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Modern genomic tools reveal the structural and cellular diversity of cnidarian nervous systems

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Cnidarians shared a common ancestor with bilaterians more than 600 million years ago. This sister group relationship gives them an informative phylogenetic position for understanding the fascinating morphological and molecular cell type diversity of bilaterian nervous systems. Moreover, cnidarians display novel features such as endodermal neurogenesis and independently evolved centralizations, which provide a platform for understanding the evolution of nervous system innovations. In recent years, the application of modern genomic tools has significantly advanced our understanding of cnidarian nervous system structure and function. For example, transgenic reporter lines and gene knockdown experiments in several cnidarian species reveal a significant degree of conservation in the neurogenesis gene regulatory program, while single cell RNA sequencing projects are providing a much deeper understanding of cnidarian neural cell type diversity. At the level of neural function, the physiological properties of ion channels have been described and calcium imaging of the nervous system in whole animals has allowed for the identification of neural circuits underlying specific behaviours. Cnidarians have arrived in the modern era of molecular neurobiology and are primed to provide exciting new insights into the early evolution of nervous systems.

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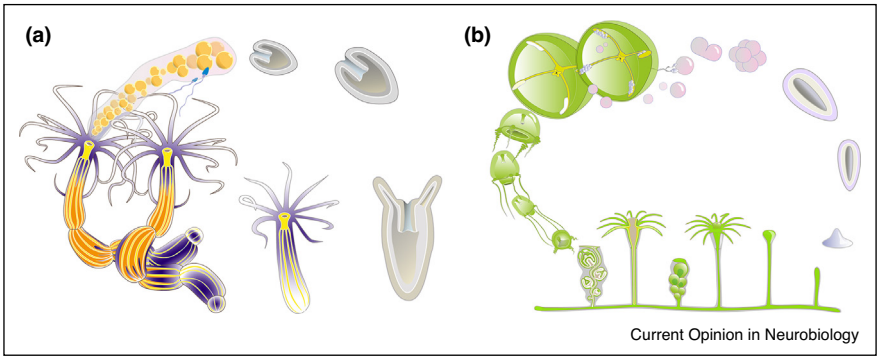
Introduction

Cnidarian nervous systems have attracted the interest of zoologists and neurobiologists since the middle of the 19th century, when first 'neuro-muscular-epithelial cells' and nematocytes [1,2], and a few decades later neurons [3–5] were identified. Central to this interest was the apparent simplicity of the cnidarian body plan, which was soon realized to reflect a long evolutionary distance to other animal groups. It is now well established that cnidarians are the sister group to the bilaterians [6–8] and thus occupy a key position in the animal tree of life for understanding early stages in nervous system evolution. Cnidarians are carnivores that are found in two strikingly different morphotypes: sessile polyps, generally attached to a substrate, and free-swimming medusae. In a typical cnidarian life cycle, a swimming planula larva is derived from sexual reproduction and continues to develop into a sessile polyp (Figure 1). Cnidarians comprise two principle groups, anthozoans and medusozoans; the polyps of these groups share a similar anatomy but differ in their developmental potential and their role in the life cycle. In anthozoans, polyps are the sexually mature stage that completes the life cycle by generating gametes that give rise to new planulae (Figure 1a). In medusozoans, polyps asexually generate medusae, and the medusae are the sexually mature stage that produces gametes to close the life cycle (Figure 1b). However, there is variation in the life cycle among medusozoan species, many of which have a truncated life cycle, having lost one or multiple stages [9].

Nervous system complexity and organization differ between the sessile polyp and free-swimming medusae, which is likely due to differences in life style. Polyps have a bi-layered, tube-shaped body with a single opening, surrounded by a ring of tentacles. The tentacles are used for catching prey and the single opening serves as both mouth and anus. The nervous system in the outer layer, named epidermis or ectoderm, is organized as a nerve net, though the density of neurons is typically higher at both the oral and aboral ends. In some species, a nerve ring at the oral end coordinates the feeding response, which involves tentacle motility and the 'mouth' opening. The inner tissue layer, named gastrodermis or endoderm, can be a plain epithelial sheet (as in many medusozoans), or it can bear folds (the mesenteries) that carry gonadal tissue and longitudinal musculature (as in many anthozoans). The gastrodermal nervous system is also a nerve net, but in some species prominent tracts of neurites run along the base of the mesenteries [10–13].

Figure 1



Cnidarian life cycles.
(a) Life cycle of an anthozoan exemplified by *Nematostella vectensis* in clockwise order. Mature polyps (on the left) release gametes into the water. After blastula and gastrula stages, the animals develop into free-swimming planulae, which settle on their aboral pole and develop into sessile polyps that start feeding. **(b)** Life cycle of a medusozoan, exemplified by the hydrozoan *Clytia hemispherica*. Gametes are released by medusae and develop after fertilization into planulae. The planulae settle and form colonies of different polyps, including feeding polyps and reproductive polyps. The reproductive polyps asexually generate medusae to complete the life cycle. Note that, there are many variations to medusozoan life cycles. For example, freshwater *Hydra* polyps propagate asexually by budding or the polyps reproduce sexually by forming gametes; fertilized *Hydra* embryos develop directly into polyps without producing a swimming larval form. Therefore, *Hydra* lack both the planula and medusa stages. Artwork by Johanna Kraus.

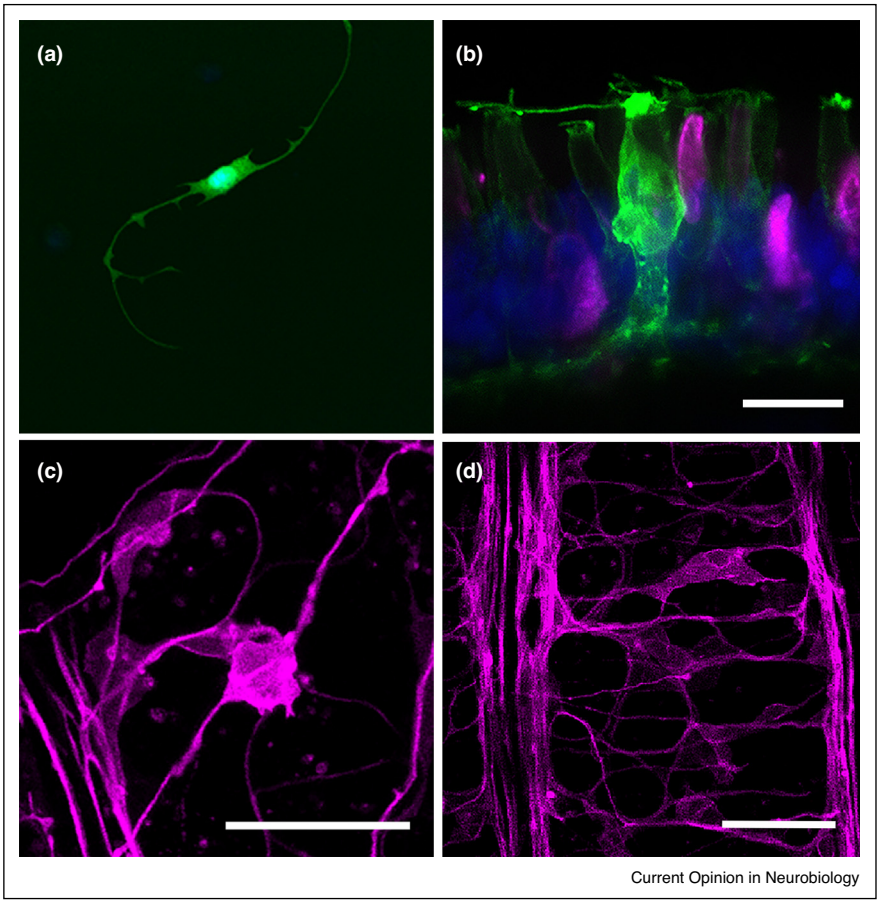
Medusae display a much higher degree of nervous system centralization as compared to polyps. A prominent nerve ring is often located at the margin of their bell and many medusae contain well-developed eyes that can be integrated into sophisticated light and gravity-sensing organs, the rhopalia [14,15]. The nerve ring and the sensory structures contribute to the control of swimming behavior, for example the avoidance of obstacles or escape from predators [16–18]. Studies of different medusae revealed interesting features in the regulation of their locomotion. In scyphomedusae, contraction of the bell musculature is coordinated with the help of bidirectional synapses in the motor nerve net [19,20]. In the hydromedusae *Aglantha digitale*, slow swimming and escape swimming are mediated by the same motor neurons, which can generate two types of action potentials. Weak depolarization triggers small and slow, calcium-driven spikes, whereas strong depolarization causes the large and fast, sodium-driven spikes that result in escape swimming [21,22]. The ongoing characterization of neuronal ion channels in several cnidarian species will help to relate such physiological properties of neurons to their molecular constitution [23–26].

