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Title

Vestibulospinal circuits and the development of balance in fish

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Synopsis

The ability to maintain balance and adjust posture through reflexive motor control is vital for animal locomotion. The vestibulospinal nucleus residing in the hindbrain is responsible for relaying inner ear vestibular information to spinal motoneurons and is remarkably conserved from fish to higher vertebrates. Taking the advantage of relatively simple body plan and locomotor behavior, studies in fish have significantly contributed to our understanding of the anatomy, connectivity, and function of the vestibulospinal circuits and the development of balance control.

Abstract

During locomotion, animals engage reflexive motor control to adjust posture and maintain balance. The vestibulospinal nucleus responsible for transmitting vestibular information to the spinal cord is vital for corrective postural adjustments and is remarkably conserved from fish to higher vertebrates. However, little is known about how the vestibulospinal circuitry contributes to balance control. Over the past 50 years, fish have proved to be an ideal model for studying balance behavior because of their relatively simple body plan and locomotor activity. In this chapter, we summarize early works examining anatomical structures and functions of vestibulospinal neurons in different species of fish and recent studies on the kinematics of zebrafish balance behavior. Furthermore, we discuss how future investigations will link neural activity, circuit functions, and behavioral outputs to decipher the role of vestibulospinal circuits in balance control.

Introduction

Effective locomotion in animals requires active righting from unstable postures and balancing against externally imposed forces (Dickinson *et al.*, 2000). Therefore, the ability to engage reflexive motor control that corrects posture and maintains balance is vital for locomotor behavior (Webb and Weihs, 2015). In vertebrates, instability detected by the otolith organs and/or semicircular canals in the inner ear are transformed into corrective motor outputs in the spinal cord through vestibulospinal circuits (Pompeiano, 1972; Highstein, 1991; Witts and Murray, 2019; see Chapter 26). The vestibulospinal circuitry consists of five main elements: vestibular receptors (hair cells), vestibular ganglion neurons, vestibulospinal neurons, and spinal motoneurons/interneurons. Vestibulospinal neurons located in the hindbrain/rhombencephalon convey afferent signals to downstream motoneurons through conserved medial and lateral descending tracts (Jones, 1979; Auclair *et al.*, 1999; Büttner-Ennever and Gerrits, 2003; Kasumacic *et al.*, 2010; Lambert *et al.*, 2016; Witts and Murray, 2019). In most vertebrates, the medial pathway descends bilaterally along the medial longitudinal fascicle (mlf) and innervates mostly neck motoneurons in the cervical cord (Wilson and Schor, 1999; Büttner-Ennever and Gerrits, 2003; Chapter 26). In contrast, the lateral vestibulospinal tract projects ipsilaterally, terminates in all spinal cord segments, and is primarily involved in the vestibulospinal reflex (VSR) (Büttner-Ennever and Gerrits, 2003; de Lahunta and Glass, 2009; Rea, 2015). The medial pathway, together with some lateral vestibulospinal neurons terminating in the upper cervical cord, form the neural substrates underlying the vestibulo-collic reflex (VCR) which stabilizes the animal head (Wilson and Schor, 1999). Functional roles of vestibular neurons underlying the VCR, as well as the vestibulo-ocular reflex (VOR) introduced in the next chapter, have been extensively studied in various mammalian species because behaviors related to these vestibular reflexes are relatively simple and well-described. However, it is extremely challenging to study how the vestibulospinal circuit contributes to balance control and VSR because of the complexity of limb movements and the large number of muscles involved.

