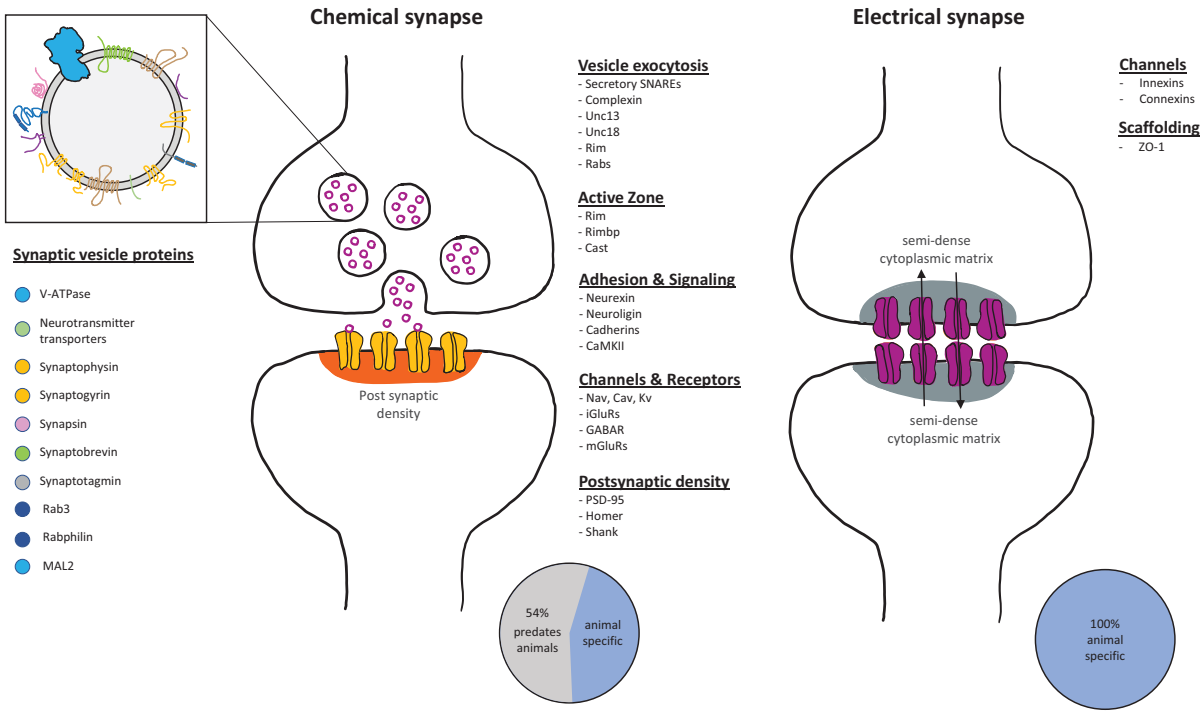

Figure 1. Major components of chemical and electrical synapses

Chemical synapses are composed of a rich repertoire of conserved proteins involved in bringing the pre- and post-synapse into close proximity, tightly regulating vesicle exocytosis, and clustering of receptors on the post-synapse. Many of these proteins originated before animal multicellularity [33–3533–35]. Electrical synapses are primarily established through the interaction of the innexins or connexins, neither of which have been identified outside of animals.

channels (VGCC) at synapses allows for rapid and localized increases in calcium concentration, triggering synchronize vesicle release [15,24]. VGCC predate animal multicellularity, including orthologs of $Ca_v1/2$ and Ca_v3 found in the choanoflagellate (the sister group to animals) *Salpingoeca rosetta* [25]. The activity of these channels and their function in these organisms are yet to be studied. In animals, clustering of these channels is achieved by Cast/ELKS proteins [15], which are animal specific [23]. However, loss-of-function studies show a decrease, but not complete ablation of calcium-dependent fusion events [26]. The major Ca^{2+} sensor in neurons, synaptotagmin 1, has not been identified in unicellular eukaryotes [27,28], though, closely related Ca^{2+} sensors, extended-synaptotagmins, have been identified in animals, yeasts, and plants [29,30]. These proteins have a similar domain organization, but with an additional Synaptotagmin-like, Mitochondrial and Lipid-Binding protein (SMP) domain, as well as different subcellular localization and function in studied organisms [30–32]. With the presence of so many active zone proteins and of polarized and enriched vesicle pools in choanoflagellates [33], it is tempting to say these are sites of regulated exocytosis. However, this remains to be functionally tested.