The cells that comprise cnidarian nervous systems are traditionally grouped into three broad classes: sensory/sensory-motor neurons, ganglion neurons, and mechanosensory cells called cnidocytes. Sensory neurons are defined by their upright position in the epithelium and the presence of an apical cilium. Ganglion neurons are considered a morphological equivalent of interneurons; their somata are located basally within the epithelium. Consistent with their classification as neurons, both sensory and ganglion cells extend neurites on their basal side that form a basi-epithelial meshwork (Figure 2). Distinction of neurites as axons and dendrites has not been shown yet. Cnidocytes ('stinging cells') are cnidarian-specific cells that can contain structures resembling presynaptic sites [27,28], a cnidocil and a highly sophisticated extrusive organelle, the cnidocyst, that discharges by fast Ca^{2+}-dependent exocytosis for catching prey [29,30]. These features have led to the suggestion that cnidocytes are highly derived neural cells (reviewed in Ref. [11]). Morphological and molecular analyses reveal that each neuronal class contains several subpopulations, characterized for example by the expression of particular neuropeptides or by the presence of a specific type of cnidocyst [31–34]. In recent years, the genomes of several cnidarians have been sequenced [35–39], transgenic reporter lines have been established [40•,41•,42•], and gene-knockdown or genome-editing technologies have been implemented for *Hydra* ([43,44], *Hydractinia* [45,46•], *Clytia* [47•,48] and *Nematostella* [49,50•,51]. These technologies open the door to a comprehensive understanding of the composition, the development, and the function of cnidarian nervous systems [9,52,53].

Transgenic reporter lines and single cell RNA sequencing as new tools for studying the diversity of cnidarian neurons

Histological and ultrastructural observations have provided many insights into the morphology of neurons in different cnidarians [54–57], but identifying defined subpopulations of neurons and capturing the dynamics of their development has been a major challenge. The generation of transgenic reporter lines in several cnidarian species [40•,41•,42•] was an important step in overcoming this

Figure 2



Labeling of neurons in transgenic cnidarians.
(a) A GFP-labeled bipolar neuron after dissociation of a *Hydra* polyp. **(b)** A sensory cell in the body wall of a *Nematostella* late planula (green). The apical surface of the ectoderm is oriented to the top. The capsules of cnidocytes (magenta) are labeled with a different transgene [62•]. **(c)** A multipolar neuron close to the oral opening of a young *Nematostella* polyp, labeled by an *NvElav1::mOrange* transgene. **(d)** The gastrodermal nervous system of a *Nematostella* polyp includes prominent tracts of neurites along the mesenteries and a nerve net between these tracts. The transgene is *NvElav1::mOrange*. Scale bars in (b, d) 20 μm, in (C) 10 μm. Image credits: (a) Stefan Siebert; (b–d) Océane Tournière.

problem. Through the use of general neuronal promoters, the first transgenic lines were used to obtain a broad picture of cnidarian nervous system structure and development [58•,59•]. However, there is now a growing number of transgenic lines with fluorescent proteins expressed in specific neural subpopulations [60•,61,62•,63••,64••]. These revealed, for example, that neurons with stereotypic projection patterns and positions in the body column contribute to the *Nematostella* nervous system, thus suggesting that the seemingly diffuse and random cnidarian nerve nets have reproducible elements [60•].

Understanding the complexity and organization of cnidarian nerve nets requires uncovering neuronal diversity, which is now possible using single cell RNA sequencing (scRNA-seq). This technology combined with transgenic reporter lines has the potential to provide a detailed picture of cnidarian nervous systems. Thus far, scRNA-seq has been applied to both the *Nematostella* and *Hydra* adult polyp to characterize neuronal diversity [63••,64••]. Using transgenic lines with neuronal GFP expression, several thousand neurons were collected and sequenced from each animal, giving significant insights into cnidarian nervous systems and providing rich datasets for future exploration. Analysis of sequenced *Nematostella* neurons revealed 32 clusters with unique gene markers. In addition, the *Nematostella* neurons could be split broadly into two unique transcriptional states defined by the expression of unique sets of transcription factors. Transgenic lines were created to study these two neuronal states and this revealed differences in morphology and position [63••]. In *Hydra*, 12 neuronal subtypes were identified with distinct molecular signatures. Using both transgenic reporter lines to highlight neural subtypes and in situ hybridization, the location of

each neuronal subtype was identified thus creating a spatial and molecular map of the *Hydra* nervous system, including the identification of distinct neuronal subtypes in the epidermal and gastrodermal nerve nets [64••].