Over the past few decades, studies in early vertebrates, predominantly fishes, have greatly contributed to our understanding of vestibulospinal circuits underlying balance control. Although fish have distinct segmental patterning that differs from that of tetrapods, the anatomy and physiology of the vestibular system is remarkably conserved from fish to higher vertebrates (Büttner-Ennever and Gerrits, 2003; Straka and Baker, 2013; Yamamoto, Nakayama and Hagio, 2017; Bagnall and Schoppik, 2018; Witts and Murray, 2019). The topographical organization of vestibulospinal tracts has been comprehensively examined in a wide variety of fish species, from lampreys to lungfish (Nieuwenhuys, 1982; Nieuwenhuys and Oey, 1983; Ronan and Northcutt, 1985; Bussi eres, Pflieger and Dubuc, 1999). Importantly, the body plan and locomotor behavior in fish are much simpler compared to tetrapods, which facilitates decoding of externally imposed forces and the understanding of stabilizing reflexive movements (Webb and Weihs, 2015). Moreover, zebrafish has become a popular vertebrate model in recent years because it offers unprecedented imaging capability and genetic accessibility (Meyers, 2018). These unique features of zebrafish have greatly facilitated new approaches to understanding of vestibular behaviors and the responsible neural circuits (Bagnall and McLean, 2014; Ehrlich and Schoppik, 2017, 2019; Roberts *et al.*, 2017; Schoppik *et al.*, 2017; Favre-Bulle *et al.*, 2018; Migault *et al.*, 2018). The purpose of this chapter is to summarize the anatomical structures and functional roles of the vestibulospinal nucleus in fish and discuss how zebrafish as an emerging model can further contribute to our understanding of the vestibulospinal reflex.

Anatomical structures

Segmental organization and descending tracts of the vestibulospinal nucleus

The anatomy of vestibulospinal neurons has been described in many species of fish and is highly conserved across vertebrates (Nieuwenhuys, ten Donkelaar and Nicholson, 1998; Büttner-Ennever and Gerrits, 2003; Straka and Baker, 2013). The vestibular nucleus develops within the rhombomere (r) scaffold (see Chapter 4), a segmental framework in the developing hindbrain, and sends projections to spinal cord motoneurons and interneurons. Similar to tetrapods, descending vestibulospinal tracts in fish originate from two subgroups of cells located in r4-6: the anterior vestibulospinal nucleus with descending tracts predominantly to the ipsilateral spinal cord, and the posterior nucleus projecting bilaterally to both anterior oculomotoneurons and the upper spinal cord (Figure 1) (Büttner-Ennever and Gerrits, 2003; Straka and Baker, 2013).

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The vestibulospinal circuit is an evolutionarily ancient system that emerged before the appearance of the earliest jawless vertebrates (Gilland and Baker, 1993, 1995). Studies in lampreys, a member of the living jawless fish Cyclostomata (Potter *et al.*, 2015), identified subdivisions of vestibular nuclei, including the anterior, intermediate, posterior octavomotor nucleus (AON, ION, and PON, respectively) in r4-6 (Rovainen, 1979; Gilland and Baker, 1995; Bussièrès, Pflieger and Dubuc, 1999). Among these subgroups of vestibular nuclei, the ION and the PON form descending projections to the spinal cord. Stefanelli was the first to observe that the ION gives rise to an ipsilateral vestibulospinal tract and PON project bilaterally (Stefanelli, 1934). The morphology of descending neurons in ION and PON were further examined by Bussièrès and Dubuc using retrograde tracing, anterograde labeling, and intracellular labeling in silver lampreys (*Ichthyomyzon unicuspis*) and sea lampreys (*Petromyzon marinus*) (Bussièrès, Pflieger and Dubuc, 1999). They found that the ION, which contains 100-

150 neurons, projects in a loose bundle of axons dorsal-medially to the ipsilateral spinal cord, whereas axons from the PON project almost horizontally, cross the midline, and bifurcate into ascending and descending tracts (Bussi eres, Pflieger and Dubuc, 1999). Descending axons originating from the ION finally innervate motoneurons in the ipsilateral spinal cord but their projections were confined to the first 10 segments in the upper spinal cord (Rovainen, 1979; Zelenin *et al.*, 2003). Notably, the anatomical structure and projection of the ION resemble that of the lateral vestibulospinal neurons in tetrapods (Chapter 26), where they both originate from the anterior/rostral subgroup of the vestibular nucleus and project mostly to the ipsilateral spinal cord. Similarly, the axonal tract originating from the PON is homologous to the medial vestibulospinal tract responsible for the VCR (Bussi eres, Pflieger and Dubuc, 1999; Glover, 2000; B uttner-Ennever and Gerrits, 2003; Straka and Baker, 2013).

