Connecting Ears to Eye Muscles: Evolution of a 'Simple' Reflex Arc

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Abstract

Developmental and evolutionary data from vertebrates are beginning to elucidate the origin of the sensorimotor pathway that links gravity and motion detection to image-stabilizing eye movements – the vestibulo-ocular reflex (VOR). Conserved transcription factors coordinate the development of the vertebrate ear into three functional sensory compartments (graviception/translational linear acceleration, angular acceleration and sound perception). These sensory components connect to specific populations of vestibular and auditory projection neurons in the dorsal hindbrain through undetermined molecular mechanisms. In contrast, a molecular basis for the patterning of the vestibular projection neurons is beginning to emerge. These are organized through the actions of rostrocaudally and dorsoventrally restricted transcription factors into a 'hodological mosaic' within which coherent and largely segregated subgroups are specified to project to different targets in the spinal cord and brain stem. A specific set of these regionally diverse vestibular projection neurons functions as the central element that transforms vestibular sensory signals generated by active and passive head and body movements into motor output through the extraocular muscles. The large dynamic range of motion-related sensory signals requires an organization of VOR pathways as parallel, frequency-tuned, hierarchical connections from the sensory periphery to the motor output. We suggest that eyes, ears and functional connections subserving the VOR are vertebrate novelties that evolved into a functionally coherent motor control system in an almost stereotypic organization across vertebrate taxa.

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Introduction

Almost all extant vertebrates have eyes and ears that are interconnected so that vestibular sensory input can stabilize retinal image motion by guiding coordinated eye movements – the vestibular-ocular reflex (VOR) [Baker, 1998; Fritzsch, 1998; Straka, 2010]. In contrast, neither a vertebrate-like brain nor vertebrate-like eyes or ears exist as specific organs in other chordates [Fritzsch and Glover, 2007] indicating that the entire system that mediates the VOR is a vertebrate novelty. The 'skin-brain' [Fritzsch and Glover, 2007; Pani et al., 2012] of nonvertebrate deuterostomes lacks vestibular neurons, extraocular motoneu-

Abbreviation used in this paper	
VOR	vestibulo-ocular reflex

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rons, and the lateral eyes and ears found in vertebrates [Fritzsch, 1996; Fritzsch and Glover, 2007] even though molecular data identify sensory precursor cells in deuterostome outgroups of vertebrates [Pierce et al., 2008; Candiani et al., 2011; Vopalensky et al., 2012; Joyce Tang et al., 2013]. While the functional organization of the three-neuron VOR arc that links vestibular sensory organs in the inner ear to eye muscles has long been established [Szentagothai, 1950], it has only recently become apparent how such a complex system of two sensors interconnected by spatially specific neural pathways may have evolved.

Here, we propose that evolution introduced: (1) a set of conserved transcription factors that orchestrate the development of visual and vestibular sensory organ development; (2) an initial direct link from vestibular afferents to extraocular motoneurons; (3) a set of interneurons that relays vestibular signals to the hindbrain/spinal locomotor system; (4) spinal interneurons that relay an efference copy of locomotor output to the extraocular motoneurons to generate compensatory movements of the eyes during self-motion; (5) elaboration of the neck to make the head more movable relative to the body, and (6) a secondary set of interneurons that relays vestibular signals to the extraocular motoneurons, to provide additional compensatory eye movements made necessary by the appearance of less predictable head movements. Accordingly, we suggest that during evolution a simple reflex arc (sensory afferents directly contacting motoneurons) has been transformed into a three-neuron reflex arc (sensory afferents to interneurons to motoneurons) that builds on and supplements an even older eye motion control system that relied on intrinsic spinal locomotor efference copies.

Evolving an Ear and Connecting It to the Hindbrain

Evolution has recruited specific transcription factors to control the development of specific sensory organs. For example, the *Pax* gene family of transcription factors apparently evolved with multicellular organisms such as sponges [Hill et al., 2010] and later multiplied into several families associated with the eye and statocyst [Kozmik et al., 2003]. The single *Paxb* gene apparently evolved into the *Pax6* and *Pax2/5/8* genes found in all triploblasts. These *Pax* genes appear to be superorganizers for sensory organ development across phyla, indicating a deep molecular homology of structurally different sensory organs. Whereas *Pax6* is uniquely associated with eye development [Gehring, 2011], *Pax2/5/8* is related to multiple or-

gan systems such as kidney and ear [Bouchard et al., 2010; Christophorou et al., 2010]. While *Pax* genes are important for ear placode formation and development, additional transcription factors are also required [Chen and Streit, 2013; Fritzsch and Straka, 2014].