Communication through a chemical synapse requires tight regulation of the release of the signaling molecule as well as tightly ordered receptors to detect it at the post synapse. In early animals, these post synaptic structures likely arose from receptor clusters on sensory cells. As with active zone proteins, many post-synaptic proteins predate animals, such as Homer, Shank, PSD-95, and CamKII [34, 36–38] (Figure 1), as well as a wide repertoire of ion channels, transient receptor potential channels (TRP), and G-protein coupled receptors (GPCRs) [39–41], which could function in detection of signal. However, the function of these proteins in the close relatives to animals remains unresolved.

Flagella/cilia are found throughout eukaryotes and can serve as a major guide in polarity and polarized secretion. In animals, cilia (both motile and non-motile) have specialized sensory function in a variety of cell types, both neuronal and non-neuronal. Examples include flow sensation [42,43], hedgehog signaling [44], photoreception [44,45], and a wide variety of sensory neurons [46,47]. Though not nearly as well conserved as the core set of structural proteins, signaling modules associated with cilia also appear to be ancient, with the function as a sensory organelle arising alongside motility in early eukaryotic evolution [48–51]. Apart from a flagellum, choanoflagellates also have a

microvilli collar which functions in prey capture. Though structurally distinct from cilia, actin-based microvilli and stereovilli represent cellular projections with high surface area to volume, making ideal domains for receptor presentation. In animals, these structures can serve to increase nutrient absorption as in the brush border epithelial cells [52] but are also found as sensory structures in cell types such as vertebrate lateral line and cochlear hair cells [53–55], cnidarian hair cells and cnidocytes [56–58], and ctenophore sensory neurons [59]. Though specialized feeding cells, sponge choanocytes express many markers of sensory microvilli in bilaterians [60] and may have a sensory function [61]. The similarity of these structures with those of choanoflagellates has long suggested homology [62–65], though it remains unclear whether subtle differences are the result of large evolutionary distances or convergence [66–68]. The presence of both of these cellular structures in non-animal eukaryotes raises the possibility that the sensory modules associated with them are also ancient. Consistent with this, TRP channels, often associated with ciliary signaling [69–71], localized to the cilia of *Chlamydomonas* have been shown to function in mating behavior [72] as well as mechanosensation [73]. Technical limitations on functional studies of these channels and signaling molecules in non-animal holozoans (ichthyosporea, pluriformea, filasterea, and choanoflagellatea) are lessening [74–77], making it an exciting time to better understand how organized the sensory components of these structures may have been in the first animals.

At the root of neuronal signaling is cell–cell communication. Cell–cell communication exists in all domains of life and is facilitated by a variety of means (reviewed in [78]). In neurons, small molecules like glutamate and GABA, neuropeptides, and diffusible gases, like nitric oxide, are released at chemical synapses [79,80]. In the mammalian central nervous system glutamate is the most highly utilized chemical messenger (reviewed in [81]). Glutamatergic signaling is likely very ancient and arose out of a balance of osmotic regulation, metabolism, and damage response (reviewed in [41]). Peptidergic signaling involves the release of small peptides, which are processed from large prepropeptide proteins by a variety of well conserved enzymes [82]. One common modification of neuropeptides is the amidization of glycine by peptidylglycine α-amidating monooxygenase (PAM) [82,83]. A functional PAM enzyme has been identified in *Chlamydomonas*, where it was shown to function in ciliogenesis [84,85]. Amidated peptides have also been identified in ciliary exosomes, which function in mating behavior, suggesting the presence of peptidergic signaling between cells [86]. Orthologs for animal neuropeptide-like molecules have also been identified in choanoflagellates, though functional studies have not yet been performed [87]. Nitric oxide is a small diffusible signal that plays an important role in both plant and animal multicellularity [88–92]. It has been shown to serve a variety of functions in eukaryotes [93] and was recently shown to control collective contraction in a colonial choanoflagellates [94].