Homologs of the lateral vestibulospinal nucleus have also been examined in many actinopterygians, or ray-finned fish, which make up about half of all known vertebrate species. Early studies in primitive actinopterygii, chondrostei fish, reported the finding of four subgroups in the octaval nuclei: the nucleus anterior, the nucleus vestibularis magnocellularis (VEM), the nucleus descendens and the nucleus posterior (which appears to coincide with the tangential nucleus) (McCormick, 1982; New and Northcutt, 1984; New and Bodznick, 1985). The VEM, located lateral to the Mauthner cell, appears to be homologous to the vestibular nucleus in tetrapods. Descending axons from the VEM project both contralaterally through the medial vestibulospinal tract and ipsilaterally through the lateral vestibulospinal tract (Nieuwenhuys, ten Donkelaar and Nicholson, 1998).

Teleosts, a more evolutionarily advanced type of actinopterygii, comprise the majority of all extant species of fish. Topographical maps of the vestibular system have been studied in many species of teleosts, including salmon, goldfish, and zebrafish. Using retrograde labelling, Oka *et al.* (1986) examined descending pathways from the hindbrain in sockeye salmon (*Oncorhynchus nerka*) and identified four subgroups in the

octavolateral area that project to the spinal cord, including the VEM, the vestibularis descendens (VED), the tangential nucleus (TAN), and the intermedius nucleus (Oka, Satou and Ueda, 1986). According to Oka et al., (1986) axons from neurons in the VEM project to the spinal cord both ipsilaterally through the lateral vestibulospinal tract (so called “tractus bulbospinalis”) and bilaterally along the mlf; those from the VED and the TAN form the medial vestibulospinal tract. Notably, and unlike the VEM identified in other species, the salmon VEM reported by Oka et al. (1986) consists of neurons of various size, indicating possible heterogeneities within the subdivision (Kimmel, 1982; Oka, Satou and Ueda, 1986; Prasada Rao, Jadhao and Sharma, 1993; Yamamoto, Nakayama and Hagio, 2017). Moreover, axon terminations of these octavolateral neurons have not been examined in sockeye salmon. It is currently unknown whether the VEM nucleus identified by Oka et al. (1986) is homologous to the vestibulospinal nucleus in other species of fish and tetrapods.

In goldfish (*Carassius auratus*), the magnocellular octaval nucleus (MON) predominantly projects to the ipsilateral lateral tract with a subset entering the contralateral mlf (Lee, Eaton and Zottoli, 1993; Prasada Rao, Jadhao and Sharma, 1993; Straka and Baker, 2013). A small population of neurons in the dorsal (d) part of the descending octaval nucleus (DO), which is considered as caudal vestibulospinal nucleus in the goldfish, projects bilaterally to the spinal cord with predominantly ipsilateral projections (Prasada Rao, Jadhao and Sharma, 1993; Straka and Baker, 2013). Using classical retrograde tracing, Prasada Rao et al. (1993) examined the distribution of descending axons in adult goldfish and found that 70 out of 134 MON neurons and 20 out of 24 DO neurons project up to the 20th segment of the spinal cord but none of them reached the caudal-most 25th segmental level (Prasada Rao, Jadhao and Sharma, 1993).

