Review article

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# Neurons interact with the microbiome: an evolutionary-informed perspective

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**Abstract:** Animals have evolved within the framework of microbes and are constantly exposed to diverse microbiota. Microbes colonize most, if not all, animal epithelia and influence the activity of many organs, including the nervous system. Therefore, any consideration on nervous system development and function in the absence of the recognition of microbes will be incomplete. Here, we review the current knowledge on the nervous systems of *Hydra* and its role in the host–microbiome communication. We show that recent advances in molecular and imaging methods are allowing a comprehensive understanding of the capacity of such a seemingly simple nervous system in the context of the metaorganism. We propose that the development, function and evolution of neural circuits must be considered in the context of host–microbe interactions and present *Hydra* as a strategic model system with great basic and translational relevance for neuroscience.

**Keywords:** antimicrobial peptides; evolution; *Hydra*; metaorganism; nerve nets.

**Zusammenfassung:** Tiere sind in einer Welt voller Mikroben entstanden und sind ständig verschiedenen Mikrobiota ausgesetzt. Mikroben besiedeln die meisten, wenn nicht gar alle tierischen Epithelien und beeinflussen die Aktivität vieler Organe, einschließlich des Nervensystems. Jede Betrachtung der Entwicklung und Funktion des Nervensystems ohne Berücksichtigung des Mikrobioms bleibt daher unvollständig. Hier fassen wir das aktuelle Wissen über das Nervensystem von *Hydra* und insbesondere seine Rolle in der Wirt-Mikrobiom-Kommunikation zusammen. Wir zeigen, dass moderne molekulare und bildgebende Verfahren ein umfassendes Verständnis der Leistungsfähigkeit eines solchen scheinbar einfachen Nervensystems im Kontext des Metaorganismus ermöglichen. Wir schlagen vor, künftig die Entwicklung, Funktion und Entwicklung neuronaler Schaltkreise im Kontext von Wirt-Mikroben-Wechselwirkungen zu betrachten und präsentieren *Hydra* als strategisches Modellsystem mit großer grundlegender und translatorischer Relevanz für die Neurowissenschaften.

**Schlüsselwörter:** Antimikrobielle Peptide; Evolution; *Hydra*; Metaorganismus; Nervennetz.

## Introduction: neurons interact with the microbiome

Nervous systems allow animals to perceive signals from the environment and to respond to them. Novel technologies including sequencing and imaging has unveiled that an important component of the immediate environment of many if not all organisms is a coevolved and resident microbiota (Baquero and Nombela, 2012; Blaser et al., 2016). Microbes shaped the Earth since billions of years before the “invention” of any nervous system, and they continue to shape the Earth. In animals, including humans, microbes are colonizing all epithelia. Animal evolution therefore appears intimately linked to the presence of microbes. Considering organisms as “metaorganisms” or “holobionts” (Bosch and Mcfall-Ngai, 2011, 2021) incorporates this impact of the microbial world and attempts to drive the neurosciences to the next level of enquiry.

Previous studies on germ-free (GF) animals, i.e., organisms treated with broad-spectrum antibiotics to completely eliminate their microbes or animals born and raised in absolutely axenic conditions, show that specific microbiota can impact central nervous system (CNS) physiology and neurochemistry (Sharon et al., 2016). GF mice that are devoid of associated microflora exhibit neurological deficiencies in learning, memory, recognition, and emotional behaviours (Foster et al., 2017; Gareau, 2014). In developing mice embryos, proliferation of neurons in the dorsal

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hippocampus is greater in GF mice than in conventionalized mice. However, post-weaning exposure of GF mice to microbial clones did not influence neurogenesis, suggesting that neuronal growth is stimulated by microbiota at an early stage (Ogbonnaya et al., 2015).

It is now well established that microbiota not only affect the CNS but also influence the enteric nervous system (Cryan et al., 2019). For example, mice kept under sterile conditions show reduced excitability of enteric neurons, resulting in slower gut peristalsis and protracted intestinal transit time. Interestingly, colonization of adult GF mice with microbes taken from the intestine of animals kept under standard laboratory conditions restores the peristaltic activity to normal levels (De Vadder et al., 2018; Obata et al., 2020), indicating that the gut monitors continuously the contents of the lumen and responds to potential changes. It also has been shown that intestinal microbiota directly affects transcriptional programs in enteric neurons (Obata et al., 2020). These observations are medically relevant because changes in the composition of microbiota (known as dysbiosis) are also observed in common gastrointestinal disorders, including those characterized by changes in intestinal motility, such as irritable bowel syndrome (De Palma et al., 2017).