Direct intercellular communication also occurs through gap junctions at electrical synapses. Gap junctions are established through the extracellular interaction of innexins/connexins (see below) of two cells forming intercellular channels [95] (Figure 1). So far, orthologs for these proteins have not been identified outside of animals, suggesting gap junctions are a metazoan innovation [96]. However, intercellular bridges are seen between cells of choanoflagellate colonies [68,97,98], which could serve a signaling function. These bridges contain electron dense structures near the body of each cell, though the molecular composition of these is unknown.

Non-bilaterian animals provide key insights into the evolutionary origin of synapses and neurons

Neurons are very ancient cell types, with complex nervous systems present across bilaterians. However, the precise origin is difficult to resolve, partly due to the contentious placement of sponges or ctenophores as the sister group to all other animals [99–105]. Of the non-bilaterian lineages, neurons are present in ctenophores and cnidarians, while they appear to be absent from sponges and placozoans. Despite the absence of bona fide neurons in these two lineages, the molecular toolkits for developing a nervous system are largely present [106–109]. Depending on the placement of sponges or ctenophores, it is assumed that either ctenophores independently gained their nervous system [110], the nervous system was lost in the placozoan (and possibly sponge) lineage(s) [111], or a combination of these two [101,112].

Traditionally thought of as 'simple' nerve-nets, cnidarian nervous systems are now considered highly complex (reviewed in [113]). There is evidence that both classical small molecule neurotransmitters, such as glutamate, GABA, and acetylcholine, and neuropeptides [70,114–117] play a role in neurotransmission. However, diverse studies have found that small molecule transmitters or genes involved in their processing do not always localize to neurons and may play a role in developmental patterning [118–120]. Furthermore, many of the genes involved in pre- and post-synaptic

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https://doi.org/10.1042/EBC20220042

guidance and organization in bilaterians are not well conserved and the synaptic toolkit of cnidarians remains ambiguous [113]. On the other hand, cnidarians have a rich repertoire of neuropeptides [121] and the presence of dense core vesicles [122] suggests an important role of peptidergic signaling at chemical synapses.

Ctenophores, fierce predators in marine environments all over the world, use long cilia to propel their body through the water column [67,123,124]. The ctenophore nervous system consists of a polygonal subepithelial nerve net, mesogleal neurons, an aboral-sensory organ, and a variety of putative sensory cells with cellular protrusions (cilia or filopodia) covering the epidermis, the pharynx, and the tentacles [110,125–133]. Although there is no direct experimental evidence yet, the interplay of these neuronal cell types is thought to allow the animal to quickly react to different physical and environmental stimuli. In agreement with this, the aboral-sensory organ acts as a gravity sensor and also can detect changes in light intensity and pressure, as putative pressure sensors and photoreceptor cells have been described in this sensory organ [125,134–137]. Their polygonal nerve net displays a unique structure, as neurites from the same cell occur through a continuous membrane and thus are anastomosed [132]. There is evidence of only very few classical small molecule neurotransmitters in ctenophores [110,138]. Glutamate and glycine are candidates for the main neurotransmitters, as some ionotropic glutamate receptors tested are sensitive to glutamate and glycine [139,140]. In addition to small molecule neurotransmitters, many neuropeptides have recently been identified by *de novo* predictions in the ctenophore *Mnemiposis leidyi*, some of which showed a functional effect on swimming speed and muscle contraction [132,141]. Furthermore, there is evidence that neurons and colloblast (specialized secretory cells) share common progenitor cells, strengthening the link between these two cell types [142].