Kimmel was the first to describe vestibulospinal descending tracts in zebrafish (*Danio rerio*). The zebrafish vestibulospinal nucleus originates from r4-5 (Straka and Baker, 2013; Bagnall and Schoppik, 2018). Unlike other salmon and goldfish, zebrafish

vestibulospinal axons project exclusively to the ipsilateral spinal cord (Kimmel, 1982). Similarly, the MON in the toadfish (*Opsanus tau*) also give rise to the lateral, ipsilaterally descending vestibulospinal tract which terminates on extensor motoneurons. Cells in the zebrafish vestibulospinal and toadfish MON are likely homologous to the Deiters' nucleus of e.g. mammals (Highstein *et al.*, 1992; Sarkisian, 2000; Rea, 2015; Witts and Murray, 2019; Chapter 26).

Roman and Northcutt investigated the descending spinal projections in south American lungfish (*Lepidosiren paradoxa*) which belongs to the class of sarcopterygii, or lobe-finned fish. In the lungfish MON, the majority of neurons project ipsilaterally to the spinal cord with axons from several neurons terminating contralaterally (Ronan and Northcutt, 1985), resembling the lateral vestibulospinal spinal nucleus reported in most actinopterygians and other vertebrates (Sarkisian, 2000; Rea, 2015; Yamamoto, Nakayama and Hagio, 2017).

Given minor discrepancies in the topographical organization of descending projections among different species, fish vestibulospinal nuclei reside in r4-6, consist of subgroups projecting to the ipsilateral and contralateral spinal cord, and innervate interneurons and motoneurons at different segmental levels.

Afferent connections of the vestibulospinal nucleus

The vestibulospinal nucleus in fish receives inputs from the vestibular system as well as the lateral line. According to Rovainen, the lamprey “octavomotorius nucleus” form mixed synapses with vestibular afferents extending from the ipsilateral inner ear labyrinth (Rovainen, 1979). The “octavomotorius nucleus” identified by Rovainen is likely the ION, or the vestibulospinal nucleus, because it sends ipsilateral descending fibers to the spinal cord and is involved in the vestibulospinal reflex (C. M. Rovainen, 1979; Gilland and Baker, 1995). Pflieger and Dubuc further investigated the relationship between vestibular afferents and the vestibulospinal nucleus using fluorescent tracers and found that vestibular afferents form bulb-like synapses with neurons in the ION and

the PON (Pflieger and Dubuc, 2000). Moreover, axons from the anterior branch of the vestibular nerve predominantly innervate with the ION and those from the posterior branch terminate in the PON (Pflieger and Dubuc, 2000).

Similarly, studies in teleosts have shown that the MON, or the vestibulospinal nucleus, receives ipsilateral projections from both the inner ear vestibular organs, including the saccule, the lagena, the utricle, and semicircular canals, as well as the posterior lateral line (McCormick, 1983; Meredith and Butler, 1983; Zottoli and Van Horne, 1983).

Afferent fibers from each inner ear endorgan in teleosts have preferred terminations in different regions of the MON, resembling the projection of vestibular afferent signals in tetrapods (Straka and Dieringer, 2004). For example, afferents from the semicircular canals and the utricle generally terminate in ventral cells in the MON; endings from the sacculus and the lagena are found among dorsal cells (Meredith and Butler, 1983). In goldfish, the lateral dDO, which contains a small population of vestibulospinal neurons, also receives inputs from the lateral line (McCormick *et al.*, 2016).

Function and behavior

Electrophysiological properties of the vestibulospinal nucleus

The vestibulospinal nucleus relays sensory inputs from inner ear vestibular organs to spinal motoneurons and interneurons. Early studies investigated the electrophysiology of neurons in the toadfish lateral vestibulospinal nucleus and lamprey ION and PON (Lowenstein, 1970; Korn, Sotelo and Bennett, 1977; C. M. Rovainen, 1979). These studies demonstrated that stimulation of the vestibular nerve evokes compound short-latency excitatory postsynaptic potentials (EPSP) in both ipsilateral and contralateral vestibulospinal nuclei, consistent with the observation that presynaptic afferent fibers form both chemical and electrical synapses on the cell bodies and dendrites of vestibulospinal neurons (Korn, Sotelo and Bennett, 1977; C. M. Rovainen, 1979). Additionally, electrophysiological studies of the lateral vestibulospinal nucleus in toadfish demonstrated the presence of strong electronic coupling between these

neurons, indicating that vestibulospinal neurons can be rapidly excited by spreading activity from neighboring neurons (Korn, Sotelo and Bennett, 1977).

