Ontogenetic rules and constraints of vestibulo-ocular reflex development
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Vestibulo-ocular reflexes (VOR) assist retinal image stabilization during vertebrate locomotion thereby ensuring accurate visual perception. The importance of this motor behavior for animal survival requires that the underlying circuitry and all individual components are fully developed and functional as soon as post-embryonic animals initiate self-motion. Recent progress on the genetic, molecular, and activity-dependent regulation of placode development, vestibular sensory organ formation, circuit assembly, and acquisition of neuronal properties revealed rules and restrictions that give insight into how hindbrain VOR neuronal networks are assembled and become functional during ontogeny. Major crucial steps that correlate with early/delayed functional VOR onsets concern the maturation of cellular properties (precocial/altricial species) and the acquisition of minimal semicircular canal dimensions (small-sized vertebrates).

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Introduction
Accurate perception of the visual world during locomotion is an important aspect for animal survival. In all vertebrates, self-generated body motion causes retinal image displacements with a resultant degradation of visual information processing. To maintain visual acuity, the image drift must be compensated by counteracting eye and/or head-adjustments that are classically considered to derive from sensory-motor transformation of vestibular, optokinetic, and proprioceptive feed-back signals [1,2]. Thus, image stabilization results from the concerted action of several synergistically acting reflexes (Figure 1). In addition, intrinsic locomotor efference copy signals [3] have been shown in larval amphibians to potentially contribute to compensatory eye movements [4].

The sensory-motor transformation of head motion occurs in brainstem circuits with few synaptic elements that have been phylogenetically conserved throughout evolution [5,6]. Semicircular canal (angular) and otolith-mediated (gravitoinertial) vestibulo-ocular reflexes (VOR) are relayed by three-neuronal reflex arcs that include vestibular nerve afferent fibers, central vestibular neurons, and extraocular motoneurons (Figure 2a). Cervico-ocular reflexes, activated by neck proprioceptive signals supplement the VOR [7], although to variable extents in different species. These open-loop reflexes are closed by feed-back visual signals arising from the residual retinal image slip (Figure 1), through activation of optokinetic reflexes (OKR).

Given the large dynamic working range of vestibular sensors for head motion detection, semicircular canal and otolith-evoked VOR play the major role for retinal image stabilization. This requires that VOR pathways and their individual elements are functional as soon as animals start to actively locomote during ontogeny. The developmental period for acquisition of coordinated locomotor activity after hatching/birth however varies considerably between different vertebrates. The offspring of precocial species are relatively mature and mobile from the moment of birth or hatching, while those of altricial species are rather helpless and unable to execute respective species-specific locomotor patterns for extended postnatal periods. The two categories form a continuum with different degrees of altriciality/precociality between extreme endpoints. Superprecocial animals develop a nearly adult locomotor performance within minutes to hours after hatching/birth, while the most extreme altricial animals need weeks to months for acquisition of adult locomotor dynamics. Given the tight functional link between locomotion and retinal image stabilization, the delayed postnatal maturation of locomotor performance in altricial animals might partly correlate with the completion and maturation of vestibular pathways and/or properties of VOR neuronal elements.

Ontogeny of vestibular sensors and afferent pathways
Vestibular sense organs in the inner ear arise during embryogenesis from bilateral epithelial thickenings of the cranial ectoderm (placodes) at the level of rhombomeres (r) 5 and 6 [8]. The formation of otic placodes is
Ontogeny of central vestibulo-ocular circuitry

Brainstem vestibular neurons and extraocular motoneurons are formed very early during vertebrate embryogenesis as shown in chicken [17] and mouse [18]. Vestibulo-ocular neurons develop as distinct subgroups of functionally specific phenotypes in unique hindbrain segmental compartments [19,20] that are defined by the combinatorial expression of evolutionary conserved genes [21,22]. These genetic regulatory elements are expressed in both transverse and longitudinally restricted domains to create a mosaic of unique neuroepithelial regions that give rise to the different vestibular phenotypes.

Recent advances in deciphering the developmental dynamics of VOR circuit formation in chicken and mouse by the group of J. Glover indicated that the synaptic connectivity of the VOR pathway is established in reversed order to the signaling direction [17,18]. Accordingly, the first synaptic connection is formed between extraocular motoneurons and eye muscles, which is necessary for vestibulo-ocular neurons to connect with hindbrain target neurons. The overall projection is formed by molecular means but is probably followed by activity-dependent refinement [15,16].

Oculomotor and trochlear nucleus-projecting vertical semicircular canal vestibular neurons are essentially located in r2, r3, and r6 (Figure 2a), whereas abducens nucleus-projecting horizontal canal vestibular neurons are located in r5 in mouse and frog (Figure 2a) and in r5/r6 in chicken [19]. Utricular-related vestibulo-ocular neurons originate largely from r5 (blue projection in Figure 2a) with variable contributions from adjacent segments in different vertebrate groups. The latter r5 group forms the tangential nucleus in chicken and fish that serves as gravito-inertial relay center [23,24]. The segmental origins of the different subgroups are rather conserved among vertebrates and major organizational variations exist mainly for vestibular subgroups in r5/r6 [17,19,20,22,23]. This spatial preservation also allows linking the classically described superior (SVN), medial (MVN), descending (DVN), and lateral vestibular nucleus (LVN) with the hindbrain segmental framework (Figure 2b) [25].