The application of scRNA-seq to uncover neuronal diversity in cnidarians is very promising; however, more work is required to determine whether the full transcriptional diversity has been uncovered in *Nematostella* and *Hydra*. Importantly, the clustering parameters, number of cells sequenced, and sequencing depth are all important factors contributing to the number of clusters reported in a given study. In addition, the degree of similarity between cell types is an important consideration. Sequencing a small number of transcripts from relatively few cells is sufficient to discern two very different cells types due to large transcriptional differences. Delineating cell types by cluster analysis is more difficult when considering cell types with similar transcriptional profiles; we don't yet fully understand the level of sampling required to distinguish two neuronal subtypes, which likely have a high level of transcriptional overlap. Finally, it is unclear what level of transcriptional differences between two cell types warrants classification as two different neuronal subtypes. Unlike in cnidarians, the full diversity of *Caenorhabditis elegans* neurons is known from a large body of previous work and therefore attempts to identify the transcriptional signatures of *C. elegans* neurons using scRNA-seq are informative in defining benchmarks. In *C. elegans*, nearly 7000 single neurons were sequenced with a median of approximately 700 transcripts per cell; this revealed 40 of the 118 known neuronal subtypes [65]. It is likely that increasing the number of sequenced cells and/or increasing the sequencing depth would ultimately uncover the transcriptional signatures of all 118 subtypes. The *Nematostella* and *Hydra* single cell datasets were sequenced at different depths – a median of approximately 550 transcripts per cell for *Nematostella* and a median of approximately 5650 transcripts per cell for *Hydra*. In addition, different clustering methodologies were used, so it is likely too early to make direct comparisons between the neuronal diversity of *Nematostella* and *Hydra* [63••,64••]. Ultimately, scRNA-seq experiments describing the neuronal diversity of any organism should be validated at the bench. Regardless, scRNA-seq is clearly a very valuable tool that will allow us to uncover the neuronal diversity in a large array of cnidarian species and life stages. This information can be used to gain an understanding of the organizational principles underlying the cnidarian nervous system and will provide molecular handles for the functional manipulations required to test nervous system development and function.

The developmental basis for the generation of neural cell types

Approaches to manipulate gene function using morpholinos, CRISPR/Cas9, RNAi, and shRNAs have been implemented in several cnidarian species [43–45,47•,48,49,50•,51,66,67]. In combination with transgenic lines and data collected from scRNA-seq, the ability to test gene function allows for detailed analysis of cnidarian nervous system development and function, thus providing a basis for evolutionary comparisons.

Surprisingly, the stem cells that give rise to neurons and cnidocytes might be quite different between medusozoans and anthozoans. In hydrozoans (a class of medusozoans), the multipotent interstitial stem cells give rise to all cells of the nervous system, as well as gland cells and germ cells [68–72]. By contrast, in anthozoans, interstitial stem cells have not been found and the nervous system may instead arise from epithelial-like stem cells [59•,73], suggesting that interstitial stem cells might be a hydrozoan-specific or medusozoan-specific innovation [74].

Candidate gene approaches used to study the molecular control of neurogenesis suggest a significant degree of conservation in the broad specification of neurons between cnidarians and bilaterians (reviewed in Refs. [13,73]). For example, Notch and Wnt signalling, *soxB*, *atonal/neurogenin* and *achaete-scute* family genes play central roles in *Nematostella* neurogenesis [59•,75•,76–78] and *soxB* and *nanos* genes function in *Hydractinia* neurogenesis [79,80••]. However, in contrast to *Nematostella* and most bilaterians, Notch signaling appears not be involved in the regulation of neurogenesis in both adult *Hydra* and embryonic *Hydractinia*, in which neurons derive from the non-epithelial interstitial cells [46•,81•,82•]. Currently, only a few functional studies have addressed the development of specific neural cell types. In adult *Hydra*, apical neurons require the ParaHox gene *gsx/cnox-2* for their development [83] and in *Nematostella*, *PaxA* and *Mef2* are involved in the formation of cnidocytes [84•,85]. Now, with new tools and resources, like those provided by scRNA-seq, it is possible to move beyond candidate gene approaches to obtain a less biased view of the regulatory networks that underlie cnidarian neurogenesis.

In adult cnidarian organisms, the nervous system is continuously replaced in homeostatic animals and is capable of regeneration, thus providing a platform for understanding the regulation of adult neurogenesis. In polyps, the whole nervous system readily regenerates after significant loss of the body column, while in medusae, regeneration is restricted to some organs such as the manubrium or the eyes [86–88]. Comparative analysis of progenitor behaviours between cnidarians and bilaterian model organisms showing similar regenerative potential (e.g. planarians), will allow us to determine possible common molecular mechanisms [89]. Such comparisons can be made using amputation paradigms or ablation of specific structures like the eyes of jellyfish and planarians [88,90].

Conserved molecular basis for neurotransmission in cnidarians and bilaterians

Even though neuroid conduction, that is electrical conduction across non-neuronal cells, is observed across a wide repertoire of organisms, that is plants, protists, porifers, cnidarians and bilaterians [91], electrophysiological studies performed on the giant axon from the hydromedusae *Aglantha*, have demonstrated that cnidarian and bilaterian synapses exhibit similar properties, with their activity relying on the formation of presynaptic and postsynaptic potentials [92,93]. Genomic sequencing from choanoflagellates, poriferans and placozoans has actually demonstrated that the molecular components of the post-synaptic density are already almost complete in phyla that do not differentiate nerve cells [94,95]. Genomic sequencing and transcriptomic sequencing from cnidarians have confirmed pharmacological studies showing that most chemical neurotransmitters used in bilaterians are also active in cnidarian neurons, acetylcholine, glutamate, GABA, glycine for fast transmission, catecholamines and serotonin for slow transmission [96,97•,98]. Signaling through these neurotransmitters is required for coordinated behaviors such as the rather complex feeding response [99].

The role of peptides in neural signaling

Neuropeptides and epitheliopeptides are a prominent feature of the cnidarian nervous system. The expression of numerous G protein-coupled receptors and the discovery of peptide-gated ion channels suggests that these peptides play a key role in both slow and fast neurotransmission [31,100,101]. In mammals, peptidergic signaling is neuromodulatory, involved in slow neurotransmission and interacting with fast neurotransmission driven by small molecules such as GABA and glutamate. This neuromodulatory role of neuropeptides might represent a typical synapomorphy of nervous systems or alternatively, a convergent evolutionary trait in cnidarians and bilaterians.

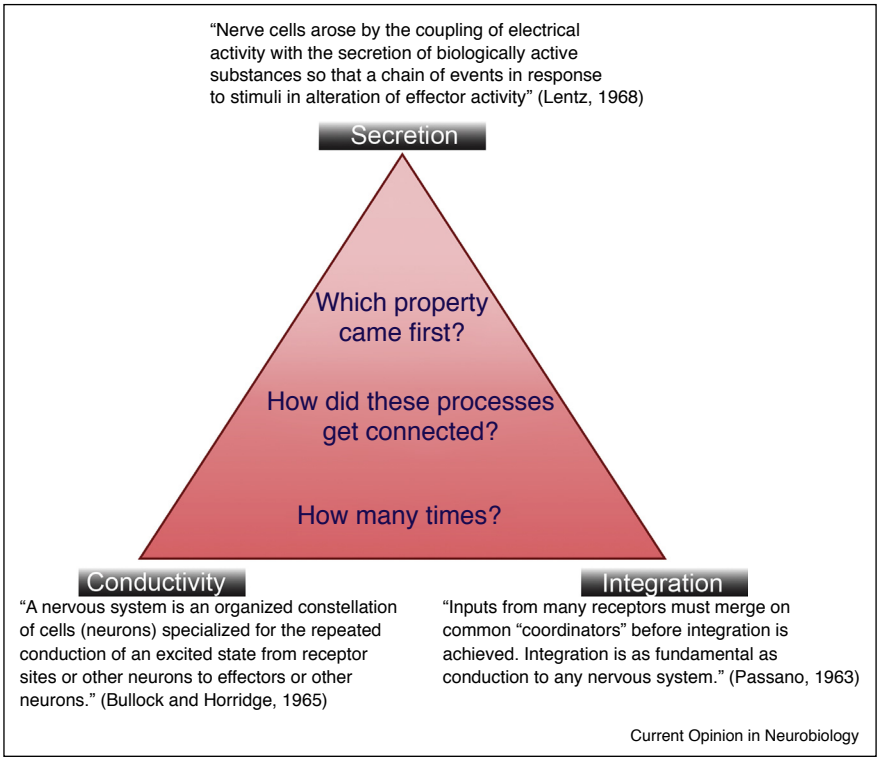
Recent studies highlight the power of functional genomics to test the function of neuropeptides in cnidarians. For example, disruption of the expression of a GLWamide neuropeptide in *Nematostella* [102•] resulted in a subtle delay in the progression from swimming to sessile life stages under laboratory conditions. Two other recent studies analyzed how light cues regulate the release of gametes, a feature common to many animals. A group of cells required for spawning in the gonad of the *Clytia hemispherica* medusa are both light sensitive and secrete neuropeptides. Opsin9 is expressed in these cells and mutating the *Opsin9* gene blocks light induced oocyte maturation and spawning. The same cells express a neuropeptide that functions as the maturation-inducing hormone (MIH), and secretion of this neuropeptide requires the stimulation of Opsin9 by blue-cyan light [103••,104••]. These elegant experiments provide an interesting example of direct coupling of sensory and neurosecretory functions in one cell, a situation that may have been more common early in animal evolution [105]. Future studies should address the role of neuropeptides in nervous system function using similar approaches.