How the vestibulospinal nucleus relays vestibular information to motoneurons and evoke muscle contractions was first studied in lampreys. Rovainen demonstrated that stimulation of the anterior vestibular nerve mostly results in contractions of the dorsal ipsilateral myotome, whereas activation of posterior vestibular afferents evoke contractions of the ventral contralateral muscles (C. M. Rovainen, 1979). These results agree well with the topographical mapping of the vestibulospinal circuit: the anterior vestibular afferents innervate the ION which project ipsilaterally and the posterior nerve innervate the PON which projects contralaterally (Gilland and Baker, 1995; Pflieger and Dubuc, 2000). Moreover, the axonal projection pattern of vestibulospinal neurons is related to rhythmic fluctuations of their membrane potential. By recording vestibulospinal neurons during fictive locomotion, Bussièrès and Dubuc found that the membrane potential of the majority of vestibulospinal neurons had peak depolarization during ipsilateral ventral root discharges, whereas the rest of them showed the opposite pattern (Bussièrès and Dubuc, 1992). Additionally, a small population of vestibulospinal neurons showed a complex pattern of activity: two waves of depolarization could occur during one locomotor cycle. Unsurprisingly, such rhythmic fluctuations of membrane potential in subgroups of vestibulospinal neurons are correlated with different projection patterns of their descending axons (Bussièrès and Dubuc, 1992).

The physiological and anatomical features of the vestibulospinal nucleus mentioned above allow a rapid response to rotations in all three body axes (Figure 2A) and excite motoneurons to produce corrective reflexes. Lowenstein first examined responses of fish vestibulospinal neurons to rotatory stimulation in lampreys (*Lampetra fluviatilis*) by mounting them on a torsion swing (Lowenstein, 1970). Rovainen further demonstrated how the vestibulospinal nucleus response to rotatory and mechanical stimulation by physical manipulation of the inner ear labyrinth (C. M. Rovainen, 1979). Together, they determined that roll, pitch and yaw evoke responses in subgroups of lamprey

vestibulospinal neurons. Interestingly, under each type of stimulation (pitch, yaw, or roll), several vestibulospinal neurons did not show a tuning preference and could be excited by rotations to either directions (Lowenstein, 1970; C. M. Rovainen, 1979). These neurons appeared to be the small population of vestibulospinal neurons that exhibit a complex pattern of membrane potential fluctuation mentioned above (Bussi eres and Dubuc, 1992).

Our understanding of the vestibulospinal neurons in response to rotatory stimulation has been brought to the next level by recent studies using advanced imaging techniques in larval zebrafish (Favre-Bulle *et al.*, 2018; Migault *et al.*, 2018). These works, together with the use of zebrafish as a model for vestibulospinal behaviors, will be summarized below.

Vestibulospinal circuits in balance behaviors

Freely swimming fish explore the environment with three-dimensional (3D) maneuvers. The ability to perform controlled and efficient locomotion in 3D environment is critical for the survival of the organism (Webb and Weihs, 2015). However, most fish are inherently unstable. A classic example is that anesthetized fish mostly lay on their sides. Fish generally have denser heads than tails, which generates a front-heavy body plane and anteriorly-located center of mass (Webb and Weihs, 1994, 2015). In addition, the center of buoyancy is offset posteriorly from the center of mass in most species of fish, resulting in unstable posture (Figure 2B) (Webb and Weihs, 1994, 2015; Ehrlich and Schoppik, 2017). Therefore, fish need to adjust their vertical posture to maintain balance or to reach different depth (Ehrlich and Schoppik, 2017). Although many studies have investigated the kinematics of directional and postural control in zebrafish larvae, how the vestibulospinal circuit contributes to balance control is not well understood (Li *et al.*, 2012; Nair, Azatian and McHenry, 2015; Ehrlich and Schoppik, 2017, 2019).