Most if not all of these studies were done in laboratory mice. How relevant are they for our understanding of neurobiology in general? From our evolutionary point of view, the discovery of an interaction of microbes with the mouse nervous system(s) comes as no surprise. Below we show that similar interactions between the microbiota and the neurons are in place already at the beginning of animal evolution, suggesting that animal–bacteria interactions are likely as ancient as animals themselves.

## *Hydra*, a model to study neuron–microbiome interactions

*Hydra*, a member of the animal phylum Cnidaria, is close to the earliest animals in evolution that had nervous systems (Figures 1, 2A and B). Cnidaria occupy a sister phylogenetic position to bilaterians. The fact that they encode most of the gene families found in bilaterians makes them a suitable model to study the "genetic tool kit" present in the cnidarian–bilaterian ancestor. This includes their repertoire of ion channels, synaptic proteins, small neurotransmitters, receptors and the corresponding processing machinery. In *Hydra*'s simple tube-like body structure, the single layer of ectodermal epithelial cells covered by a multilayered glycocalyx represents a physical barrier toward the environment, whereas a single layer of endodermal epithelial cells separates the body from the content of the gastric cavity. Very much to our surprise, and made possible by novel sequencing technologies, we discovered very early that *Hydra*'s ectodermal epithelial surface is densely colonized by a stable multispecies bacterial community (Fraune and Bosch, 2007) (Figure 2C). Since that discovery, *Hydra* has proven itself as an excellent model for studying host–microbe interactions and how metaorganisms function *in vivo* (Bosch, 2013, 2014; Klimovich and Bosch, 2018; Schröder and Bosch, 2016). The presence and composition of *Hydra*'s microbiota is critical for the tissue homeostasis and health of the polyps (Rathje et al., 2020). Remarkably, each *Hydra* species supports long-term associations with a different set of bacteria, suggesting that the host imposes specific selection pressure onto its microbiome (Franzenburg et al., 2013; Fraune and Bosch, 2007). Both, ecto- and endodermal epithelial cells produce a rich repertoire of antimicrobial peptides that regulate the microbiome (Franzenburg et al., 2013).

In addition to ectodermal and endodermal epithelial cells, *Hydra* has an anatomically simple nervous system (Figures 1, 2D–G) which consists of approximately 3000 neurons in an adult and 70 neurons in a newly hatched polyp (Klimovich and Bosch, 2018; Martin et al., 1997). Although in the early studies, we had considered the epithelial cells as prime regulators of the microbiome (Bosch, 2013,

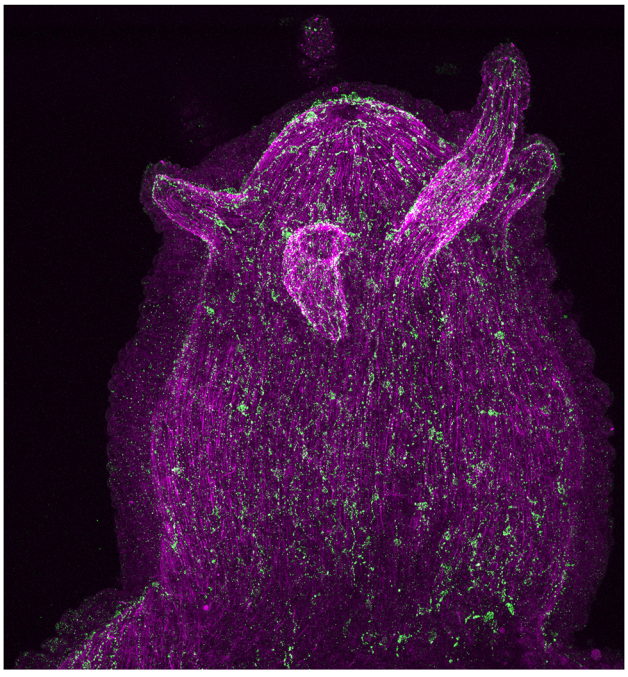

**Figure 1:** The nervous system of *Hydra* is a diffuse nerve net. Here, a population of neurons expressing a *Hydra*-specific Hym355 neuropeptide (green) and muscular fibers of epithelial cells (magenta) are visualized in a juvenile polyp.