In *Hydra*, epitheliopeptides can act either positively or negatively on neurogenesis and/or neurotransmission [101]. This epitheliopeptide signaling points to a tightly regulated cross-talk between the myoepithelial cells and the nervous system. The physiological function and the regulation of this crosstalk are currently not well understood. The elimination of the interstitial stem cells, and consequently neurons, leads to the up regulation of taxon-specific epitheliopeptides in the epithelial cells in *Hydra* [97•]. This may suggest a proto-neuronal function for the epithelial cells, which are widely recognized as the cells from which synaptic conduction likely emerged [106]. A differentiated nervous system could repress the proto-neuronal function of the epithelial cells, whereas in the absence of neurogenesis, this potential could be expressed. While this hypothesis remains to be tested, one possible interpretation is that peptides played a key role in the emergence of neurons from epithelial cells, that is cells that integrated three major functions: secretion, integration, and conductivity (Figure 3).

The wiring of cnidarian nervous systems

Electrophysiological recordings have been instrumental for understanding conduction and function in cnidarian nervous systems [22,107,108]. Genetically encoded reporters of neural activity and tools for optogenetic manipulations now also allow system-wide analyses. This has recently been accomplished in *Hydra* by transgenic expression of the fluorescent calcium sensor protein GCaMP6s in the entire nervous system [109••]. This allowed for imaging of nervous system activity in a whole animal and the identification of neuronal populations whose activities correlate with two main types of previously described electrical activity – contraction bursts (CBs) and rhythmic potentials (RPs) [107,110,111]. As previously postulated, CBs are associated with longitudinal contraction. Unexpectedly, two non-overlapping RP networks were identified, one in the epidermis and one in the gastrodermis. While the gastrodermal RP is related to radial contraction as previously thought, the epidermal RP is related to longitudinal elongation as a response to light stimulation. Apart from their functional and spatial separation, the two RP neuron populations also displayed differences in cellular morphology [109••]. These findings-coupled with the new molecular and spatial map of the *Hydra* nervous system [64••], the recent classification of the *Hydra* behavioral repertoire [112•], and the development of new technologies to measure *Hydra* nervous system activity [113•], means we are now

Figure 3



The three properties that are necessary and sufficient to build a nervous system. Quotations are from Refs. [117–119].

poised to gain a comprehensive understanding of the *Hydra* nervous system form molecules to behavior.