<Figure 2 near here>

The main function of the vestibulospinal reflex is to generate corrective movements of the head and body to maintain balance. Zelenin et al. (2003) examined the role of individual vestibulospinal neurons located in the lamprey ION. They found that activation of vestibulospinal neurons leads to a gentle flexion of the rostral body, resulting in head turning in different planes (Zelenin *et al.*, 2003). Recently, the role of the vestibulospinal circuit in balance control and posture adjustment has been more comprehensively studied in larval zebrafish.

During the first week post fertilization, larval zebrafish develop the self-righting behavior required for efficient locomotion. At the beginning of a swim bout, a larva laying on its side will change to its preferred dorsal-up posture by rolling (Figure 2C). This asymmetric posture adjustment requires engagement of dorsal-and ventral-projecting spinal cord motoneurons (Bagnall and McLean, 2014). Bagnall and McLean examined the role of vestibular signals in this righting behavior using a mutant line, *rock solo* (*otogelin*^{-/-}), that have no anterior otolith at early stages (Mo *et al.*, 2010). *rock solo* mutants failed to develop the self-righting behavior and their motoneurons did not exhibit asymmetric activity, indicating that the vestibular signal from the anterior otolith organ is critical to asymmetric activity of motoneurons (Bagnall and McLean, 2014). These results confirm that the vestibulospinal circuitry responsible for transmitting vestibular information to the spinal cord is required for the self-righting behavior in zebrafish larvae. However, little is known about how the vestibulospinal circuit transforms sensory information to generate motoneuron activity.

A recent study from Ehrlich and Schoppik demonstrated the role of the vestibular system in stabilizing pitch-axis rotation (nose-up/nose-down) during zebrafish spontaneous vertical locomotion using the same *otogelin* mutant. They found that wild-type larvae are able to coordinate fin and body movements to climb with stable postures, whereas mutants exhibited impaired balance control in climbing behavior, indicating that sensory information from the vestibular system is required for pitch

adjustment and efficient locomotion (Ehrlich and Schoppik, 2019). Excitingly, ongoing works from the Schoppik lab have demonstrated that photoablation of vestibulospinal neurons interferes with the ability of larvae to maintain their preferred posture in the pitch axis, suggesting that the vestibulospinal nucleus is necessary for pitch adjustment in zebrafish (Hamling and Schoppik, unpublished).

Development of balance behavior and circuitry in zebrafish

Similar to most vertebrates, zebrafish larvae are inherently unstable and have to learn to stabilize posture during development (Dickinson *et al.*, 2000; Bagnall and Schoppik, 2018). Accordingly, a normal development of vestibulospinal circuitry is critical for balance adjustment and ultimate survival. Because sensory development usually requires environmental inputs during critical periods, Moorman *et al.* (2002) examined whether such a “critical period” exists during development of the vestibular system. In order to minimize the sensation of gravity, Moorman *et al.* (2002) exposed zebrafish embryos at different stages with microgravity by putting them in a bioreactor. Results from their experiments suggest a critical period for zebrafish vestibular development between 30 to 66 hours post fertilization (hpf) (Moorman, Cordova and Davies, 2002). Embryos exposed to microgravity within the critical period had long-lasting vestibular defects, whereas those with microgravity experience outside that timeframe only showed transient phenotypes (Moorman, Cordova and Davies, 2002). This critical period includes the stages when vestibular afferent neurons are born and innervate their targets (Haddon and Lewis, 1996; Whitfield *et al.*, 2002).