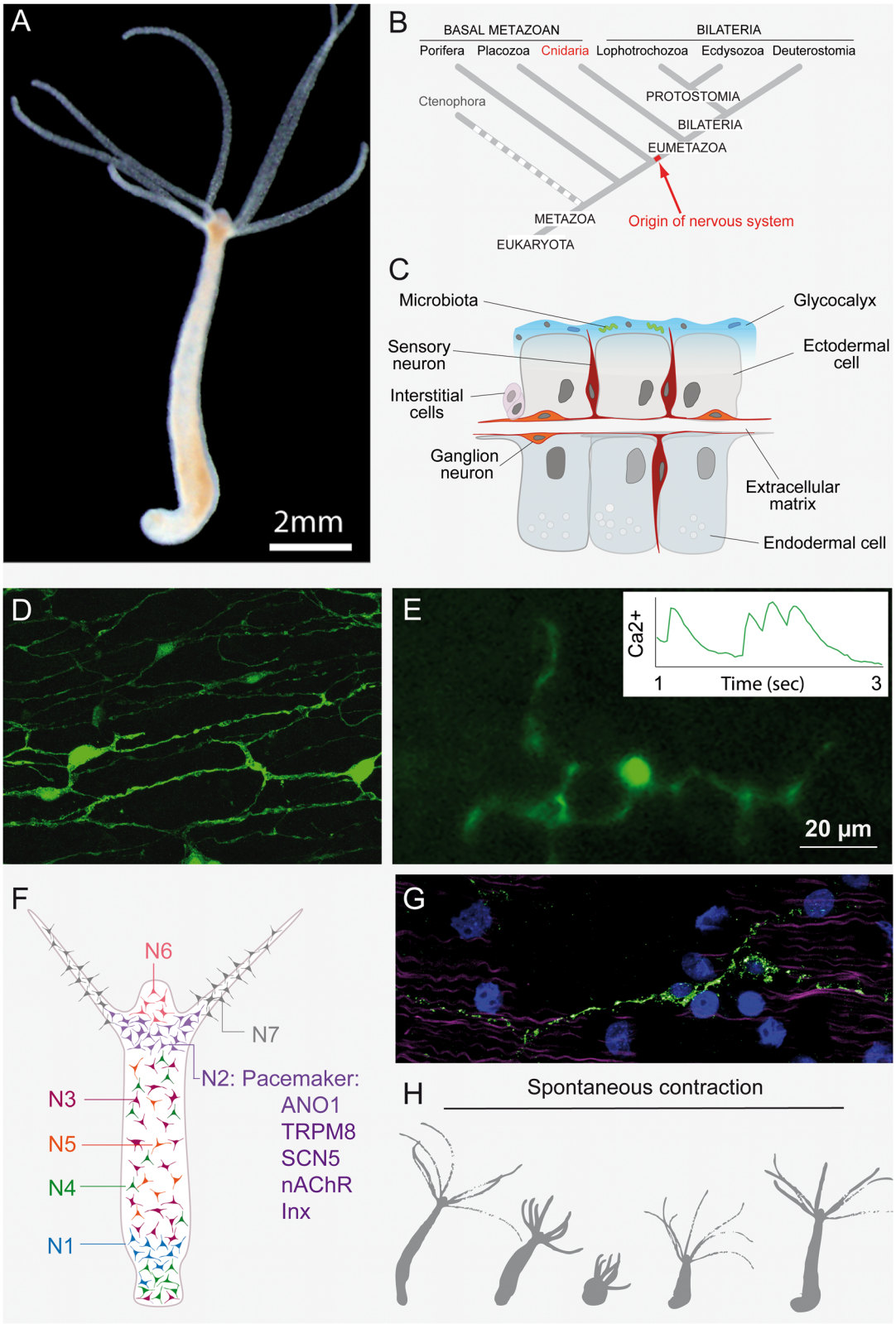


**Figure 2:** *Hydra* as a model to study neuron–microbe interactions.
**A.** An adult polyp contains a nervous system comprising approximately 3000 neurons. **B.** *Hydra* belongs to the phylum Cnidaria, the sister group to the Bilateria. Their common ancestor developed the first nervous system. **C.** The tissue structure of *Hydra* with the microbiota in the glycocalyx. **D.** The nerve net of *Hydra*. **E.** *In vivo* recording and quantification (inset) of neuronal activity using a genetically-encoded $Ca^{2+}$-sensor GCaMP expressed specifically in neurons in transgenic *Hydra*. **F.** The spatial distribution of the seven distinct neuronal populations in *Hydra*. N2 as the pacemaker population with the conserved signature of ion channels. **G.** A pacemaker neuron of *Hydra,* visualized using an antibody against SCN5-like channel (green). Nuclei are stained with TO-PRO (blue) and actin filaments of epitheliomuscular cells are stained with Phalloidin (magenta). **H.** Schematic illustration of the spontaneous contraction behaviour.