Conclusions and outlook

Cnidarian neurobiology is enjoying a renaissance with several species being amenable to genetic manipulations, thus allowing for the visualization and interrogation of nervous system development and function. Calcium imaging will likely soon be used together with optogenetic tools for the activation and inhibition of individual neurons, which will lead to new insights into the logic of neural circuits in nerve net-based nervous systems. Studies of neural development currently focus on the specification of different neural cell types; in the near future, we expect these studies to expand to cellular aspects of the formation of neural connectivity via neurites and synapses. Basic questions about the nature of cnidarian neurons, including the identification of distinct dendrites and axons and the molecular composition of chemical synapses remain unanswered. The development of cell culture protocols has so far eluded cnidarian researchers, but would be useful for addressing these questions. Another emerging topic is the crosstalk between neurons and the microbiome that recently has been evidenced in *Hydra*, pointing to previously overlooked functions of the nervous system [114], possibly maintained in bilaterians [115,116]. At the organismal level, adding genetically tractable model systems from other classes of cnidarians would allow, for example, studying the development of convergently evolved eyes and centralizations of the nervous system (in scyphozoans and cubozoans), or the neural basis of the exquisite behavioural repertoire of box jellies (cubozoans). Extrapolating from the recent progress summarized here, it is likely that many new insights into the fascinating neurobiology of this diverse group of animals are on the horizon.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kleinenberg N: *Hydra. Eine Anatomisch-Entwicklungsgeschichtliche Untersuchung*. Leipzig: Verlag Wilhelm Engelmann; 1872.
2. Parker TJ: **On the histology of Hydra fusca**. *Proc R Soc Lond* 1879, **30**:61-66.
3. Hertwig O, Hertwig R: *Das Nervensystem und die Sinnesorgane der Medusen*. Leipzig: F.C.W. Vogel; 1878.
4. Schneider KC: **Histologie von Hydra fusca mit besonderer Berucksichtigung des Nervensystems der Hydropolypen**. *Arch Mikroskopischer Anatomie* 1890, **35**:321-379.
5. Hadzi J: **Ueber das Nervensystem von Hydra**. *Arbeiten aus den zoologischen Instituten der Universitaet Wien* 1909, **17**:1-44.
6. Zapata F, Goetz FE, Smith SA, Howison M, Siebert S, Church SH, Sanders SM, Ames CL, McFadden CS, France SC *et al.*: **Phylogenomic analyses support traditional relationships within Cnidaria**. *PLoS One* 2015, **10**:e0139068.
7. Dunn CW, Giribet G, Edgecombe GD, Hejnol A: **Animal phylogeny and its evolutionary implications**. *Ann Rev Ecol Evol Syst* 2014, **45**:371-395.
8. Telford MJ, Budd GE, Philippe H: **Phylogenomic insights into animal evolution**. *Curr Biol* 2015, **25**:R876-R887.
9. Leclere L, Copley RR, Momose T, Houliston E: **Hydrozoan insights in animal development and evolution**. *Curr Opin Genet Dev* 2016, **39**:157-167.
10. Galliot B, Quiquand M: **A two-step process in the emergence of neurogenesis**. *Eur J Neurosci* 2011, **34**:847-862.
11. Galliot B, Quiquand M, Ghila L, de Rosa R, Miljkovic-Licina M, Chera S: **Origins of neurogenesis, a cnidarian view**. *Dev Biol* 2009, **332**:2-24.
12. Watanabe H, Fujisawa T, Holstein TW: **Cnidarians and the evolutionary origin of the nervous system**. *Dev Growth Differ* 2009, **51**:167-183.
13. Kelava I, Rentzsch F, Technau U: **Evolution of eumetazoan nervous systems: insights from cnidarians**. *Philos Trans R Soc Lond B Biol Sci* 2015, **370**.
14. Garm A, Ekstrom P, Boudes M, Nilsson DE: **Rhopalia are integrated parts of the central nervous system in box jellyfish**. *Cell Tissue Res* 2006, **325**:333-343.
15. Koizumi O, Hamada S, Minobe S, Hamaguchi-Hamada K, Kurumata-Shigeto M, Nakamura M, Namikawa H: **The nerve ring in cnidarians: its presence and structure in hydrozoan medusae**. *Zoology* 2015, **118**:79-88.
16. Satterlie RA: **Neuronal control of swimming in jellyfish: a comparative story**. *Can J Zool* 2002, **80**:1654-1669.
17. Petie R, Garm A, Nilsson DE: **Visual control of steering in the box jellyfish *Tripedalia cystophora***. *J Exp Biol* 2011, **214**:2809-2815.
18. Garm A, Oskarsson M, Nilsson DE: **Box jellyfish use terrestrial visual cues for navigation**. *Curr Biol* 2011, **21**:798-803.
19. Anderson PA: **Physiology of a bidirectional, excitatory, chemical synapse**. *J Neurophysiol* 1985, **53**:821-835.
20. Katsuki T, Greenspan RJ: **Jellyfish nervous systems**. *Curr Biol* 2013, **23**:R592-594.
21. Mackie GO: **Central neural circuitry in the jellyfish Aglantha: a model 'simple nervous system'**. *Neurosignals* 2004, **13**:5-19.
22. Satterlie RA: **Cnidarian neurobiology**. In *The Oxford Handbook of Invertebrate Neurobiology.* Edited by Byrne JH. Oxford University Press; 2017:1-60.
23. Li X, Liu H, Chu Luo J, Rhodes SA, Trigg LM, van Rossum DB, Anishkin A, Diatta FH, Sassic JK, Simmons DK *et al.*: **Major diversification of voltage-gated K+ channels occurred in ancestral parahoxozoans**. *Proc Natl Acad Sci U S A* 2015, **112**:E1010-E1019.
24. Martinson AS, van Rossum DB, Diatta FH, Layden MJ, Rhodes SA, Martindale MQ, Jegla T: **Functional evolution of Erg potassium channel gating reveals an ancient origin for IKr**. *Proc Natl Acad Sci U S A* 2014, **111**:5712-5717.
25. Assmann M, Kuhn A, Durrnagel S, Holstein TW, Grunder S: **The comprehensive analysis of DEG/ENaC subunits in Hydra reveals a large variety of peptide-gated channels, potentially involved in neuromuscular transmission**. *BMC Biol* 2014, **12**:84.
26. Gur Barzilai M, Reitzel AM, Kraus JE, Gordon D, Technau U, Gurevitz M, Moran Y: **Convergent evolution of sodium ion selectivity in metazoan neuronal signaling**. *Cell Rep* 2012, **2**:242-248.
27. Holtmann M, Thurm U: **Mono- and oligo-vesicular synapses and their connectivity in a Cnidarian sensory epithelium (Coryne tubulosa)**. *J Comp Neurol* 2001, **432**:537-549.
28. Thurm U, Brinkmann M, Golz R, Holtmann M, Oliver D, Sieger T: **Mechanoreception and synaptic transmission of hydrozoan nematocytes**. *Hydrobiologia* 2004, **530**:97-105.
29. Ozbek S, Balasubramanian PG, Holstein TW: **Cnidocyst structure and the biomechanics of discharge**. *Toxicon* 2009, **54**:1038-1045.
30. Nuchter T, Benoit M, Engel U, Ozbek S, Holstein TW: **Nanosecond-scale kinetics of nematocyst discharge**. *Curr Biol* 2006, **16**:R316-318.
31. Takahashi T, Takeda N: **Insight into the molecular and functional diversity of cnidarian neuropeptides**. *Int J Mol Sci* 2015, **16**:2610-2625.
32. Hansen GN, Williamson M, Grimmelikhuijzen CJ: **Two-color double-labeling in situ hybridization of whole-mount Hydra using RNA probes for five different Hydra neuropeptide preprohormones: evidence for colocalization**. *Cell Tissue Res* 2000, **301**:245-253.
33. Fautin DG: **Structural diversity, systematics, and evolution of cnidae**. *Toxicon* 2009, **54**:1054-1064.
34. Rachamim T, Morgenstern D, Aharonovich D, Brekhman V, Lotan T, Sher D: **The dynamically evolving nematocyst content of an anthozoan, a scyphozoan, and a hydrozoan**. *Mol Biol Evol* 2015, **32**:7-753.
35. Leclère L, Horin C, Chevalier S, Lapébie P, Dru P, Peron S, Jager M, Condamine T, Pottin K, Romano S *et al.*: **The genome of the jellyfish Clytia hemisphaerica and the evolution of the cnidarian life-cycle**. *bioRxiv* 2018 http://dx.doi.org/10.1101/369959.
36. Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, Salamov A, Terry A, Shapiro H, Lindquist E, Kapitonov VV *et al.*: **Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization**. *Science* 2007, **317**:86-94.
37. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T, Rattei T, Balasubramanian PG, Borman J, Busam D *et al.*: **The dynamic genome of Hydra**. *Nature* 2010, **464**:592-596.
38. Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M, Fujiwara M, Koyanagi R, Ikuta T *et al.*: **Using the *Acropora digitifera* genome to understand coral responses to environmental change**. *Nature* 2011, **476**:320-323.
39. Baumgarten S, Simakov O, Esherick LY, Liew YJ, Lehnert EM, Michell CT, Li Y, Hambleton EA, Guse A, Oates ME *et al.*: **The genome of Aglantha, a sea anemone model for coral symbiosis**. *Proc Natl Acad Sci U S A* 2015, **112**:11893-11898.
40. • Kunzel T, Heiermann R, Frank U, Muller W, Tilmann W, Bause M, Nonn A, Helling M, Schwarz RS, Plickert G: **Migration and differentiation potential of stem cells in the cnidarian Hydractinia analysed in eGFP-transgenic animals and chimeras**. *Dev Biol* 2010, **348**:120-129.

Refs. [40–42] describe the establishment of transgenic reporter lines in Hydractinia (40), Hydra (41) and Nematostella (42). This was essential for subsequent descriptive and functional nervous system analyses.

41. • Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F, Bosch TC: **Transgenic Hydra allow in vivo tracking of individual stem cells during morphogenesis**. *Proc Natl Acad Sci U S A* 2006, **103**:6208-6211.
Refs. [40–42] describe the establishment of transgenic reporter lines in *Hydra* [40•], *Nematostella* [41•], and *Hydractinia* [42•]. This was essential for subsequent descriptive and functional nervous system analyses.