Otolith organs are the first vestibular organ formed in larval zebrafish. The utricular and saccular otoliths begin development around 19 to 22 hpf (Haddon and Lewis, 1996; Abbas and Whitfield, 2010). Zebrafish mutants with developmental defects in utricular otoliths on both sides failed to maintain proper posture during locomotion (Malicki *et al.*, 1996; Riley and Moorman, 2000). Roberts *et al.* noticed that development of the anterior otolith in zebrafish *otogelin* mutants is delayed by almost two weeks. Compared to wild type siblings, larvae without anterior otoliths have abnormal swimming postures

including upside-down swimming, sideways rolling, and vertical pitching in the dark (Roberts, Elsner and Bagnall, 2017). Interestingly, after the late development of utricular otoliths at about 2 weeks post fertilization, *otogelin* mutants achieve wild-type like postural control within 48 hours (Roberts, Elsner and Bagnall, 2017). One explanation is that vestibulospinal circuits controlling balance behavior retain their plasticity and are able to readjust functional connectivity after otolith development. However, this conflicts with the critical period in vestibular system development which starts from 30 hpf (Moorman, Cordova and Davies, 2002). Other possibilities are: (A) afferent inputs from other vestibular organs are sufficient for the development of the vestibulospinal circuitry; (B) the development of vestibulospinal neurons does not require sensory inputs from inner ear endorgans but environmental cues during the critical period determine the formation and proper function of the vestibular organ and afferent nerves. Further studies combining functional analysis of the vestibulospinal circuit and manipulation of different circuit components during development may be able to address this question.

After the development of the otoliths, zebrafish larvae learn to maintain a horizontal posture using discrete swim bouts (Ehrlich and Schoppik, 2017). Because of their inherent instability, larvae tend to pitch nose downwards between bouts. Stability requires the initiation of subsequent bouts to correct their posture in the pitch axis. Studies from Ehrlich and Schoppik demonstrated that larvae at 4 days post fertilization (dpf) are already capable of using bouts to maintain horizontal posture, but their pitch angle has little influence on bout initiation (Ehrlich and Schoppik, 2017). In older larvae (7 to 21 hpf), bout initiation becomes posture dependent. They are more likely to initiate bouts as they rotate away from their preferred posture (Ehrlich and Schoppik, 2017). Other works from Ehrlich and Schoppik showed that zebrafish larvae also change the way of fin and body movement coordination as they develop, which allows them to climb with increasingly stable postures and to compensate for instability (Ehrlich and Schoppik, 2019). Together, these findings confirm that older larvae have higher posture sensitivity, suggesting that the function of vestibulospinal—or other vestibular—circuits

underlying balance control improves with age. However, the neural basis of balance improvement during development requires further investigation.

New approaches to functional analysis of vestibulospinal circuits

In recent years, larval zebrafish has become a popular and powerful model for studying neural circuits (Meyers, 2018). Because of their rapid development, accessible genome modification, and near-transparency, many new techniques have been developed and applied in larval zebrafish. These unique advantages of zebrafish open new approaches to understand the role of vestibulospinal neurons in balance control.

One limitation of the traditional methods to study neuronal functions is the constraint on the number of neurons that can be recorded at the same time. Thanks to recent advancements of functional imaging, one can now perform long-term, non-invasive recording of neuron activity across the entire zebrafish brain (Ahrens, Michael B Orger, *et al.*, 2013; Ahrens, Michael B. Orger, *et al.*, 2013; Leung, Wang and Mourrain, 2013; Panier *et al.*, 2013; Keller and Ahrens, 2015). In a recent work, Migault *et al.* took the advantage of light-sheet microscopy and performed calcium imaging of the whole zebrafish brain under vestibular stimulation (Migault *et al.*, 2018). In order to provide roll stimulation and image the brain simultaneously, they mounted larval zebrafish on a rotating platform together with a miniaturized light-sheet microscope and delivered controlled rotation to the whole system. Using this unique apparatus, Migault *et al.* identified three clusters of neurons with distinct tuning preference to rotation angle and angular speed (Migault *et al.*, 2018).