2014), we recognized over time that neurons per se are involved and that they somehow interact with microbes. When assessing antibacterial activity in *Hydra*, we observed a strong correlation between the number of neurons present and the antibacterial activity (Kasahara and Bosch, 2003). Moreover, when we removed all neurones from the epithelium, we observed significant changes in *Hydra*'s microbial community (Fraune et al., 2009). Based on these observations, meanwhile, we have established an experimental platform to investigate the neuron–microbe interactions in *Hydra* not only at a descriptive level, but also to uncover the underlying molecular mechanisms (causalities).

## What neuronal circuits do in *Hydra*

Nervous systems are commonly interpreted as information processing input–output devices. They receive environmental information from their sensors as input, subsequently process or integrate this information, and use the result to control effectors, providing some kind of output. Although this interpretation seems beyond dispute, from an evolutionary perspective, it requires some clarification and reflection. First, neuronal circuits evolve by the Darwinian processes of variation and selection. Given the extremely high costs of building and maintaining nervous systems, selective optimization seems to shape neuroanatomy rather than neutral evolutionary processes (Jékely, 2011). Second, in evolutionary ancient neuronal circuits, input is provided by sensory cells having receptors for neurotransmitters, neuropeptides and other signaling molecules in the neural membrane which may still have been incompletely genetically individualized compared to more complex animals (Schlosser, 2018). In animals such as *Hydra*, the few neuronal cell types are multifunctional, and later during a long evolutionary history may have diversified and specialized for different sensory modalities and larger and more complex sense organs. Third, as emphasized by Jékely (2011), behaviour evolved before nervous systems. Various single-celled eukaryotes (protists) and the ciliated larvae of sponges devoid of neurons can display sophisticated behaviours, including phototaxis, gravitaxis or chemotaxis. Fourth, animal behaviour allows individuals to adapt to the environment at a time scale that is much faster than natural selection, and therefore drives the rapid evolution of the nervous system (Anderson and Perona, 2014). Last, there is increasing evidence that human and animal's social behaviour, hyperactivity and maybe even anxiety are influenced by microbes (Bruckner et al., 2020; Vuong et al., 2017).

*Hydra* behaviour has been studied for centuries. It was first described by Trembley (1744) and consists of both spontaneous and stimulus-evoked, reactive behaviours (Trembley, 1744). Spontaneous behaviours include the rhythmic spontaneous body contractions that are correlated with a specific electrophysiological activity termed contraction bursts and are modulated by light, among other stimuli (Passano and McCullough, 1964). The stereotypical feeding response, a typical reactive behaviour induced by food-associated stimuli consists of three distinct stages: tentacle writhing, tentacle ball formation and mouth opening (Koizumi et al., 1983; Lenhoff, 1968). This reactive and elaborate reflex-like behaviour is fundamental to the survival of *Hydra* and sensitive to its needs: well-fed animals do not appear to show feeding behaviour when exposed to a food stimulus (Grosvenor et al., 1996; Koizumi and Maeda, 1981; Loomis, 1955). In addition, feeding behaviour can be robustly induced by small molecules, such as glutathione (Lehoff, 1961). Mounting evidence suggests that all these behaviours represent a motor output of neuronal circuits that may comprise sensory and ganglion neurons which are controlling the effectors, i.e. the multifunctional epitheliomuscular cells. However, the architecture of these circuits, the principles of neuronal connectivity and signal transduction within the *Hydra* nerve net and the neural mechanisms underlying the behaviour changes under environmental, physiological, nutritional or pharmacological manipulations are still largely in the dark.

## Ontogeny, architecture and activity of *Hydra*'s nervous system

In a *Hydra* embryo, the first neurons appear to develop relatively late and just before hatching (Martin et al., 1997). A juvenile polyp (hatchling) emerges with a few dozens of neurons. After hatching, the growth of a polyp is accompanied by a gradual increase in the number of neurons, which reaches approximately 3000 two weeks after hatching. Since *Hydra* proliferates asexually continuously by budding, in an adult *Hydra*, neurogenesis also takes place continuously to maintain tissue homeostasis. The simplicity and difference in neural architecture between an embryo and an adult polyp offer great potential for understanding the basic design principles and minimal size of a nervous system to still perform basic sensory information processing tasks.

