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Sensory neurons in the spinal cord of zebrafish and their local connectivity

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Sensory neurons of the spinal cord provide critical information on the internal and external environment. We review recent work that has made significant strides in elucidating the structure and function of two such neurons, excitatory Rohon-Beard neurons and inhibitory cerebrospinal fluid contacting neurons. These studies provide newfound appreciation for the diversity within each of these cell types and for the high complexity of sensory inputs to motor circuits in the spinal cord. We highlight research that links sensory neurons and spinal circuits to behaviors, leveraging the zebrafish as a powerhouse system to interrogate whole, intact circuitry in a behaving animal.

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Introduction

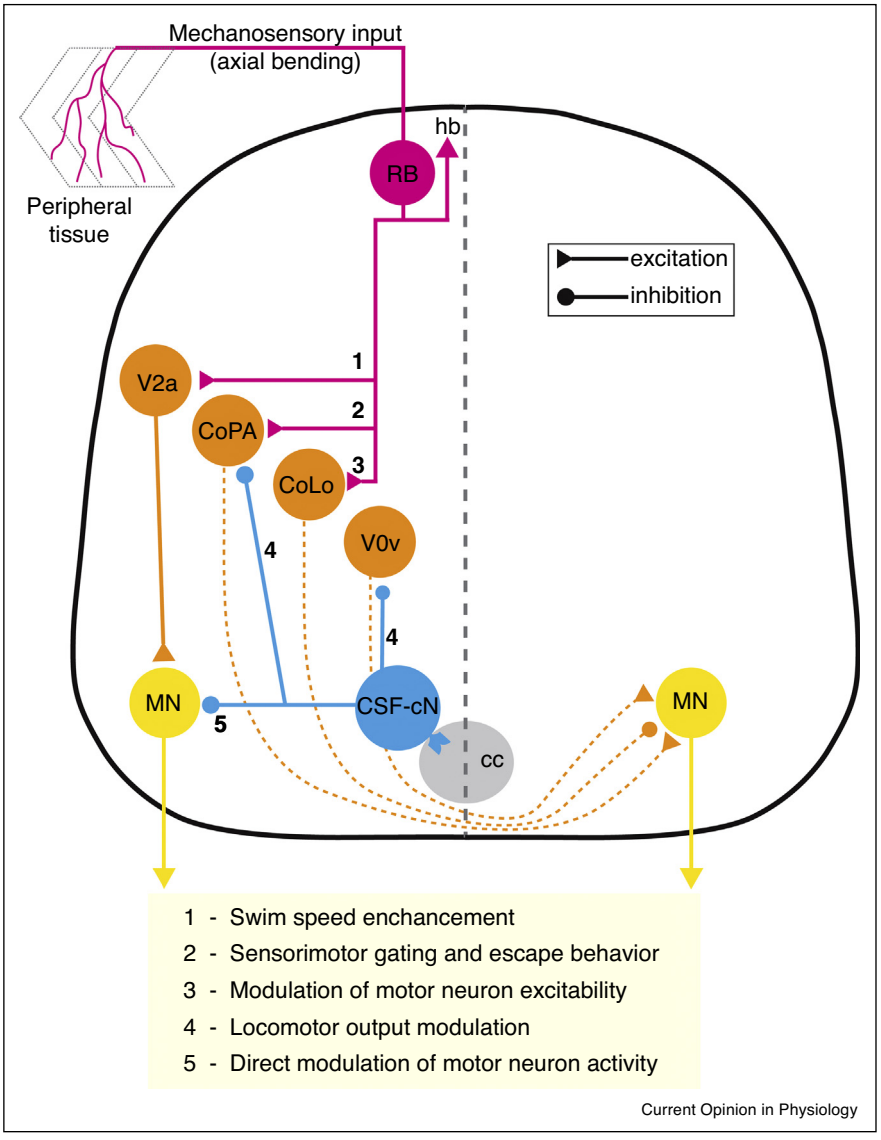
Sensory inputs to the spinal cord are critically important for coordinated locomotion, providing feedback from axial and limb musculature during movement. Foundational work in *Xenopus laevis* and lamprey described two intraspinal sensory neurons: Rohon-Beard neurons (RBs), and cerebrospinal fluid contacting neurons (CSF-cNs) [1–5]. Recent studies incorporating novel techniques have led to a more nuanced view of these neurons and their synaptic partners within spinal circuits. Many technical advances in vertebrate model systems have been critical for this work. Genetic tools [16–22], transsynaptic tracing techniques [23–25] and optogenetics [26] allow labeling and silencing of sensory neuron subtypes, mapping of neural circuits, and assessment of their functional roles in behavior. Through innovative and combinatorial experimental paradigms, it is now possible to study model organisms with improved cellular resolution and in more biologically relevant contexts [14•,27•,28•]. These new approaches are advancing our knowledge of sensory feedback from primary mechanosensors. Experiments in RBs and CSF-cNs in larval zebrafish, discussed below, integrate these approaches to provide a tractable system for detailing intraspinal sensory neuron morphology and function in a whole, intact organism in ethologically relevant contexts.

