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Evolution: Neuronal control of an archaic mouth

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Cnidarians (corals, hydras, jellyfish, sea anemones) are prey-devouring creatures with a simple nervous system, the function of which is largely unknown. A new study on the freshwater polyp *Hydra* has now uncovered the neuronal circuits that control its feeding behavior.

The freshwater polyp *Hydra* sp. stands out for its almost unlimited regeneration capacity, which was first described by Abraham Trembley almost 300 years ago, but there is more to it than that. As a member of the Cnidaria, *Hydra* is also one of the earliest animals with a nervous system. Trembley began his ingenious regeneration experiments by testing the hypothesis that polyps were plants and could develop into complete organisms when bisected[1]. He found that they did indeed regenerate, but when he observed them capturing and eating prey, it became evident that they are in fact animals, not plants. "*Fortunately, he did not make this observation until after he had conclusively shown that hydras could regrow missing parts. As Trembley said, if he had seen his subjects eat earlier, he might never have decided to study regeneration*"[2]. Today, we are beginning to get a mechanistic and molecular view of the function of *Hydra*'s head during regeneration because we now understand how it acts as a developmental organizer with a Wnt signaling center[3–5]. However, it is still a mystery how *Hydra*'s head, with its neurons, sensory and stinging cells as well as gland cells, is functioning. Understanding its function is particularly challenging, given that the mouth of the *Hydra* and other cnidarians must be considered an archaic mouth — in terms of developmental lineage — corresponding to the embryonic blastopore. This mouth is fully functional in sea anemones and corals as early as the late gastrula stage, allowing them to catch and ingest prey[6]. A study by Giez, Bosch and colleagues in this issue of *Current Biology* now describes the neuronal modules controlling mouth opening and food intake in *Hydra*, and how microbes can interfere with this process[7].

Despite their simplicity and unlike the situation in most other animals, our knowledge of the neural basis of *Hydra*'s and other cnidarians' behavior is still in its infancy. Previous work has shown that *Hydra*'s nervous system arises from interstitial stem cells[8] and is composed of sensory and ganglion cells, forming a nerve net in both the ectoderm and endoderm[9]. There is no cephalization or ganglion formation in the head region with its mouth and tentacles (Figure 1), only regions along the body column with higher or lower densities of specific non-overlapping neuronal networks[9]. *Hydra*'s behavior is characterized by a number of distinct spontaneous or stimuli-evoked behavioral patterns, all linked to multiple neuropeptides that in part also act in *Hydra* pattern formation[10–12]. One example is *Hydra*'s somersaulting movement along the substrate, which was recently shown to be under the control of a specific neuronal population that secretes the RP1 peptide and increases its activity before movement[1,13]. Another example is *Hydra*'s feeding reaction[1], which can be induced by glutathione (GSH) and consists of three different stages: tentacle writhing, tentacle ball formation, and mouth opening[14,15]. This sophisticated behavior also reflects the internal neuronal state of the animals because a food stimulus given to well-fed animals cannot induce the feeding behavior and polyps without nerve cells do not react at all[16]. A unique aspect of cnidarian neurobiology is that these simple and ancient animals exhibit different neuronal networks forming integrated neuronal circuits that control a complex behavior without any central nervous system[9]. These neural networks act on epitheliomuscular cells that determine the well-defined body mechanics of polyps in a combination of neuronal and muscular excitation dynamics[17,18].

The recent study by Giez, Bosch and colleagues now pushes *Hydra* biology to the next level by combining transgenesis and calcium imaging with cell ablation approaches[7]. They show that *Hydra*'s eating behavior is controlled by multiple subpopulations of neurons that are activated in a temporally and spatially ordered manner, ultimately leading to mouth opening. Based on single cell transcriptomics, the authors identified three neuronal lineages in the head region: the ectodermal N3 and N6 neurons, which produce the neuropeptides Hym-355 and RFamide, respectively, and the endodermal N4 neurons, which produce the marker neurogenic differentiation factor 1-like. The individual subpopulations have a clear spatial distribution, creating a complex neuronal network with an ordered structure in the head region. N6 neurons are composed of sensory cells at the top of the head and ganglion cells at the base of the head. N3 and N4 neurons are found throughout the body, but with increased densities in the foot and head region. N4 neurons reach their highest density in the head at the base and between the tentacles.