Currently, it is unknown which level of neuronal organization is required for *Hydra*'s spontaneous and stimulus-evoked, reactive behaviour. Moreover, we do not know the essential components of the nerve net that enable

spontaneous and reactive behaviour as an indication of functional neuronal circuit growth. A rich repertoire of methods including *in vivo* labelling and tracking of transgenic neurons, cell cycle analysis and expression analysis at the protein and transcriptome level is available to explore how neuronal circuits are established and maintained by continuously integrating stem-cell-derived migratory neuronal precursor cells into the nervous system. In addition, improved imaging techniques enable neuroscientists to observe the entire activity of certain nervous systems at a glance, providing completely new insights in neural circuit architecture and functioning (Figure 2E). This becomes obvious in the recent work by Dupre and Yuste (Dupre and Yuste, 2017) who demonstrated by calcium imaging technology in *Hydra* the existence of multiple circuits within these nerve nets. They proposed that three major functional networks extend through the entire animal and are activated selectively during longitudinal contractions, elongations in response to light, and radial contractions. From these and others studies, it is obvious that the advent of novel imaging technologies leverages the great potential of *Hydra* as a model system to get in-depth insight into the functional sophistication of apparently simple nerve nets.

## Rhythmic spontaneous body contractions require both pacemaker cells and the presence of microbes

Spontaneous contractions of the digestive tract play an important role in almost all animals. From simple invertebrates to humans, there are consistently similar patterns of movement, through which rhythmic contractions of the muscles facilitate the transport and mixing of the bowel contents. The triggers for the spontaneous contractions of the muscle tissue are so-called pacemaker cells of the nervous system. In a specific rhythm and without any external stimulation, they emit electrical impulses, that ultimately reach the smooth muscles of the intestinal wall, and cause them to contract. Although the impulses as such occur autonomously, their frequency, regularity and intensity are subject to external influences. The factors underlying the control of these impulses are not known yet.

*Hydra* turned out to be a very suitable system for understanding the control of this simple spontaneous behaviour and the underlying neuronal circuits. An undisturbed adult *Hydra* polyp will contract spontaneously with a frequency in the order of 5–10 contractions per hour (Figure 2H). The regularity and frequency of these contractions are highly sensitive to environmental conditions—such as light intensity and spectrum, presence of food, and osmolarity (Benos and Prusch, 1973; Kanaya et al., 2019, 2020; Passano, 1963; Rushforth et al., 1963; Yamamoto and Yuste, 2020). The contractions are electrically induced by central pattern generator neurons or pacemaker cells (Figure 2F and G). Previous extracellular electrophysiological recordings (Passano and McCullough, 1964) suggest that the pacemaker cells operate at a higher intrinsic frequency than the contraction burst frequency, and thus imply existence of an additional mechanism or cell type that relays their motor output. To identify additional components of the control machinery, we remembered that the *Hydra* epithelium is colonized by a specific microbiota, and compared normal *Hydra* which had typical bacterial colonisation with those that had their microbiome completely removed (Murillo-Rincon et al., 2017). In comparison, organisms without bacterial colonisation exhibited a reduction in contractions by about half. At the same time, the rhythm of the movements became disrupted, and some of the breaks between the contractions were much longer. Thus, the absence of the specific microbiome in *Hydra* compromised the peristaltic movements in the body cavity. In a further step, we restored the specific bacterial colonisation in the germ-free organisms. Initially, we introduced each of the five most common bacterial species found in the *Hydra* microbiome individually back into the sterile polyps. It turned out that this individual bacterial colonisation has no appreciable effect on the frequency and timing of contractions. Only the joint reintroduction of the five main representatives of the microbiome led to a marked improvement in peristalsis, although even then, the pattern of contractions was not fully normalised. The point was made even stronger by the fact that an extract produced from the colonising bacteria had a similarly positive influence (Murillo-Rincon et al., 2017). Taken together, these observations indicate that only the natural and complete *Hydra* microbiome is indispensable for regular spontaneous contractility. Not yet identified molecules secreted by the bacteria can intervene in the control mechanism of the pacemaker cells. As such, bacterial metabolites or signals can have a decisive effect on the pattern of spontaneous peristaltic contractions. In sum, the microbiome appears to have an indispensable function in the frequency and timing of tissue contractions.