42. • Renfer E, Amon-Hassenzahl A, Steinmetz PR, Technau U: **A muscle-specific transgenic reporter line of the sea anemone, *Nematostella vectensis***. *Proc Natl Acad Sci U S A* 2009, **107**:104-108.
Refs. [40–42] describe the establishment of transgenic reporter lines in *Hydra* [40•], *Nematostella* [41•], and *Hydractinia* [42•]. This was essential for subsequent descriptive and functional nervous system analyses.

43. Lohmann JU, Endl I, Bosch TC: **Silencing of developmental genes in Hydra**. *Dev Biol* 1999, **214**:211-214.

44. Chera S, de Rosa R, Miljkovic-Licina M, Dobretz K, Ghila L, Kaloulis K, Galliot B: **Silencing of the hydra serine protease inhibitor Kazal1 gene mimics the human SPINK1 pancreatic phenotype**. *J Cell Sci* 2006, **119**:846-857.

45. Duffy DJ, Plickert G, Kuenzel T, Tilmann W, Frank U: **Wnt signaling promotes oral but suppresses aboral structures in Hydractinia metamorphosis and regeneration**. *Development* 2010, **137**:3057-3066.

46. • Gahan JM, Schnitzler CE, DuBuc TQ, Doonan LB, Kanska J, Gornik SG, Barreira S, Thompson K, Schiffer P, Baxevanis AD *et al.*: **Functional studies on the role of Notch signaling in Hydractinia development**. *Dev Biol* 2017, **428**:224-231.
By using pharmacological inhibition and CRISPR/Cas9 genome editing in F0 animals, this paper shows that Notch signaling is not involved in the regulation of neurogenesis in*Hydractinia*.

47. • Momose T, De Cian A, Shiba K, Inaba K, Giovannangeli C, Concordet JP: **High doses of CRISPR/Cas9 ribonucleoprotein efficiently induce gene knockout with low mosaicism in the hydrozoan Clytia hemisphaerica through microhomology-mediated deletion**. *Sci Rep* 2018, **8**:11734.
In this paper, Momose*et al.* show that CRISPR/Cas9 is an efficent tool for genome editing in *Clytia*.

48. Momose T, Houliston E: **Two oppositely localised frizzled RNAs as axis determinants in a cnidarian embryo**. *PLoS Biol* 2007, **5**: e70.

49. Magie CR, Daly M, Martindale MQ: **Gastrulation in the cnidarian *Nematostella vectensis* occurs via invagination not ingression**. *Dev Biol* 2007, **305**:483-497.

50. • Ikmi A, McKinney SA, Delventhal KM, Gibson MC: **TALEN and CRISPR/Cas9-mediated genome editing in the early-branching metazoan Nematostella vectensis**. *Nat Commun* 2014, **5**:5486.
Establishes TALENs and CRISPR/Cas9 as tools for genome editing in*Nematostella*.

51. He S, Del Viso F, Chen CY, Ikmi A, Kroesen AE, Gibson MC: **An axial Hox code controls tissue segmentation and body patterning in Nematostella vectensis**. *Science* 2018, **361**:1377-1380.

52. Technau U, Steele RE: **Evolutionary crossroads in developmental biology: Cnidaria**. *Development* 2011, **138**:1447-1458.

53. Rentzsch F, Technau U: **Genomics and development of Nematostella vectensis and other anthozoans**. *Curr Opin Genet Dev* 2016, **39**:63-70.

54. Bullock TH: **Coelentarata and ctenophora**. In *Structure and Function of the Nervous systems of Invertebrates.* Edited by Bullock TH, Horridge GA. W.H. Freeman and Co; 1965:459-534.

55. Thomas MB, Edwards NC: **Cnidaria: Hydrozoa**. In *Microscopic Anatomy of Invertebrates.* Edited by Harrison FW, Westfall JA. Wiley-Liss; 1991:91-183.

56. Lesh-Laurie GE, Suchy PE: **Cnidaria: Scyphozoa and Cubozoa**. In *Microscopic Anatomy of Invertebrates.* Edited by Harrison FW, Westfall JA. Wiley-Liss; 1991:185-266.

57. Fautin DG, Mariscal RN: **Cnidaria: Anthozoa**. In *Microscopic Anatomy of Invertebrates.* Edited by Harrison FW, Westfall JA. Wiley-Liss; 1991:267-358.

58. • Nakanishi N, Renfer E, Technau U, Rentzsch F: **Nervous systems of the sea anemone Nematostella vectensis are generated by ectoderm and endoderm and shaped by distinct mechanisms**. *Development* 2012, **139**:347-357.
This paper characterizes neural development in*Nematostella* using an *NvElav1* reporter line. By transplantation experiments, it is shown that both ectodermal and endodermal cells generate neurons.

59. • Richards GS, Rentzsch F: **Transgenic analysis of a SoxB gene reveals neural progenitor cells in the cnidarian Nematostella vectensis**. *Development* 2014, **141**:4681-4689.
This work uses a*NvSoxB(2)* reporter line to identify a population of proliferative cells that gives rise to cnidocytes, sensory neruons, and ganglion neurons. Knockdown experiments show that *NvSoxB(2)* is required for the development of these cell types.

60. • Havrilak JA, Faltine-Gonzalez D, Wen Y, Fodera D, Simpson AC, Magie CR, Layden MJ: **Characterization of NvLWamide-like neurons reveals stereotypy in Nematostella nerve net development**. *Dev Biol* 2017, **431**:336-346.
In this paper, a transgenic reporter line is used to characterize several subpopulations of neurons expressing the neuropeptide NvLWamide. It is shown that some of these subpopulations have specific projection patterns and positions in the body column, suggesting previously unrecognized stereotypic features in the nervous system of*Nematostella*.

61. Busengdal H, Rentzsch F: **Unipotent progenitors contribute to the generation of sensory cell types in the nervous system of the cnidarian Nematostella vectensis**. *Dev Biol* 2017, **431**:59-68.

62. • Sunagar K, Columbus-Shenkar YY, Fridrich A, Gutkovich N, Aharoni R, Moran Y: **Cell type-specific expression profiling unravels the development and evolution of stinging cells in sea anemone**. *BMC Biol* 2018, **16**:108.
This study uses transgenic reporters to purify cnidocytes at different stages of maturity. Subsequent RNAseq and functional experiments identify cnidarian-specific c-Jun and c-Fos paralogs as regulators of cnidocyte development.

63. •• Sebe-Pedros A, Saudemont B, Chomsky E, Plessier F, Mailhe MP, Renno J, Loe-Mie Y, Lifshitz A, Mukamel Z, Schmutz S *et al.*: **Cnidarian cell type diversity and regulation revealed by whole-organism single-cell RNA-Seq**. *Cell* 2018, **173**:1520-1534 e1520.
Refs. [63] and [64] are the first studies using single cell RNA seq in cnidarians. The repertoire of neurons in*Nematostella* [63••] and *Hydra* [64••] is described based on sequencing of single cells derived from whole animals and from sorted neurons. They also assess chromatin accessibility by ATAC seq to identify regulatory regions with possible functions in neuron specification. In *Hydra*, developmental trajectories were built that describe the gene expression changes that occur during neurogenesis.