Using calcium indicators (GCaMP6s) under the control of specific promoters for N6, N3 and N4 neurons, Giez, Bosch and colleagues identified the functional circuits that control mouth opening[7]. (A similar approach has been previously used to identify the neuronal circuits of the gastric region, which are associated with longitudinal and radial contraction patterns, and also light sensing[19].) When feeding behavior was triggered by GSH, mouth opening started with the response of N6 sensory cells, followed by N6 ganglion cells and endodermal N4 neurons, whereas the response of N3 neurons was delayed[7]. The entire mouth

opening took less than a minute. During this time, the neuronal activities of endodermal N4 and ectodermal N6 ganglionic cells were strongly correlated with the epitheliomuscular movement of both layers, while N6 sensory cells were only active during the perception of sensory inputs[7]. The contribution of these neuronal subpopulations was further validated using ablation experiments. In this approach, GFP-labeled transgenic cells express nitroreductase, which, upon incubation of animals in metronidazole, forms a toxic product that induces apoptosis in the target cells. This eliminates (neuronal) target cell populations in *Hydra* within 12 hours[7,13]. To completely inhibit mouth opening, the combined ablation of endodermal N4 and ectodermal N6 populations was necessary; ablation of just one of these two populations led only to a shortening in the duration of mouth opening.

The synergistic effects between endodermal N4 and ectodermal N6 neurons strongly suggest a physical interaction between both networks. Strikingly, however, the authors did not identify any synapses or contact zones between the ectodermal and the endodermal neurons or epitheliomuscular cells. They searched for potential synaptic connections using antibodies directed against RFamide-expressing neuronal subpopulations (N1, N6, and N7) or using GFP-expressing transgenic lines[7]. While the ectodermal networks, such as the N3 neurons, are connected to these neuronal subpopulations in the ectoderm (N1, N6, and N7), such contacts were not found with the endodermal N4 neurons[7]. This is remarkable because it leaves the question of synaptic contacts unanswered, although multiple contacts of the ectodermal and endodermal circuits should exist, based on the cascade of neuronal activation and the coordinated behavior of both epithelia during mouth opening. Also, N6 sensory neurons, which lead the cascade of neuronal activation after GSH triggering, do not exhibit any contact with endodermal neurons[7]. In another recent study using a pan-neuronal marker, no contacts of endodermal to ectodermal nerve nets could be identified[17]. Thus, it remains unclear how neuronal communication between the ectoderm and endoderm actually occurs,