## The molecular signature of pacemaker cells

Based on the differential expression of transcripts encoding neurotransmitter receptors, ion channels, neuropeptides, and transcription factors, the neuronal population of *Hydra* can be subdivided into distinct clusters that are likely to include neurons with unique functions (Klimovich et al., 2020; Siebert et al., 2019). By using cluster-specific transcripts as molecular markers, certain neuronal classes were found to be restricted to specific domains in the *Hydra* body column. One such neuronal subpopulation is located at the base of the tentacles and expresses nicotinic acetylcholine receptors as well as genes encoding SCN-like sodium channels, ANO1-like chloride channels and TRPM-like cation channels (Figure 2F and G). When we blocked the activity of these genes in *Hydra*, this immediately led to a drastic reduction in rhythmic body contractions. Modulation of the activity of these "pacemaker" channels disturbed both the rhythm and the frequency of the spontaneous contractions of the *Hydra* body, indicating that they depend on the unique combination of ion channels. For this reason, we are convinced that these neurons are indeed the pacemaker cells that control the peristalsis; and that they are able to perceive signals from microorganisms and react to them. Interestingly, the human orthologs of these channels are expressed by the intestinal pacemaker cells in mammals (first identified by Ramón y Cajal and called interstitial cells of Cajal) and linked to the pathogenesis of irritable bowel syndrome (Beyder et al., 2014; Mazzone et al., 2019; Strege et al., 2018). This evolutionary connection can be stretched even further since the unique molecular architecture of pacemakers appears to be conserved between *Hydra* neurons, the pharyngeal pacemaker complex of *Caenorhabditis elegans* and the above mentioned enteric nervous system of the mouse. The peristaltic activity of the gut turns out as an evolutionarily ancient neurogenic behaviour dependent on microbial signals and essential for life.

## Bidirectional communication between pacemaker neurons and the symbiotic bacteria

Our studies uncovered that *Hydra* neurons not only receive signals from the microbiome, but also actively affect the composition of the associated microbiota. A detailed molecular genetic analysis of *Hydra's* individual nerve cells using single cell RNA sequencing technology showed (Klimovich et al., 2020) that distinct subpopulations of neurons exert a direct influence on the density and composition of the symbiotic bacteria using the tools of the innate immune system. Distinct neuronal types, including the pacemakers, produce neuropeptides that display highly selective antimicrobial activity and alter the composition and spatial distribution of the microbial communities on *Hydra* body (Augustin et al., 2017; Klimovich et al., 2020). In addition, neurons in *Hydra* produce many components of microbe-associated molecular pattern (MAMP) receptors, such as Toll-like receptors, NOD-like receptors, C-type lectin, etc., indicating that neurons in *Hydra* are immunocompetent cells with critical roles in immune signalling function (Klimovich et al., 2020). Emphasizing further the role of *Hydra* neurons in immunity, bioinformatics and machine learning algorithms revealed that a large fraction of neuronal genes unique to this genus (so called taxonomically restricted genes), are capable of encoding antimicrobial peptides (Klimovich et al., 2020). To our surprise, we uncovered that a number of *Hydra*-specific neuropeptides known to mediate neuroeurotransmission and motor control have a second function; they act as antimicrobial peptides and shape the microbiome (Augustin et al., 2017). Intriguingly, a bidirectional interaction between neurons and microbes can also be observed in vertebrates. While defensin family AMPs are expressed in the murine enteric neurons (Klimovich et al., 2020), a plethora of other peptides, produced in the mammalian brain, may also play a role in controlling resident beneficial microbes (Holzer and Farzi, 2014). Moreover, similar to dual-function neuropeptides of *Hydra*, a neuropeptide PACAP known to regulate neurodevelopment, emotion and stress responses in the mammalian brain has been recently identified as an antimicrobial peptide (Lee et al., 2021). Strikingly, antimicrobial peptides have structural features that make them prone to aggregation into plaques similar to those characteristic for the amyloids in the brain (Lee et al., 2020). Even more intriguingly, the β-amyloid protein also has antimicrobial potential and may normally function in the innate immune system (Soscia et al., 2010). These observations provide an exciting perspective that the accumulation of amyloid, considered a toxic waste product, may in fact be an immune reaction of the brain to the presence of microbes or their products (Abbott, 2020). Taken together, these observations uncover the existence of a common evolutionary conserved principle and support an emerging paradigm that the communication between the nervous system(s) and the microbiota are indeed bidirectional. The nervous system receives signals from the gut (gut–brain axis) affecting host behaviour and

development; and on the other hand is producing neuropeptides with antimicrobial activity and a possible role in controlling the microbiota.