Rohon Beard neurons

First described in the 1800s [1,2], RBs are now known to be present in a number of aquatic species. They are a transient population of neurons present during early life history before development of sensory neurons in the dorsal root ganglia [6–11]. RBs are located in the dorsal spinal cord and exhibit a broad spectrum of primary afferent branching patterns in the skin (Figure 1) [3,12,13]. Initial functional studies identified RBs as mechanosensors that respond to touch on the skin in *X. laevis* [3,12]. Earlier anatomical and electrophysiological studies in fish and *Xenopus* found that RBs provide input to dorsally located spinal interneurons that excite interneurons and motor neurons in the contralateral spinal cord [29,39] as well as relaying information to the brain via their ascending axons, forming contacts with the reticulospinal Mauthner cells involved in the escape behavior (Figure 1) [32,40]. The classical views of RB function is that these glutamatergic cells mediate initiation of motor output in response to tactile stimulation [12].

While RBs were traditionally thought to be a homogeneous population [3,29], recent experiments have identified subpopulations of RBs with different ion channel compositions and gene expression patterns [30–32]. The heterogeneity of ion channels, specifically the expression of different classes of sodium channels, suggests differences in sensory roles across the RB population [30]. Heterogeneity of gene expression has revealed subsets of RBs express mechanosensory piezo2b, a homolog to the stretch-activated piezo2 in mammals [33], and others that express chemosensory transient receptor potential, trpA1 ion channels [34]. In mammalian systems, it is known that primary sensory cells of the dorsal root ganglia are separated into subtypes based on firing properties, mechanosensory ion channels, soma positions, and projections to the spinal cord [35–38]. Taken together, the diversity of ion channels expressed in subtypes of RBs suggests that similar organizational principles might also exist for RBs in zebrafish and other aquatic organisms with RB subtypes responding to different sensory inputs. Zebrafish offer a unique opportunity to

Figure 1



Integration of mechanosensory inputs in the zebrafish spinal cord.
The primary afferents of Rohon Beard neurons (RBs) innervate peripheral tissues while cerebrospinal fluid contacting neurons (CSF-cNs) receive direct sensory input from the central canal (cc). Both RBs and CSF-cNs are activated during spinal cord bending providing mechanosensory inputs to spinal circuits during active locomotion. Spinal circuit diagram shows the known spinal neuron targets of RBs and CSF-cNs. RBs exert excitatory modulation onto V2a neurons, commissural primary ascending (CoPA) neurons and commissural local (CoLo) neurons and CSF-cNs provide inhibitory control directly onto motor neurons, premotor V0v neurons and CoPAs. Mechanosensory signals are integrated in the spinal cord and the numbers 1–5 indicate our current understanding of the modulatory role of sensory inputs from RBs and CSF-cNs during active locomotion. The connectivity diagram depicts only connections within the spinal cord and does not include inputs to supraspinal structures. Each cell type indicated is representative of a whole population and not an individual cell. RBs: pink; CSF-cNs: blue; spinal interneurons: orange; motor neurons: yellow; midline: grey dashed line; central canal: grey circle; commissural axons: dashed lines; hb: hindbrain. References: 1: [28•]; 2: [48]; 3: [41•]; 4: [27•,46]; 5: [27•].

investigate *in vivo* how primary sensory signals are encoded by subtypes of sensory neurons in the spinal cord.

Connecting circuit structure and function to RB subtypes is further refining our understanding of their role in behavior. Knafo *et al.* [28•] combined optogenetic activation of RBs and whole-cell recordings to demonstrate that RBs provide inputs to a subset of excitatory *Chx10*+ V2a premotor interneurons. Elimination of these contacts via toxin-induced silencing of synaptic release from RB terminals

revealed that RBs modulate the speed of swimming by interacting directly with motor circuits (Figure 1) [28•]. These findings suggest that RBs modulate excitatory gain through local spinal circuits, thus providing sensory feedback during ongoing locomotion. In another study, Liu and Hale employed multi-electrode electrophysiological approaches to demonstrate that RBs connect with commissural local (CoLo) pre-motor inhibitory interneurons to precondition motor neuron excitability (Figure 1) [41•]. These results revealed a far more complex role for RB neurons in generating startle behaviors by driving both local sensory circuits in the caudal spinal cord, resulting in caudal bending, and providing excitatory input to startle circuits in the hindbrain [41•]. RBs also innervate ipsilateral glutamatergic commissural primary ascending (CoPAs) which drive contralateral bending behaviors and are important in the escape behavior [42]. These studies demonstrate that RBs have an intricate role in influencing motor neuron activation and modulating locomotor output.