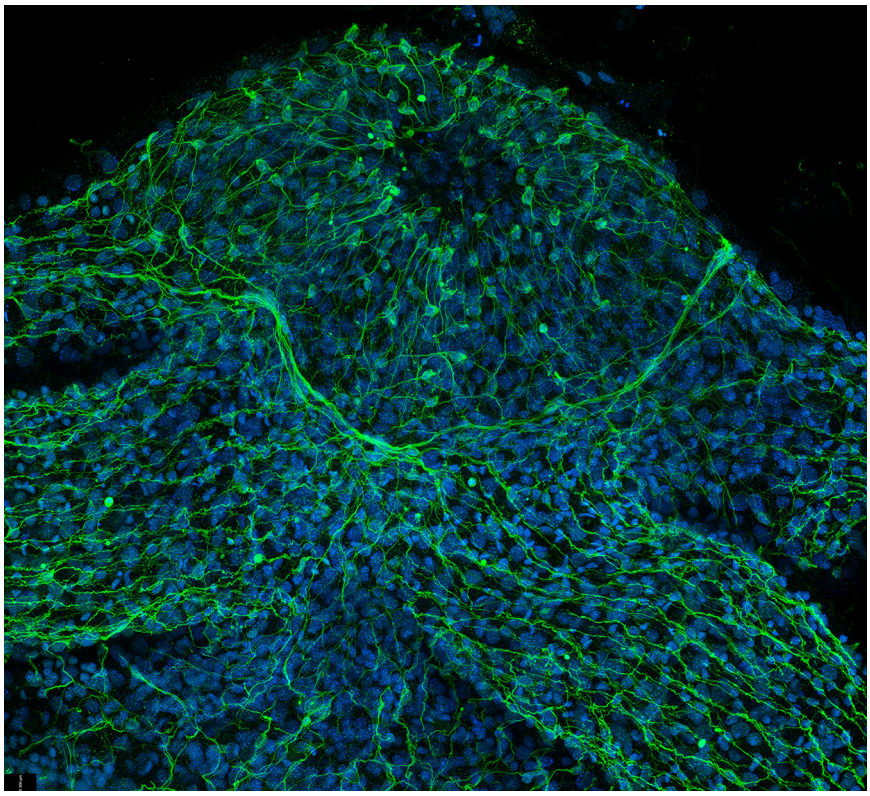

Figure 1. Nerve net of the head and mouth region of *Hydra oligactis*.
Neurons were stained with a pan-neuronal antibody used by Keramidioti *et al.*[17] (green) and the cell nuclei were stained with Höchst (blue). (Photo: Ira Maegele, Ulrike Engel and Thomas W. Holstein.)

but it may be realized by direct contact of the ectodermal and endodermal epitheliomuscular cells that are passing through the mesoglea — the extracellular matrix that separates the ectoderm and the endoderm[17].

What are the N6 sensory neurons sensing? To address this question, Giez, Bosch and colleagues cultured polyps under germ-free conditions[7]. The mouth opening time was shorter in these animals, but this deficiency was easily corrected when the polyps were grown again in the presence of the *Hydra* core microbiota[7]. With this approach, the influence of individual bacterial species could be tested. Surprisingly, upon association with only *Curvibacter* sp., the duration of mouth opening was reduced to almost zero. This inhibitory effect was unexpected because *Curvibacter* sp. constitutes the majority of *Hydra*'s bacterial microbiota (70%) and had not been associated with negative effects on the host so far, but this effect could be reversed by providing *Hydra*'s complete microbiota cocktail[7]. Further experiments indicated that a reduction in the duration of mouth opening is due to the secretion of bacterial glutamate. Although the regulatory mechanisms behind this eating behavior are not yet fully clear[7], it suggests a model whereby glutamate secreted by *Curvibacter* sp. is sensed by the glutamate receptors present in *Hydra*'s major neuronal subpopulations. Interestingly, only endodermal N4 neurons respond to glutamate by reducing their spike frequency, generating a more global effect of *Curvibacter* sp. on the nervous system. How endodermal N4 and ectodermal N6 neurons integrate the glutamate signal to affect downstream signaling[7] remains to be shown, however.

In summary, new findings on neuronal circuits in Hydra[7,13] show in remarkable detail how the molecular basis of fundamental behavioral patterns in *Hydra* can be unraveled through a combination of simple behavioral analysis with advanced imaging in elegantly designed transgenic lines. This progress brings us closer to deciphering the complex

neural mechanisms governing *Hydra*'s behaviors. A further exciting question will be how the formation of this neuronal network is controlled in such an archaic mouth. Here, a picture is emerging from work in the sea anemone *Nematostella*[20] that, at least in cnidarians, early neurogenesis is controlled by β-catenin and Wnt signaling.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Cell polarity: How to build an asymmetric bridge

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A new study provides key insights into planar cell polarity (PCP) establishment through the discovery of molecular asymmetries in the homotypic adhesive interactions of the PCP cadherin, Flamingo, resulting in the formation of asymmetric, intercellular bridges.

Most, if not all, cells have the ability to sense direction. Using dedicated 'molecular compasses', known as polarity pathways, cells sense directional cues that convey information about their orientation in the body and, in response, assemble polarized structures that align with the body axes. The asymmetric positioning and alignment of mechanosensory cilia, hairs and bristles across the surfaces of epithelia are particularly striking examples of this phenomenon, referred to as planar cell polarity (PCP). PCP is controlled by a set of membrane-associated proteins that localize asymmetrically in the cell[1]. What sets PCP apart from other types of polarity is its long-range, collective alignment across tissues, made possible through intercellular binding of its transmembrane components across cell