Sponges and placozoans both lack nervous systems and muscles, though both display coordinated body wide behaviors. Placozoans are small, generally flat marine animals with a limited number of identifiable cell types [143]. There is currently strong support for their placement as sister group to cnidarians and bilaterians suggesting their morphological simplicity arises from a secondary reduction [108,144]. The genome of *Trichoplax adhaerens* contains genes for major components of both small molecule and peptidergic neurosecretion and transmission [108]. Functional work has also shown specific responses to treatment with different neuropeptide-like molecules as well as non-overlapping expression of the endogenous peptides [145,146]. Single cell sequencing reveals overlapping expression of neuropeptide-like precursors, with peptide processing genes, and pre-synaptic regulatory proteins in putative peptidergic cells, which are distinct from digestive gland cell [143]. The occurrence of these two populations of cell types presents a powerful system for understanding subtle differences in secretory cell types.

Sponges are sessile filter feeders, which use ciliary action to draw water through their aquiferous system where bacterial prey and nutrients are filtered out primarily by choanocytes [147]. Like placozoans, sponge genomes and transcriptomes have revealed the presence of proteins involved in generation and secretion of classic neurotransmitters and peptides [107,148–151]. Even with the absence of a nervous system and muscles, sponges have been shown to undergo full body contractions following changes in internal flow rate [152,153]. This behavior is complex, but in general terms it appears the changes in flow are detected by non-motile cilia on cells lining the osculum, major excurrent canal, which results in production of the diffusible molecule NO [154]. Glutamate and GABA treatments have also been shown to induce or inhibit contractions in sponges [155,156]. Apart from small molecule transmitters, recent bioinformatic study has shown the same neuropeptide-like molecules identified in choanoflagellates appear to be conserved in some sponges [87]. Single-cell RNA sequencing has also identified a unique neuroid-like cell in the freshwater sponge *Spongillia lacustris*. This cell type moves through the choanocyte feeding chambers, expresses many proteins involved in regulated secretion, contains putative secretory vesicles and seems to form neurite-like extensions to microvilli of the choanocytes, though no clear synapse-like structure is present [106].

Action potentials have been recorded in glass sponges (hexactinellid), which triggers the arrest of the feeding system [157]. This electrical signaling is achieved through the syncytial nature of glass sponge tissue, and is dependent on Ca^{2+} and K^{+}, but not Na^{+} [158]. At the sites of incomplete abscission there are cytoplasmic bridges, which contain electron dense plugged junctions [159]. These structures appear morphologically different from the densities seen in choanoflagellate bridges, though like them, they remain to be characterized at the protein/molecular level [160,161].

Did neurons evolve multiple times?

There are two evolutionary points to consider in unraveling the origin of fundamental animal cell types: (1) the transition to constitutive multicellularity in the lineage that gave rise to animals and (2) the last common ancestor of all extant animal lineages (LCAA). In the late 19th century, Ernst Haeckel proposed a sequence of transition to animal multicellularity in which the first step was a hollow ball of flagellated cells (Blastea), followed by the invagination, which created evolutionary space for differentiation of endo- and ectoderm as well as specific cell types [162]. In a general sense, this suggests that cell types arose after the onset of stable multicellularity. Many recent studies of

closely related unicellular or transiently multicellular relatives of modern animals have revealed complex life cycles, with morphologically distinct cell states [68,97,163–166]. In modern choanoflagellates, for example, physical confinement leads to the retraction of collar and flagellum and a switch to amoeboid-like motility [165]. Reconstructions of colonies have also revealed striking differences in cell morphologies, suggesting different cell states/types within the colony [68]. These findings suggest that the ability to dramatically shift cell state existed prior to the onset of stable animal multicellularity. While differing in the basis from which they arose, these two ideas are not mutually exclusive and the transition to animal multicellularity may have involved a combination of the two.