Developmental formation of extraocular motoneurons, including establishment of axonal connections with target eye muscles occurs very early during embryogenesis [17]. All extraocular motoneurons develop at conserved locations in the midbrain and hindbrain (Figure 2a) with the exception of taxon-specific segmental variations of abducens motoneurons in r5/r6 (Figure 2a) [26].

Functional onset of vestibulo-ocular reflexes

Completion of the VOR pathway during embryogenesis suggests that after birth/hatching, semicircular canal, and
Otolith-driven image stabilization is functional. This is a necessary requirement for precocial animals such as frog (tadpoles) or chicken hatchlings, which start to freely locomote almost immediately after hatching. By contrast, the limited postnatal mobility and unstable posture in altricial animals such as mice is accompanied by largely immature vestibular reflexes, as seen by the low VOR gains even after eye opening around P13 [28].

The embryonic establishment of neuronal circuitry is a prerequisite for VOR function early after birth/hatching but not sufficient for temporally precise sensory-motor transformation. The processing of naturally occurring head motion and transformation into dynamically adequate extraocular motor commands requires appropriate tuning of intrinsic membrane properties of all VOR neuronal elements. However, the postnatal maturity of vestibular cellular properties appears to be a major difference between precocial and altricial species [29]. Immature intrinsic properties of vestibular afferents [30,31] and central neurons [32] are correlated with modest vestibulo-motor performance and reduced locomotor capacities of early postnatal altricial animals. Cellular properties of mouse vestibular neurons are not mature before P30 [32], compatible with impaired VOR gains of juvenile mice [28]. The process of postnatal maturation of cellular properties appears to be independent of vestibular-driven afferent activity as indicated by the results from KCNE1(−/−) mutant mice [33]. Nonetheless, Eugène et al. [34∗] suggested a ‘critical period’ during which suppression of vestibular inputs can induce permanent locomotor deficits. However, it remains unclear so far, which of the developmental steps of vestibulo-motor circuit formation and refinement are critical. By contrast, principal tangential vestibular neurons of newly hatched chicken have similar firing properties as adults [35,36], consistent with the fast acquisition of locomotor ability of this precocial species.
The early embryonic establishment of cellular and network properties in precocial vertebrates complies with the activation of compensatory eye movements during low frequency or static pitch and roll head position changes very soon after hatching in larval zebrafish, goldfish, Medaka [37], and Xenopus [38,39]. In the latter species, utricular-evoked extraocular motor responses have robust gains immediately after hatching (Figure 3b) [39]. By contrast, the functional onset of semicircular canal-evoked extraocular motor responses is delayed after hatching in fish [37] and amphibian larvae [39] and the first measurable angular VOR coincides with significant body growth and increase of semicircular canal size (Figure 3a). Similarly, the increased afferent sensitivity to angular acceleration in postnatal rats correlates with fast semicircular canal growth, even though, part of the initial low discharge modulation is due to immature neuronal properties [30,31]. However, independent of the degree of maturation of the cellular properties, angular VOR onset crucially depends on semicircular canal dimensions, a parameter that is particularly important in small-sized vertebrates.

**Functional constraints of angular VOR onset**

Semicircular canal dimensions and fluid inertia determine angular VOR onset and the poor gain thereafter. Experimental data [31,39,40,41*,42*] and theoretical considerations [43,44,45,46,47] show that semicircular canal sensitivity augments with increasing canal lumen and circuit radius. The fluid flow resistance by the canal lumen is inversely proportional to the fourth power of the tube radius [44]. Thus, small increases in lumen radius considerably decrease the resistance to endolymph movement. Because small canal dimensions render the forces during self-motion insufficient to overcome fluid resistance, a minimal semicircular canal dimension must be reached during ontogeny to elicit an angular VOR (Figure 4). This threshold dimension plays a particularly important role for vertebrates that develop through very small post-embryonic stages such as fish or amphibians (Figure 3) [37,39**]. For the angular VOR to become functional these animals must grow rapidly to quickly increase semicircular canal dimensions beyond a particular threshold (oblique red line in Figure 4).

Delayed semicircular canal function and poor angular VOR performance in small fish and amphibian larvae [37,39**] render visual field stabilization during undulatory swimming, which starts almost immediately after hatching, almost impossible. Moreover, since semicircular canal function is essential to resolve the ambiguity of otolith signals related to translational linear acceleration...
and head tilt [48], the spatial tuning of otolith-related reflexes [49] is also affected by restricted canal function. Nonetheless, larval frog and fish exhibit locomotor patterns with considerable angular head excursions that necessitate compensatory eye movements. Thus, mechanisms other than the semicircular canal-related angular VOR might facilitate image stabilization.