CSF-cNs

A second type of cell, CSF-cNs, also known as Kolmer–Agdur cells, are present early in development in a range of aquatic and terrestrial vertebrate species [4,5,14•,15]. Like RBs, the cell bodies of CSF-cNs are located in the spinal cord; however, they extend microvilli and a single motile kinocilium into the central canal providing feedback on fluid conditions within the central canal (Figure 1) [4,5,14•,15]. The initial work in CSF-cNs indicated that they are conserved across vertebrates; however, until very recently, they had not been well studied beyond their morphological description.

Intraspinal sensory neurons have been shown to sense axial bending. Classical work identified edge cells in lamprey as an intraspinal proprioceptor that detects stretch during spinal cord bending and modulates motor output [43–45]. These intraspinal mechanosensors have never been identified in other species outside of lamprey; however, more recent zebrafish studies have demonstrated that proprioceptive feedback enters the spinal cord from the activation of CSF-cNs during axial bending [14•,27•,46]. A subset of CSF-cNs express a conserved transient receptor potential channel, PKD2L1, which has been found in zebrafish, mice and macaque [15]. This channel mediates the mechanosensory responses of these cells in zebrafish [14•]. Interestingly, a subset of mechanosensory CSF-cNs in lamprey also have chemosensory properties. They respond to pH changes through the acid-sensing ion channel ASIC3 [47•]. Differences in firing properties between dorsal and ventral CSF-cNs in zebrafish during varying behaviors [14•] suggest that these mechanosensors can also be classified into subtypes. Investigations of these questions will require genetic manipulations and *in vivo* manipulations of sensory populations.

CSF-cNs, unlike RBs, are inhibitory, GABAergic cells that have only been shown to project locally in spinal circuits. CSF-cNs synapse onto motor neurons, CoPAs [27•], and multipolar commissural descending (MCoDs) *dbx1b*+ V0v interneurons [46] within the zebrafish spinal cord (Figure 1). Silencing CSF-cNs via toxin-induced synaptic release or via genetic mutations of the PKD2L1 channel results in dampened postural maintenance during fast swimming [27•] and decreased swimming frequency [14•], respectively. Taken together, these studies have revealed a proprioceptive mechanism whereby CSF-cNs modulate swimming behaviors through inhibitory sensory feedback.

Conclusion

As new techniques and technologies have allowed for innovative *in vivo* approaches in zebrafish, our understanding of spinal sensory feedback in relation to behavior has blossomed and knowledge of the functional and anatomical diversity across and within RBs and CSF-cNs is expanding rapidly. Despite this, there are some major gaps in our knowledge of sensory feedback from intraspinal sensory neurons in zebrafish. There is a need to further investigate RB and CSF-cN subtypes to better understand their integration into spinal circuits and their roles in behavior.

Future work also needs to consider how RBs and CSF-cNs provide complementary feedback in tandem during locomotor behaviors. With modern genetic tools in combination with electrophysiology and optogenetic approaches, it would be possible to record from either population while manipulating the other. More classical techniques using touch and chemosensory stimuli during electrophysiological experiments could also help narrow down the sensory modalities encoded by these cells. There are clear similarities in how these populations convey sensory information centrally and how they integrate into spinal circuits. RBs and CSF-cNs both target excitatory and inhibitory interneuron subtypes within the spinal cord that have possible homologues across vertebrates (e.g. CoPAs). These second order interneurons have the capacity to influence motor circuits located ipsilaterally and contralaterally, and these shared projection patterns may indicate a conserved approach to modulating motor circuits across the independent evolution of different primary sensory systems. In addition to some of the shared synaptic connectivity patterns, the identification of CSF-cN contacts onto motor neurons indicates a novel integration of monosynaptic feedback outside of the canonical muscle spindle. Furthermore, RBs and CSF-cNs are likely to be working in tandem and may make local 'decisions' within the spinal cord. With the established techniques in CSF-cNs and RBs, we are closer than ever to a model capable of investigating multimodal sensory feedback during naturalistic behaviors. The established circuits of RBs and CSF-cNs can

be utilized as a basis for functional behavioral studies evaluating how two primary sensory populations modulate motor circuits during different behaviors.

Conflict of interest statement

Nothing declared.

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