Favre-Bulle *et al.* also managed to image the whole zebrafish brain while delivering vestibular stimulation but with a different approach. Instead of rotating the larvae together with the microscope, they used optical tweezers to apply physical forces to the otoliths (Favre-Bulle *et al.*, 2018). This approach enabled them to focally target a specific vestibular organ instead of stimulating the whole vestibular system, allowing for better dissection of vestibular processing across the brain. These exciting new

techniques can now be applied to functionally analyze vestibulospinal circuits underlying balance adjustment. Using controlled vestibular stimulation and advanced imaging of the vestibulospinal circuitry, it will be possible to obtain a comprehensive understanding of how vestibulospinal neurons relay sensory information from the inner ear to downstream motoneurons.

Conclusions

All vertebrates need to sense and adjust their orientation and posture against the pervasive force on earth, gravity. Balance adjustment requires the evolutionary conserved vestibulospinal circuits to relay vestibular information to downstream motoneurons. Over the past 50 years, fish have proved to be an ideal model for studying balance control because of the simple body plan and locomotor behavior. Early studies in various species of fish focusing on anatomical structures and neural connections of vestibulospinal circuits provide a strong foundation for functional analysis. Recent works on the kinematics of locomotor behavior broaden our knowledge of balance and postural control in fish. Future investigations linking neural activity, circuit functions, and behavioral outputs will decipher how the vestibulospinal circuit develops the capacity to control balance.

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All the illustrations used in this chapter are original.

Illustrations

Figure 1

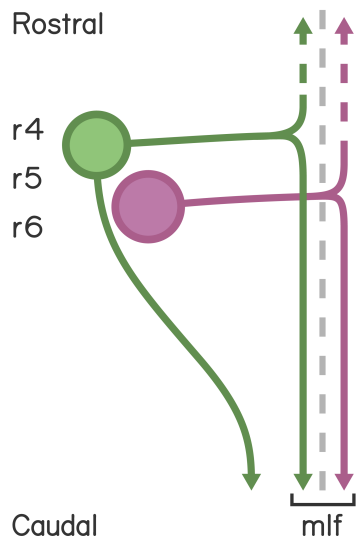


Figure 1. Schematic dorsal view of the vestibulospinal nuclei and their projections. Descending axons from the anterior vestibulospinal nucleus (green) predominantly innervate the ipsilateral spinal cord, whereas the posterior (magenta) nucleus project mainly to the contralateral spinal cord. r, rhombomere; mlf, medial longitudinal fasciculus.

Figure 2

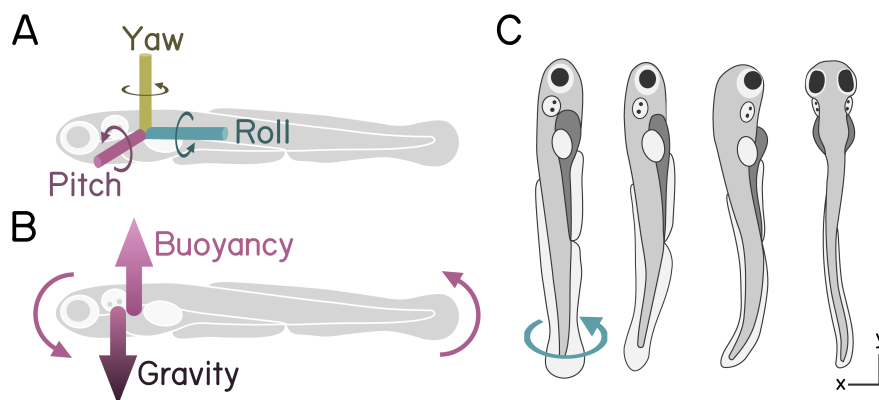


Figure 2. Illustrations of balance control in zebrafish larvae. (A) A diagram of three axes of rotation in fish: roll, pitch and yaw. (B) Forces causing instability of body position in the pitch axis. The center of gravity is offset in anterior direction from the center of buoyancy, which leads to a nose-down rotation. (C) Self-righting behavior in zebrafish larvae. A larva laying on its side changes to its preferred dorsal-up posture by rolling.