## Conclusions, open questions, and future perspectives: a new way of exploring neuronal circuits

Here we have reviewed that nerve cells are involved in controlling resident beneficial microbes in the early emerging metazoan *Hydra*, and that microbes affect the animal's behaviour by directly interfering with neuronal receptors. Recent progress in molecular and imaging analysis allows us to present *Hydra* as a powerful system for studying neural interactions and neural circuit formation which allows easy access to combined genetic, cell biological, molecular, and biocomputational tools. It is increasingly evident that bidirectional interactions exist in many vertebrates among the gastrointestinal tract, the intestinal microbiota and the enteric and central nervous systems.

The path taken so far enables us to address specific and evolutionary informative questions with regard to the evolutionary origin of host neuron–microbe interactions. Open questions include:

- How do microbes affect innate behaviour such as *Hydra*'s feeding reflex?
- What are the microbial taxa involved and the responsive neuron populations?
- How different are these signals and factors closely related but different *Hydra* species? Preliminary observations point to a surprising difference in neuroanatomy in closely-related and apparently similar *Hydra* species; yet functional consequences of these differences remain unclear.
- Does the resident microbiota influence neurogenesis in embryos and/or in adults?
- Is the resident microbiota involved in educating neuronal precursor cells/stem cells which, in turn, influence the composition of the microbiota?
- How do the commensal microbiota support the development of the complex nerve net made of distinct spatially restricted neuronal populations (Figures 3 and 4)?
- Ample histochemical, biochemical and functional data has been accumulated, indicating the presence of different small molecule neurotransmitters such as catecholamines, serotonin, acetylcholine, glutamate and GABA in *Hydra*. Do the resident microbes contribute to the repertoire of neurotransmitters?

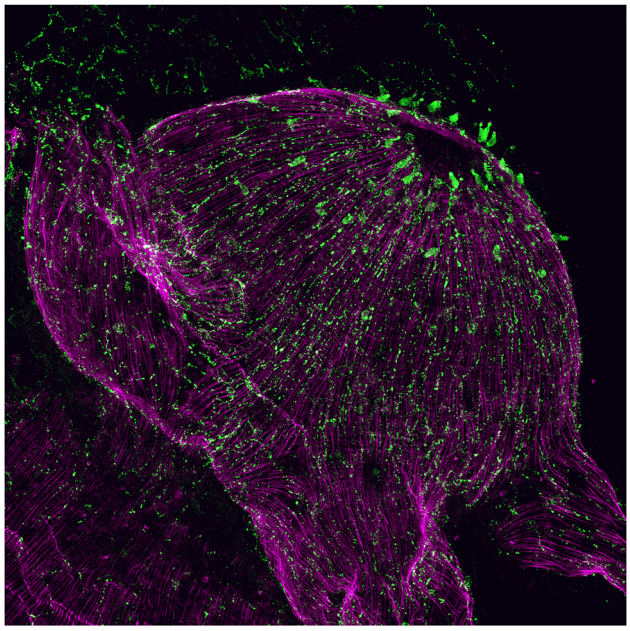

**Figure 3:** The nerve net of *Hydra* is composed of at least seven distinct spatially restricted neuronal populations. Here, one of them – a population of neurons expressing the RF-amide neuropeptide (green) in the hypostome of a polyp are visualized using specific antibodies. Muscular fibers of epithelial cells (magenta) are counterstained with Phalloidin.