64. •• Siebert S, Farrell JA, Cazet J, Abeykoon Y, Primack AS, Schnitzler CE, Juliano CE: **Stem cell differentiation trajectories in Gydra resolved at single-cell resolution**. *bioRxiv* 2018 http://dx.doi.org/10.1101/460154.
References [63] and [64] are the first studies using single cell RNA seq in cnidarians. The repertoire of neurons in*Nematostella* [63••] and *Hydra* [64••] is described based on sequencing of single cells derived from whole animals and from sorted neurons. They also assess chromatin accessibility by ATAC seq to identify regulatory regions with possible functions in neuron specification. In *Hydra*, developmental trajectories were built that describe the gene expression changes that occur during neurogenesis.

65. Cao J, Packer JS, Ramani V, Cusanovich DA, Huynh C, Daza R, Qiu X, Lee C, Furlan SN, Steemers FJ *et al.*: **Comprehensive single-cell transcriptional profiling of a multicellular organism**. *Science* 2017, **357**:661-667.

66. Fuchs B, Wang W, Graspeuntner S, Li Y, Insua S, Herbst EM, Dirksen P, Bohm AM, Hemmrich G, Sommer F *et al.*: **Regulation of polyp-to-jellyfish transition in *Aurelia aurita***. *Curr Biol* 2014, **24**:263-273.

67. Pankow S, Bamberger C: **The p53 tumor suppressor-like protein nvp63 mediates selective germ cell death in the sea anemone Nematostella vectensis**. *PLoS One* 2007, **2**:e782.

68. Gahan JM, Bradshaw B, Flici H, Frank U: **The interstitial stem cells in Hydractinia and their role in regeneration**. *Curr Opin Genet Dev* 2016, **40**:65-73.

69. Bosch TC, Anton-Erxleben F, Hemmrich G, Khalturin K: **The Hydra polyp: nothing but an active stem cell community**. *Dev Growth Differ* 2010, **52**:15-25.

70. Watanabe H, Hoang VT, Mattner R, Holstein TW: **Immortality and the base of multicellular life: lessons from cnidarian stem cells**. *Semin Cell Dev Biol* 2009, **20**:1114-1125.

71. Hobmayer B, Jenewein M, Eder D, Eder MK, Glasauer S, Gufler S, Hartl M, Salvenmoser W: **Stemness in Hydra - a current perspective**. *Int J Dev Biol* 2012, **56**:509-517.

72. Frank U, Plickert G, Muller WA: **Cnidarian interstitial cells: the dawn of stem cell research**. In *Stem Cells in Marine Organisms,* edn 1. Edited by Rinkevich B, Matranga V.Springer; 2009:33-59.

73. Rentzsch F, Layden M, Manuel M: **The cellular and molecular basis of cnidarian neurogenesis**. *Wiley Interdiscip Rev Dev Biol* 2017, **6** http://dx.doi.org/10.1002/wdev.257.

74. Gold DA, Jacobs DK: **Stem cell dynamics in cnidaria: are there unifying principles?** *Dev Genes Evol* 2013, **223**:53-66.

75. • Layden MJ, Boekhout M, Martindale MQ: **Nematostella vectensis achaete-scute homolog NvashA regulates embryonic ectodermal neurogenesis and represents an ancient component of the metazoan neural specification pathway**. *Development* 2012, **139**:1013-1022.
One of the first papers using genetic manipulations to identify the requirement of a conserved transcription factor (an*achaete-scute* gene) in embryonic neurogenesis in a cnidarian. *NvAshA* is shown to be required for the development of subsets of neural cells in *Nematostella*.

76. Watanabe H, Kuhn A, Fushiki M, Agata K, Ozbek S, Fujisawa T, Holstein TW: **Sequential actions of beta-catenin and Bmp pattern the oral nerve net in Nematostella vectensis**. *Nat Commun* 2014, **5**:5536.

77. Richards GS, Rentzsch F: **Regulation of Nematostella neural progenitors by SoxB, Notch and bHLH genes**. *Development* 2015, **142**:3332-3342.

78. Layden MJ, Martindale MQ: **Non-canonical Notch signaling represents an ancestral mechanism to regulate neural differentiation**. *Evodevo* 2014, **5**:30.

79. Kanska J, Frank U: **New roles for Nanos in neural cell fate determination revealed by studies in a cnidarian**. *J Cell Sci* 2013, **126**:3192-3203.

80. •• Flici H, Schnitzler CE, Millane RC, Govinden G, Houlihan A, Boomkamp SD, Shen S, Baxevanis AD, Frank U: **An evolutionarily conserved SoxB-Hdac2 crosstalk regulates neurogenesis in a cnidarian**. *Cell Rep* 2017, **18**:1395-1409.
A detailed study that identifies roles for the transcription factor SoxB2 and the histone deacetylase HDAC2 in regulating neurogenesis in*Hydractinia*. Crossregulation is shown both at the transcriptional and post-transcriptional levels. These observations are extended to human cells, showing that research on cnidarians has the potential to provide new insights relevant for other systems.

81. • Kasbauer T, Towb P, Alexandrova O, David CN, Dall'armi E, Staudigl A, Stiening B, Bottger A: **The notch signaling pathway in the cnidarian Hydra**. *Dev Biol* 2007, **303**:376-390.

82. • Khalturin K, Anton-Erxleben F, Milde S, Plotz C, Wittlieb J, Hemmrich G, Bosch TC: **Transgenic stem cells in Hydra reveal an early evolutionary origin for key elements controlling self-renewal and differentiation**. *Dev Biol* 2007, **309**:32-44.
References [81] and [82] show that manipulation of Notch signalling does not affect neurogenesis in adult*Hydra*. This (and the results in Ref. [46•]) is in contrast to observations in *Nematostella* and many bilaterians and is potentially related to the non-epithelial nature of hydrozoan stem cells.

83. Miljkovic-Licina M, Chera S, Ghila L, Galliot B: **Head regeneration in wild-type hydra requires de novo neurogenesis**. *Development* 2007, **134**:1191-1201.

84. • Babonis LS, Martindale MQ: **PaxA, but not PaxC, is required for cnidocyte development in the sea anemone Nematostella vectensis**. *Evodevo* 2017, **8**:14.
Babonis and Martindale use a series of double labeling experiments to describe different subpopulations of cnidocytes in*Nematostella* and demonstrate that their development is regulated by the transcription factor *paxA*.

85. Genikhovich G, Technau U: **Complex functions of Mef2 splice variants in the differentiation of endoderm and of a neuronal cell type in a sea anemone**. *Development* 2011, **138**:4911-4919.

86. Schmid V: **Regeneration in medusa buds and medusae of hydrozoa**. *Am Zool* 1974, **14**:773-781.

87. Weber C: **Structure, histochemistry, ontogenetic development, and regeneration of the ocellus of Cladonema radiatum dujardin (cnidaria, hydrozoa, anthomedusae)**. *J Morphol* 1981, **167**:313-331.

88. Stierwald M, Yanze N, Bamert RP, Kammermeier L, Schmid V: **The Sine oculis/Six class family of homeobox genes in jellyfish with and without eyes: development and eye regeneration**. *Dev Biol* 2004, **274**:70-81.

89. Atabay KD, LoCascio SA, de Hoog T, Reddien PW: **Self-organization and progenitor targeting generate stable patterns in planarian regeneration**. *Science* 2018, **360**:404-409.