The ear of vertebrates is only one of many gravity sensors found in aquatic animals [Markl, 1974; Budelmann, 1988]. Some graviceptors evolved in animals without a central nervous system and thus without the computational capability to integrate multimodal information into appropriate motor outputs. Importantly, none of the deuterostome outgroups of vertebrates have otoconia-bearing sensory organs for gravity-dependent orientation, suggesting that the vertebrate otoconia-bearing ear is a novelty within the deuterostome lineage [Fritzsch and Straka, 2014]. However, many deuterostomes have at least cellular precursors that presage the morphological evolution of the vertebrate hair cells [Fritzsch et al., 2007; Burighel et al., 2011; Fritzsch and Straka, 2014]. These primordial and definitive hair cells can be identified not only by their morphological similarities but also by their expression of a unique set of transcription factors and microRNAs [Pierce et al., 2008; Candiani et al., 2011; Joyce Tang et al., 2013]. Experimental evidence suggests that in vertebrates the respective gene products are essential for normal hair cell differentiation [Soukup et al., 2009; Pan et al., 2012]. Thus, the evolution of hair cells likely predates ear evolution, replacing previous hypotheses of ear evolution by a 'hair cell first' hypothesis [Duncan and Fritzsch, 2012; Fritzsch and Straka, 2014]. The 'hair cell first' hypothesis brings into focus the problem of the evolution of the dorsolateral placodes that give rise to ears and lateral line organs. Outgroup comparison suggests that graviceptive otocysts evolved before lateral line-like organs [Budelmann and Bleckmann, 1988]. Therefore, the otic placode can be considered a unique embryonic adaptation that assembles in space and time a cohesive interactive gene network to form ears [Streit, 2007; Grocott et al., 2012].

Comparative data suggest that the first vertebrate ear was primarily a graviceptive statocyst [Budelmann, 1988]. As in mollusks [Budelmann, 1992], angular acceleration detection was likely added later during vertebrate evolution. Graviceptive and angular acceleration sensors evolved into specific morphological patterns distinctively separating jawless from jawed vertebrates [Fritzsch and Straka, 2014]. Jawless vertebrates have either a single torus (hagfish) or two incompletely separated canals (lampreys) [Lewis et al., 1985] combined with a single otoconia-bearing epithelium [Hammond and Whitfield, 2006]. Jawed

vertebrates have two to three otoconia/otolith-bearing sensory organs for linear translational/graviceptive sensation and three perpendicularly oriented semicircular canals with cristae that detect and vectorially decompose three-dimensional head rotations [Straka and Dieringer, 2004]. The formation of specialized sensors for angular acceleration might have coevolved with an increasingly larger locomotor mobility and the appearance of flexible necks, generating less predictable head movements and/or the need to resolve ambiguities of otolith organ-mediated tilt/translational acceleration signals during fast locomotion [Angelaki and Cullen, 2008; Lambert et al., 2008]. These eco-physiological alterations during early vertebrate history might have generated the selective pressure to evolve a system for sensing body rotation at high dynamic resolution, including the generation of appropriate eye/head/body-stabilizing motor behaviors.

The transformation of a placode into a complex labyrinth with properly positioned, multiple sensory epithelia depends on many factors [Fritzsch et al., 2007; Chang et al., 2008; Fritzsch and Straka, 2014]. Lamprey and hagfish lack a lateral/horizontal canal and the expression of genes relevant for forming this canal. Experimental manipulation of gene expression can mimic this situation in mammals. For example, *Otx1* and *N-Myc* null mice have no horizontal semicircular canal [Kopecky et al., 2011], and loss of *Foxg1* abolishes horizontal canal crista differentiation [Pauley et al., 2006; Hwang et al., 2009]. Other factors, such as homeobox genes *Fgf*s and *BMP*s [Chang et al., 2008], also play a role in this process, and the inner ear shows dramatic malformations or incomplete segregation of sensory epithelia in many mutants, such as the *Lmx1a* null mutation [Nichols et al., 2008]. The emerging picture implies a progressive transformation of a simple ear, as found in jawless vertebrates, through formation of multiple recesses, each associated with its own sensory epithelium, into the complex labyrinth of jawed vertebrates [Fritzsch et al., 2013] paralleled by a concurrent segregation of sensory afferent innervation [de Burlet, 1934; Fritzsch et al., 2002].