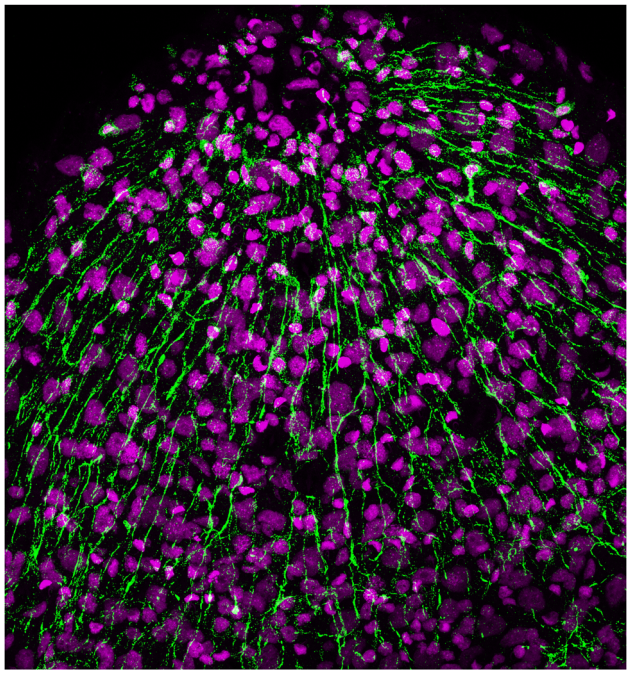

**Figure 4:** Modern microscopy technologies allow analysing the complex anatomy of the *Hydra* nerve net with unprecedented resolution. Here, the cell bodies and neurites of RF-amide-positive neurons in the hypostome (green) are visualized using a specific antibody. Cell nuclei are counterstained with TO-PRO (magenta).

– Last: what are the fundamental principles of the nerve net topology, dynamics and function that allow the simple nervous system of *Hydra* to be highly effective, multifunctional and energy-efficient? Understanding these basic rules of network design, implemented in the simple nervous systems of *Hydra* and other models, will be instrumental for development of highly efficient yet less energy-demanding microprocessors and computers (Bosch et al., 2017; Dupre and Yuste, 2017; Martinez and Sprecher, 2020).

Some of these questions are already under study in laboratories around the globe, but a more focused effort is required. The fascinating perspective is that these efforts will assist our understanding of how all of the parts of a living organism operate together within the metaorganismic framework. The comprehensive elucidation of the neural code for behaviour in an experimental system where one can have in principle access to either connectivity or functional data from every single neuron also enables the rigorous modeling of neural circuits, with simulations that are constrained completely in terms of the number of neurons, connections between the neurons and activity patterns. Model systems such as *Hydra* therefore may open new pathways to mimic basal neuronal mechanisms by electronic systems. Such studies will have relevance for understanding neural circuits in all animal species. In conclusion, the evidence is now irrefutable that "from so simple a beginning" (Charles Darwin) the neurobiology of animals has been, and is being, shaped by interactions with the microbial world.

## Glossary

**Antimicrobial peptides (AMPs):** Small molecular mass proteins with broad spectrum antimicrobial activity against bacteria, viruses and fungi. These peptides are usually positively charged and have both a hydrophobic and hydrophilic side that enable the molecule to be soluble in aqueous environments yet also enter lipid-rich membranes.

**Axenic condition:** Condition of animal culture, in which only a single species of organism is present and entirely free of all other contaminating organisms. This state is achieved by sterilizing the housing equipment, supplied food and air. Axenic culture is an essential tool for studies on symbiotic interactions in a controlled environment.

**Commensal microbe:** A bacterial, viral, fungal or archaeal organism that under normal circumstances resides in or on host tissue, does not cause disease, and forms a symbiotic relationship with the host in which one derives some benefit, while the other is unaffected.

**Conventionalized host organisms:** Carrying the full (undefined) load of organisms usually associated with this species.

**Dysbiosis:** An altered state (or disbalance) of microbiota associated with a change in species composition, abundance and/or spatial distribution and typically associated with a disease.

**Germfree:** Host organisms that are devoid of any other living germs or microorganisms.

**Holobiont:** The cnidarian host organism and all of its symbiotic algae and stably associated microbiota. While the term "meta-organism" defines a superordinate entity that is applicable to all kinds of interdependent associations, the term "holobiont" is constrained to specific taxonomic groups.

**Metaorganism:** An association composed of a uni- or multicellular macroscopic host and diverse microorganisms, including bacteria, Archaea, fungi, viruses, and various other microbial eukaryotic species including algal symbionts.

**Microbes:** Microbial life forms including bacteria, archaea, fungi and viruses.

**Microbiota:** Microbial life forms within a given habitat or host.

**Microbiome:** The totality of microorganisms and their collective genetic material present in or on the body of a macroscopic host organism or in another environment.

**Symbiotic interactions:** A close and usually obligatory association between two or more different organisms of different species that live together, often but not necessarily to their mutual benefit.

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