Within a multicellular organism, division of labor provides a strong selective advantage. Within the choanoblastea model, much like extant choanoflagellate colonies, all cells are directly engaged in feeding. In this scenario, a simple division could be represented as one cell type retaining a microvilli collar for feeding and another a motile cilium for generating movement. Coupling of these cells, either through direct cytoplasmic links or secretion, could facilitate the delivery of primary metabolites, while opening evolutionary space for specialization within each cell types (Figure 2A).

There was likely a good amount of time between the onset of multicellularity and the last common ancestor of existing animals [68,168]. During this time fundamental cell types, found across phyla, first arose. However, these were likely multifunctional and highly plastic cell types [65,169]. As single-cell RNA sequencing techniques advance and are applied to a widening variety of animals, the idea of terminally differentiated cell types is being tested, and the ability of morphologically similar cells to deploy distinct transcriptional modalities is being seen [170].

The first 'neurons' to arise were likely sensory/secretory cells, which acted at the interface of organism and environment. As stated in the first section, many of the subcellular components necessary for this type of cell can be found in non-animal holozoans. What remains to be fully understood is the capacity for linking sensory activation to tightly regulated secretion. Apart from the polarization of vesicle pools [33], this would also include the tight coupling of Ca^{2+} influx to the release of exocytic vesicle contents. It has been recently shown that presynaptic active zone organization is established through the linking of vesicle docking proteins to VGCC [171]. At the front end of this would be receptor clustering at cellular projections, either cilia or villi based [172,173].

This hypothesis assumes the first neuronal circuits relied on secretion-based signaling or chemical synapses. The presence of innexins and/or connexins in every phyla that has neurons suggests that gap junctions could have arisen during the time between the first animal and LCAA. However, absence in sponges, placozoans, anthozoan and scyphozoan cnidarians, and the pre-bilaterian bottleneck [174] suggests that losses of the genes has occurred often. Based on this, it remains unclear whether or not the LCAA utilized electrical synapses.

Chemical synapses in extant lineages rely on directed (as in between pre- and post-synaptic regions) or volumetric (peripheral release, generally of neuropeptides) transmission of signals [175,176]. Peptides are well suited for volumetric signaling systems, due to the high potential for variability, tight control of production, and ease of diffusion. This leads to the hypothesis that the first animal signaling systems consisted of peptidergic networks [167]. Well-documented sensory-responsive coupling with NO in choanoflagellates and sponges [154,155,177] suggests, early systems were not strictly peptidergic. However, since information is encoded in a single molecule, this restricts the signaling potential in a diffusion-based system. From a developmental standpoint, regional deployment of cell states and/or receptors on both sensory cells and effector cells allows for a basic wiring system for diffusion-based communication across the body. However, as body plans increase in size and complexity diffusion becomes a limiting factor. Under this hypothesis, neurite-like projections could increase precision and speed of signaling across the body, with development of synapses being an extreme example of precise coupling to an effector cell (Figure 2B).

Effector cells of first neuronal circuits

Movement is a very important aspect of animal biology (even sponges require larval settlement and control over movement of 'environment' through their aquiferous system). Two basic modes can be found throughout animals, ciliary-based motility and contractile tissue/muscle-based motility. Coordination of these systems is key to their functioning. At small size scale and large temporal scales, diffusion based signaling nicely allows for this type of coordination (i.e., positional information about signal is transmitted to all cells based on strength of signal, fast enough and with enough precision to allow a response—similar to peptidergic signaling in modern placozoans or diffusion based signaling in sponges). However, as body sizes increase and body plans diversity, targeted release of signaling molecules provides faster and more distant communicative potential (Figure 2B). In modern animals, branching neurites can be found in neuro-secretory cells [178,179], which can extend and regulate the location of signal release. This type of transmission also greatly lowers the amount of transmitter release required to act on distance cells, which could help

