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Development of vestibular behaviors in zebrafish

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Most animals orient their bodies with respect to gravity to facilitate locomotion and perception. The neural circuits responsible for these orienting movements have long served as a model to address fundamental questions in systems neuroscience. Though postural control is vital, we know little about development of either balance reflexes or the neural circuitry that produces them. Recent work in a genetically and optically accessible vertebrate, the larval zebrafish, has begun to reveal the mechanisms by which such vestibular behaviors and circuits come to function. Here we highlight recent work that leverages the particular advantages of the larval zebrafish to illuminate mechanisms of postural development, the role of sensation for balance circuit development, and the organization of developing vestibular circuits. Further, we frame open questions regarding the developmental mechanisms for functional circuit assembly and maturation where studying the zebrafish vestibular system is likely to open new frontiers.

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Introduction

Gravity is a pervasive force across Earth. Most animals learn to control their orientation with respect to gravity, engaging reflexive movements that correct and maintain posture and that also serve as organizing principles of locomotor behavior. The normal posture that results from these movements is vital for locomotion and facilitates perception by stabilizing gaze. Therefore, the development of gravity-related behaviors and their underlying neural circuitry is a general problem of vital importance. Rigorous dissection of neural circuit function is only possible in the context of well-described behaviors, which dictate the constraints on the output of neural computations [1,2]. This poses a challenge for studies of posture in tetrapods, where a large number of muscles governing both limbs and trunk are engaged to maintain the animal's orientation, defying experimental analysis of the motor output. Furthermore, behavioral capacity is constrained by the composition of the underlying neural circuits, which are built during early development and refined as animals mature. Here we focus on vestibular function in an animal with a simpler body plan, the larval zebrafish, that serves as a powerful proving ground for hypotheses about the functional development of neural circuits.

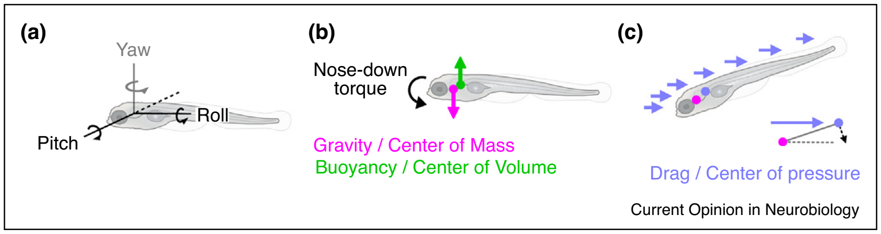
As a small model vertebrate, zebrafish have four features that uniquely facilitate the work on gravity-related behaviors and the responsible neural circuits. First, their simpler body plan facilitates decoding both destabilizing physical forces and the development of compensatory behaviors. Second, zebrafish are genetically accessible, with established mutant lines that disrupt balance and posture [3]. Third, the external development of zebrafish embryos permits continuous access, whereas in amniotes much of the development takes place *in ovo* or *in utero* [4•,5–7]. Finally, the mostly transparent bodies of zebrafish larvae permit anatomical, electrophysiological, and optical [8•,9] approaches during external development.

Vestibular circuits serve to transform sensed instability into corrective motor output. In order to produce rapid sensory-motor transformations for both posture and gaze stabilization, the nervous system relies predominantly on a short reflex arc [10]. In the inner ear, an otolith (or mass of otoconia, in later vertebrates) sits atop of hair cells. As the head tilts or translates, the otolith slides relative to the hair cells, which transduce this mechanical stimulus into electrical signals, releasing glutamate onto vestibular afferents of the eighth cranial nerve. Afferents relay this activity to central vestibular neurons, which encode head tilt in a variety of directions and project directly to motor centers, including cranial motor nuclei and the spinal cord. These feed-forward systems are remarkably well-conserved across vertebrates [11,12], reinforcing the generality of findings in the larval zebrafish.