90. Lapan SW, Reddien PW: **Transcriptome analysis of the planarian eye identifies ovo as a specific regulator of eye regeneration**. *Cell Rep* 2012, **2**:294-307.

91. Mackie GO: **Neuroid conduction and evolution of conducting tissues**. *Q Rev Biol* 1970, **45**:319-332.

92. Roberts A, Mackie GO: **The giant axon escape system of a hydrozoan medusa, Aglantha digitale**. *J Exp Biol* 1980, **84**:303-318.

93. Anderson PA, Spencer AN: **The importance of cnidarian synapses for neurobiology**. *J Neurobiol* 1989, **20**:435-457.

94. Alie A, Manuel M: **The backbone of the post-synaptic density originated in a unicellular ancestor of choanoflagellates and metazoans**. *BMC Evol Biol* 2010, **10**:34.

95. Sakarya O, Armstrong KA, Adamska M, Adamski M, Wang IF, Tidor B, Degnan BM, Oakley TH, Kosik KS: **A post-synaptic scaffold at the origin of the animal kingdom**. *PLoS One* 2007, **2**:e506.

96. Anctil M: **Chemical transmission in the sea anemone Nematostella vectensis: a genomic perspective**. *Comp Biochem Physiol D Genomics Proteomics* 2009, **4**:268-289.

97. • Wenger Y, Buzgariu W, Galliot B: **Loss of neurogenesis in Hydra leads to compensatory regulation of neurogenic and neurotransmission genes in epithelial cells**. *Philos Trans R Soc Lond B Biol Sci* 2016, **371**:20150040.
Wenger*et al.* perform extensive region- and cell type specific transcriptome analyses to characterize the nervous system of *Hydra* along the primary body axis. Lack of neurogenesis due to the elimination of the interstitial stem cells correlates with an upregulation of neural genes in epithelial cells. They also show that loss of the interstitial cells differentially affects the nervous system in homeostatic conditions vs regeneration.

98. Kass-Simon G, Pierobon P: **Cnidarian chemical neurotransmission, an updated overview**. *Comp Biochem Physiol A Mol Integr Physiol* 2007, **146**:9-25.

99. Pierobon P: **Coordinated modulation of cellular signaling through ligand-gated ion channels in Hydra vulgaris (Cnidaria, Hydrozoa)**. *Int J Dev Biol* 2012, **56**:551-565.

100. Grunder S, Assmann M: **Peptide-gated ion channels and the simple nervous system of hydra**. *J Exp Biol* 2015, **218**:551-561.

101. Takahashi T, Fujisawa T: **Important roles for epithelial cell peptides in hydra development**. *Bioessays* 2009:610-619.

102. • Nakanishi N, Martindale MQ: **CRISPR knockouts reveal an endogenous role for ancient neuropeptides in regulating developmental timing in a sea anemone**. *Elife* 2018, **7**:e39742.
CRISPR/Cas9 induced mutations of a*Nematostella GLWamide-like* gene result in a delay of the transition to the polyp stage. This phenotype can be rescued by application of synthetic GLWamide.

103. Takeda N, Kon Y, Artigas GQ, Lapebie P, Barreau C, Koizumi O, Kishimoto T, Tachibana K, Houliston E, Deguchi R: **Identification of jellyfish neuropeptides that act directly as oocyte maturation-inducing hormones**. *Development* 2018, **145**.
•• This paper takes advantage of the autonomous functionality of*Clytia* gonads to identify W/RPRPamide neuropeptides as maturation inducing hormones produced by the neurosecretory cells in the ectoderm of the gonad.

104. Quiroga Artigas G, Lapebie P, Leclere L, Takeda N, Deguchi R, Jekely G, Momose T, Houliston E: **A gonad-expressed opsin mediates light-induced spawning in the jellyfish Clytia**. *Elife* 2018, **7**:e29555.
•• This paper takes advantage of the autonomous functionality of*Clytia* gonads to identify W/RPRPamide neuropeptides as maturation inducing hormones produced by the neurosecretory cells in the ectoderm of the gonad.

105. Arendt D: **The evolution of cell types in animals: emerging principles from molecular studies**. *Nat Rev Genet* 2008, **9**:868-882.

106. Lichtneckert R, Reichert H: **Origin and evolution of the first nervous system**. In *Evolutionary Neuroscience,* edn 1. Edited by Kaas JH. Academic Press; 2009:51-78.

107. Passano LM, McCullough CB: **Pacemaker hierarchies controlling the behaviour of hydras**. *Nature* 1963, **199**:1174-1175.

108. Mackie GO, Passano LM: **Epithelial conduction in hydromedusae**. *J Gen Physiol* 1968, **52**:600-621.

109. Dupre C, Yuste R: **Non-overlapping neural networks in hydra vulgaris**. *Curr Biol* 2017, **27**:1085-1097.
•• Expression of GcaMP6s in all neurons of*Hydra* is used for neural calcium imaging in the whole animal. The activity patterns reveal neural networks correlating with contraction bursts and rhythmic potentials, respectively. The authors further identify morphological differences between the neurons constituting the different networks.

110. Passano LM, McCullough CB: **The light response and the rhythmic potentials of hydra**. *Proc Natl Acad Sci U S A* 1962, **48**:1376-1382.

111. Passano LM, McCullough CB: **Co-ordinating systems and behaviour in hydra. Ii. The rhythmic potential system**. *J Exp Biol* 1965, **42**:205-231.

112. Han S, Taralova E, Dupre C, Yuste R: **Comprehensive machine learning analysis of hydra behavior reveals a stable basal behavioral repertoire**. *Elife* 2018, **7**:e32605.
• Machine learning is used to extract and quantify behavior in freely moving*Hydra*. Despite the highly deformable body of these polyps, six basic types of behavior were identified, and these types of behavior display little variation in experimental conditions. The study provides an essential background for future manipulations of the nervous system in *Hydra*.

113. Badhiwala KN, Gonzales DL, Vercosa DG, Avants BW, Robinson JT: **Microfluidics for electrophysiology, imaging, and behavioral analysis of hydra**. *Lab Chip* 2018, **18**:2523-2539.
• Introduces three different types of microfluidic chambers for*Hydra* that allow for immobilization, chemical stimulation, and /or behavioral studies. In these settings, electrical recordings and imaging can occur over several hours.

114. Klimovich AV, Bosch TCG: **Rethinking the role of the nervous system: lessons from the hydra holobiont**. *Bioessays* 2018, **40**:e1800060.

115. Carabotti M, Scirocco A, Maselli MA, Severi C: **The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems**. *Ann Gastroenterol* 2015, **28**:203-209.

116. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK: **The central nervous system and the gut microbiome**. *Cell* 2016, **167**:915-932.

117. Lentz TL: *Primitive Nervous Systems*. Yale University Press; 1968.

118. Bullock TH: **Defining features of a nervous system**. In *Structure and Function in the Nervous Systems of Invertebrates*, vol 1. Edited by Bullock TH, Horridge GA. W.H. Freeman; 1965:5-7.

119. Passano LM: **Primitive nervous systems**. *Proc Natl Acad Sci U S A* 1963, **50**:306-313.