Essays in Biochemistry (2022) **66** 781–795
https://doi.org/10.1042/EBC20220042

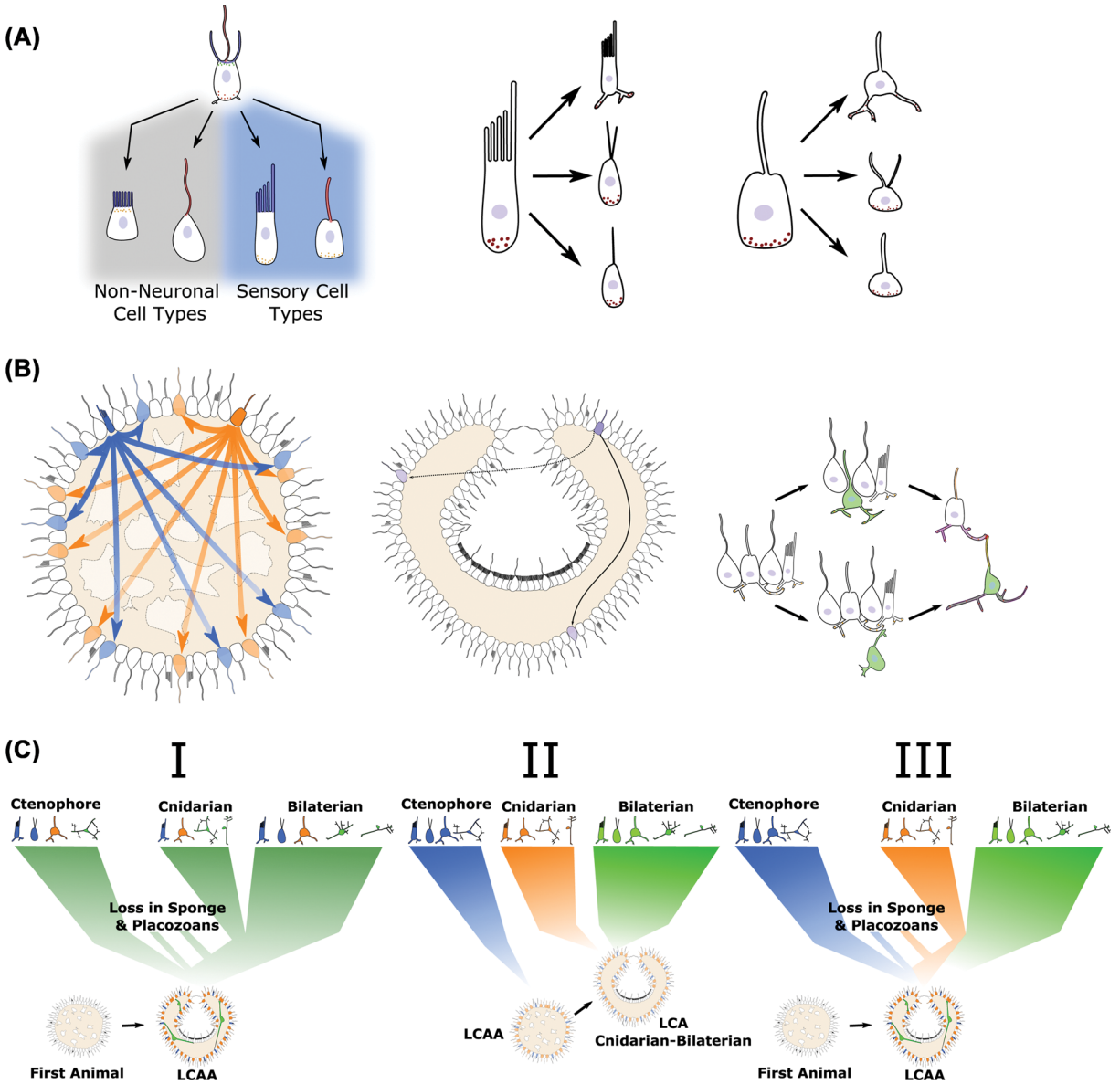

Figure 2. Origin of neurons and neuronal circuits

(**A**) Simplified diagram for division of labor of apical structures of a choanoflagellate-like ancestral cell type into villi- (blue) and cilia-based (red) structures on non-neuronal cells and sensory cells. Following the initial division, further specialization can occur under different pressures. (**B**) Establishment of early neuronal circuits based on diffusion based signaling from peptidergic sensory cells. Distinct sensory cells (blue and orange) release different peptides, which act on cells expressing specific receptors, based on [167]. As bodyplans increase in size and complexity, volumetric signaling is inefficient for full integration and rapid response. Right panel shows hypothetical transitions to directed signaling through interneurons. Synapses are indicated in red. (**C**) Hypothetical scenarios giving rise to the diversity of neurons and nervous systems seen in extant lineages. First showing a single origin, which diversified in each lineage and was lost in placozoans and possibly sponges. Next, independent origins in major lineages with neurons from a LCAA that did not have true neurons. Third, independent specialization of systems in extant lineages from different aspects of a LCAA with neurons. Better resolving the relationships between the cell types present in extant lineages will help understand which of these occurred.

balance the energetic cost of building neurite structures. The transition to feeding on other multicellular organisms and the ensuing arms race was likely a strong driver for greater speed, integration, and coordination.

Did chemical synapses evolve multiple times?

Chemical synapses bring regulated vesicle release from one cell into extremely close proximity to receptor clusters on another cells. These are highly organized and protein dense, with well conserved molecular components in bilaterians [180–182]. Interestingly, overexpression of some synaptic proteins in non-neuronal cells can induce formation of synapse-like structures [171,183,184], suggesting protein abundance may be an important factor in development. As discussed in the second section, as more detailed information about the molecular components and structure of neurons and synapses in non-bilaterians comes out, the question of a single or multiple origins becomes less clear. Ctenophores possess unique synaptic structures (so-called pre-synaptic triads) and anastomosed neurites in their nerve net, unlike anything reported in other nervous systems [124,202]. Likewise, cnidarians have an incompletely understood synaptic toolkit, many bidirectional synapses, and multifunctional neurons. It has been hypnotized that synapses arose multiple times from extensions of ER [185]. What does this mean for the evolution of neurons? Was there a single origin of neurons and neuronal circuits (or synapses), which has undergone radiations within linages giving rise to the extant phyla, or have neurons and synapses arisen multiple times independently? If the first neurons arose before the LCAA, what did these look like and how did they function (Figure 2C)?