How zebrafish learn to stabilize posture

What physical challenges destabilize larval zebrafish ($\leq$30 days old) and how do they learn to respond? Simliar to other highly maneuverable animals, zebrafish bodies are inherently unstable [13]. Vestibular-deficient animals

Figure 1



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A diagram of forces that stabilize and destabilize larval zebrafish posture. **(a)** Three axes of rotation: Roll, Pitch (nose-up/nose-down) and Yaw (left/right turns). **(b)** Forces in the pitch axis: the buoyant force acts at the center of volume (green circle) to elevate the fish; gravity acts at the center of mass (magenta circle) to pull the fish down. The center of mass sits forward of the center of gravity which leads to a nose-down torque that will rotate a passive fish nose-down. **(c)** During forward translation, or in flow, the fish will rotate (shown here in the pitch axis) to align with the direction of drag (blue lines). The center of pressure (blue circle) is displaced caudally to the center of mass (pink circle) about which the fish rotates. This displacement acts as a moment arm, schematized in the corner, that generates stabilizing torque (black arrow) to align the body.

often swim in corkscrew fashion or upside-down, suggesting instability in the roll axis (Figure 1a) [14]. Anesthetized larvae are also unstable in the pitch axis, tipping headfirst (Figure 1a). Recent work offers a physical explanation for the nose-down torques that destabilize pitch [15••]. In still water, the two main forces acting on zebrafish are gravity and buoyancy (Figure 1b). Gravity acts at a zebrafish's center of mass to pull the fish down. Meanwhile, the buoyant force acts at the center of volume to push the zebrafish up. Because the center of mass for larval zebrafish sits rostral to the center of volume, fish are subject to a constant nose-down torque. Therefore, to maintain stability, fish must govern orientation in both the pitch and roll axes.

Zebrafish exhibit both active and passive mechanisms that govern orientation, with passive contributions mitigating some external destabilization and active components providing the remaining postural control. Larval zebrafish can actively rotate their bodies by selective contractions of the dorsal (epaxial) and ventral (hypaxial) trunk musculature. Differential contraction of the dorsal and ventral muscles on the right and left sides of the body subserves roll (Figure 2a) [16••]. Conversely, contraction of dorsal or ventral muscles on the left and right sides in concert serves to rotate fish in the pitch axis, though the precise organization of this activity has not been described [17]. Importantly, asymmetries in zebrafish morphology provide directional stability in both roll and pitch axes. Just as a weathervane rotates to adopt a stable orientation relative to the wind, fish orient in roll/pitch during forward translation (Figure 1c). Thus swimming and/or facing into flowing water passively stabilizes orientation.

Separating active and passive means of orientation enabled recent work showing that larval zebrafish learn to time their swimming to stabilize posture [15••]. Larval zebrafish locomote in discrete swim bouts. Their bodies are so small that translation is constrained by viscous forces that minimize glide [18–20]. During the pause between swim bouts, larvae lose the stabilizing effects of flow and are instead pitched nose downwards by gravity. Subsequent swim bouts, made on average once a second, translate fish and correct this destabilization in concert with active reorienting movements (Figure 1c). At 4 days old, larvae time their swim bouts with comparable frequency at most postures. As they develop, the probability of making a bout becomes correlated with instability. As zebrafish rotate away too far or too quickly from their preferred posture, the probability of initiating a bout increases. Consequently, unlike younger larvae, older larvae specifically time bursts of swim bouts to compensate for instability. Emergent control of locomotor initiation thus underlies developmental improvements to postural stability.

Larval zebrafish reduce their density by inflating a gas-filled organ called the swim bladder; failure to do so is fatal. This early behavior requires larvae to reach the surface and gulp air [21]. Unlike visual cues, vestibular cues are sufficient to orient and locomote to the surface. Normal larvae raised in the dark inflate their swim bladders properly, whereas fish missing their vestibular otoliths fail to inflate their swim bladder at a normal time, even when raised in the light [22•]. Therefore to swim properly, larvae must use their vestibular system to orient and surface [23].

Vestibular input is not required for spinal circuit development

The importance of locomotion for postural stability underscores the need for rapid functional development of the spinal circuits that generate axial swimming in response to vestibular input. In larval zebrafish, the spinal circuitry responsible undergoes a change in functional configuration from 1 to 3 days old, with a shift from a spontaneous coiling behavior to the beat-and-glide swim pattern. The spinal cord of the early embryo exhibits high levels of gap junctional coupling, which is thought to