Since the VOR is an open-loop reflex, visual signals that close the loop through activation of the OKR could compensate for angular VOR deficits. In fact, both larval zebrafish and *Xenopus* have robust visual reflexes over a broad frequency range early after hatching [37,39**], similar to the presence of a robust OKR in juvenile rats [28]. Moreover, during active locomotion, spinal
efference copies of rhythmic neural motor signals in larval *Xenopus* are conveyed to the brainstem extracochlear motor nuclei (Figure 1) and potentially contribute to image stabilization [4**]. This suggests that inherent feed-forward signaling is used to counteract the visual consequences during self-motion, thereby supplementing poor or absent sensory feed-back driven retinal image stabilization. Even though, this intrinsic mechanism has been demonstrated only during undulatory swimming in amphibians that requires relatively simple bilateral alternating axial muscle coordination, it is probable that spinal locomotor circuitry also accesses VOR networks in other vertebrates, including those confronted with more complex visual disturbances resulting from flexible necks and/or limb-based locomotion.

Conclusions

The development of functional vestibulococular reflexes is characterized by three major interrelated developmental steps. 1) Formation of semicircular canal and otolith endorgans, 2) generation of distinct vestibulococular neuronal phenotypes and establishment of spatially specific connections, and 3) acquisition of appropriate intrinsic cellular properties. These events are essentially complete after embryogenesis in precocial animals, whereas some functional components mature only postnatally in altricial animals. The delayed angular VOR onset in small vertebrates might be compensated by intrinsic efference copy signals during active locomotion thereby assisting image stabilization. Future studies on the role of these signals will help understanding their evolutionary origin and functional role.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: **of special interest** or **of outstanding interest**


2. Angeliak DE, Cullen KE: Vestibular system: the many facets of a multimodal sense. *Annu Rev Neurosci* 2008, 31:125-150. This review comprehensively describes the central information processing of head motion-related inputs that originate from multiple sensory organs as well as the role of intrinsic signals related to the generation of self-motion. Vestibular sensory information after entering the CNS becomes immediately multisensory and multimodal. The multimodal functions occur at several hierarchical levels that necessitate multisensory integration of vestibular representations in several reference frames. Proprioceptive-vestibular interactions, together with corollary discharge of respective motor intentions, allow the brain to distinguish active from passive head movements. In addition, central interactions between otolith and semicircular canal are important for navigation and spatial orientation.


4. Combes D, Le Ray D, Lambert FM, Simmers J, Straka H: An intrinsic feed-forward mechanism for vertebrate gaze stabilization. *Curr Biol* 2008, 18:R241-R243. This study shows that during active locomotion in larval *Xenopus*, efference copies of rhythmic neural signals produced by locomotor pattern-generating circuitry within the spinal cord are conveyed to the brainstem extracochlear motor nuclei and potentially contribute to gaze stabilization. The copies of spinal locomotor output offer a convenient substrate for initiating eye adjustments in the fastest possible way, pre-empting the slower reactionary engagement of the various movement-encoding sensory pathways that would serve to ensure the gain and precision of the gaze-stabilizing response.


18. Pasqualetti M, Diaz C, Renaud JS, Rijli FM, Glover JC: Fate-mapping the mammalian hindbrain: segmental origins of vestibular projection neurons assessed using rhombomere-specific Hoxa2 enhancer elements in the mouse embryo. *J Neurosci* 2007, 27:9670-9681. This study assessed the contributions of individual rhombomeres to the vestibular nuclear complex. Transgenic mice with the lacZ or the enhanced green fluorescent protein reporter genes under the transcriptional control of rhombomere-specific Hoxa2 enhancer elements were used to visualize rhombomere-derived domains. Identifiable vestibular projection neurons were retrogradely labeled with conjugated dextran-amines at successive embryonic stages and the developmental fate maps were obtained through direct comparison with the rhombomere-derived domains. The fate maps show that each vestibular neuronal group derives from a unique relatively stable rostro-caudal domain. Most of the groups are multi-segmental in origin, and each rhombomere gives rise to two or more types of vestibular projection neurons. Comparison with chicken shows that the patterning of identified vestibular projections is well conserved even though species-specific differences exist in the rostro-caudal limits of particular groups.


ontogenetic rules and constraints of vestibulo-ocular reflex development Straka 695


This study used an integrated neuro-ethological approach in larval Xenopus to demonstrate that semicircular canal dimensions, and not the function of other elements, determines the onset of angular acceleration detection. Before angular VOR onset, each individual component of the vestibulo-ocular system was shown to be operational: extracocular muscles could be activated, central neural pathways were complete, and canal hair cells were capable of evoking graded responses. For Xenopus, a minimum semicircular canal radius of 60 μm was necessary to permit endolymph displacement sufficient for sensor function at peak accelerations of 400 °/s². Since size constitutes a general biophysical threshold for semicircular canal-evoked behavior, the study suggests that canal lumen and circuit radius is the limiting factor for the onset of vestibular function particularly in small-sized vertebrates.