Conclusions and future directions

In order to address these questions and start untangling the events that gave rise to the diversity of neurons we see today it is essential to establish testable hypotheses. An important aspect of both division of labor and temporal to spatiotemporal transitional origins of cell types is the regulated deployment of cellular modules. This creates questions of when the protein–protein interactions essential for neuronal signaling first arise during evolution and their function in the close relatives of animals. The increasing power of prediction software such as AlphaFold [186] coupled with advances using cryo-EM to solve protein structures *in vivo* [187,188], means addressing these questions in a wide diversity of organisms is more tangible than ever. Another important aspect of this is the co-regulation of key structural genes for building these structures. Within animals, terminal transcription factors appear to be well-conserved and can be a good indicator of cell type homology [170]. Though we have focused our discussion primarily on structural genes, an important aspect of synaptogenesis is the co-regulation and deployment of these genes. This is a multilevel process [189], terminal selector transcription factors appear to play an important role [190–193]. Animals have an extensive transcription factor repertoire, with evidence of the origin of new families and large expansions of existing families occurring within this clade [190], though this probably occurred in a stepwise fashion, from components present in their unicellular ancestors [194]. Though much is known about the gene regulatory networks involved in development of different cell and tissue types [195], less is known about how terminal selectors establish and maintain cell type identities. Recent examples for neurons have been identified and show evidence of conservation [191,196]. Building on this work and expanding into functional studies in the close unicellular relatives of animals will help understand the origin of this level of regulation of synaptic genes. The presence of so many key components of the synaptic toolkit outside of animals suggest that true chemical synapses were assemble in a stepwise manner through co-option of existing genes. As discussed above, the transition to multicellularity opens evolutionary space for deploying existing genes and modules in new cellular contexts. Functional understanding of these genes in the close unicellular relatives of animals as those lacking synapses is essential for understanding the degree in which synaptic machinery was organized prior to the occurrence of neurons.

As functional techniques in these organisms expand [74–77], it is becoming possible to truly understand the role of these factors beyond predictions based on homology. The same applies for the expanded sampling of extant lineages of animals. Single-cell RNA sequencing has proven to be an invaluable tool in understanding cell type evolution as well as the basic biology of a wide variety of animals, and recent mapping of cell atlases across species demonstrates an exciting potential [197,198]. However, at greater evolutionary distances the similarities become less clear and metacell (homogeneous groupings of cells) cutoffs need to be greatly relaxed. Increased sampling within phyla will greatly increase the resolution of this technique, as well as better understand the diversity of neurons within the phyla allowing reconstruction of what was likely present at earlier nodes of these lineages. Also moving these technologies to the close relatives of animals will provide valuable insight into the underlying transcriptional changes that give rise to morphologically different cell states. Traditional RNA sequencing has shown that distinguishable expression changes underlie different life stages in non-animal holozoans [199]. There are also evidence of morphologically distinct cells within choanoflagellate colonies [68,164]. Applying scRNA-sequencing to these organisms will provide

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https://doi.org/10.1042/EBC20220042

valuable insights into how distinct these cell states are as well as the transitional sequences between them. As powerful as scRNA-sequencing appears to be, transcription levels alone do not capture the full picture of different cell types or states. Recent multiplexing has even taken this further to understand the effects of perturbations or genetic manipulations [118], an invaluable contribution to functional studies. As briefly touched on earlier, especially with respect to the synapse, it may not just be presence or absence, but rather abundance of specific proteins that drive development of these structures. Combining transcriptional data with quantitative single cell proteomics [200,201] may be necessary to fully understand the tipping point between a 'secretory cell' and a 'neuron'. Finally, the hypothesis that the first neurons were likely sensory cells relying on peptidergic signaling requires further exploration of peptidergic signaling in non-bilaterian animals as well as their close unicellular relatives. Open questions include the functional understanding of the neuropeptides and their effectors in ctenophores [132]; the function of neuropeptide-like molecules in sponges [87,106]; and is peptidergic signaling present in non-animal holozoans and for what function? Central to addressing these questions is combining thorough study of specific organisms with broad sampling of extant lineages. The recent development of the robust technologies above, makes this more possible than ever, meaning solutions to long standing questions, such as the single or multiple origins of neurons, are finally on the horizon.

Summary

- Neurons are found in every major animal lineage except sponges and placozoans and many of the key components of the synaptic toolkit are found outside of animals, especially in their close relatives; ichthyosporeans, pluriformeans, filastereans, and choanoflagellates.
- Despite deep conservation of many of the components of neurons, there is a huge amount of diversity within the 'neuron' cell type and the traditional view of ctenophores and cnidarians having 'simple' nerve-nets underplays the complexity of these nervous systems.
- Open questions exist around the evolutionary origin of neurons and synapses. Did neurons and synapses evolve once during animal evolution or has this occurred multiple times?
- Advances in single-cell transcriptomics and proteomics as well as developing functional techniques in non-bilaterian animals and the close unicellular relatives to animals are providing new tools to address these long-debated questions.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

The authors were supported by the Sars Centre core budget.

Author Contribution

The authors contributed equally to this work.

Abbreviations

GPCR, G-protein coupled receptor; LCAA, last common ancestor of all extant animal lineages; LCA, last common ancestor; PAM, peptidylglycine α-amidating monooxygenase; SMP, Synaptotagmin-like, Mitochondrial and Lipid-Binding protein; TRP, transient receptor potential; VGCC, voltage-gated calcium channels.

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