Vestibular sensory neurons connecting the hair cells in the sensory epithelia of the labyrinthine end organs to the hindbrain evolved late [Pan et al., 2012]. The parent cell bodies of the vestibular afferent fibers (fig. 1) are located in the ganglion of Scarpa and are somewhat segregated with respect to their peripheral end organs [Fritzsch et al., 2002; Maklad and Fritzsch, 2003b]. Compatible with the necessity for a functional organization of VOR circuitry into separate, frequency-tuned pathways [Straka et al., 2009], vestibular afferent fibers differ in several

interrelated morphophysiological properties [Straka and Dieringer, 2004]. Independent of the classification scheme, these neurons form at least two functionally distinct subtypes with different cellular properties, dynamic capabilities and motion-related discharge profiles [Goldberg, 2000; Eatock et al., 2008; Cullen, 2011]. The decomposition and transformation of body motion by semicircular canal and otolith hair cells into electrical signals with different dynamic signatures is maintained at the level of the afferent fibers by connecting hair cells and afferents with matching response properties, illustrating a major functional principle of vestibular signal processing [Straka and Dieringer, 2004; Straka et al., 2009]. Different mechanisms of motion detection make the otolith organs the main origin of the pathway that encodes slow, tonic head/body movements and the semicircular canals the main origin of the pathway that processes fast motion components. Since graviceptive, otolith-like end organs encoding low-dynamic roll and pitch movements are believed to have evolved first, hair cells and afferent fibers with nonadapting response properties likely predominated within the earliest vestibulomotor pathways. The later phylogenetic arrival of semicircular canals along with the necessity to encode rapid angular head acceleration signals and to generate rapid and transient motor reactions provided a high-dynamic detection and sensory encoding system [Goldberg, 2000; Cullen, 2011].

Despite the clear morphophysiological distinction between these low- and high-dynamic vestibular pathways, little is known about the gene-regulatory networks and molecular machinery that specify the ontogenetic differentiation of the respective afferent fiber systems. Only a few factors that influence the development of the differential afferent projection pattern have been identified. For example, the absence of *Neurod1* causes a central intermingling of vestibular and cochlear afferent fibers [Jahan et al., 2010]. Nothing, however, is known about the differential targeting of vestibular afferent subpopulations to the central vestibular projection neuron subgroups related to specific motor outputs [Maklad and Fritzsch, 2003a].

During development, afferent fibers from the different inner ear end organs terminate in a characteristic pattern within the hindbrain (fig. 1a). Developmental segregation of afferents from the different inner ear or lateral line end organs generates precise projections into modality-specific, evolutionarily conserved central target regions [Rubel and Fritzsch, 2002; Fritzsch et al., 2005; Maklad et al., 2010]. Thus, vestibular afferents terminate on specific central neuronal populations, primarily within the differ-

Straka/Fritzsch/Glover

Fig. 1. Vestibular end-organ-specific afferent terminations in the vestibular nuclei and monosynaptic labyrinthine afferent signal convergence onto frog central vestibular neurons (2°VN). **a** Spatial arrangement of the classical vestibular nuclei on a horizontal section through the dorsal hindbrain (a_1) and color-coded overlay of afferent projections from the three semicircular canals (a_2) and the three otolith organs (a_3) at the same horizontal level as shown in a_1. **b** Extent of convergence of monosynaptic inputs from one (b_1), two (b_1) or all three (b_3) ipsilateral semicircular canals in 2°VN. **c** Patterns of otolith-semicircular canal convergence (c_1, c_2) illustrating the proportions of 2°VN with monosynaptic responses from one or more end organs, respectively, and the predominant combination of UT and HC (c_1) and of LA and AC or PC (c_2) afferent inputs. AC/HC/PC = Anterior vertical, horizontal, posterior vertical semicircular canal; CN = cerebellar nucleus; DVN/LVN/MVN/SVN = descending, lateral, medial, superior vestibular nucleus; LA = lagena; SA = saccule; UT = utricle. **a** Illustrations are adopted from Birinyi et al. [2001]. **b**, **c** Summaries are based on data from Straka et al. [1997, 2002b].
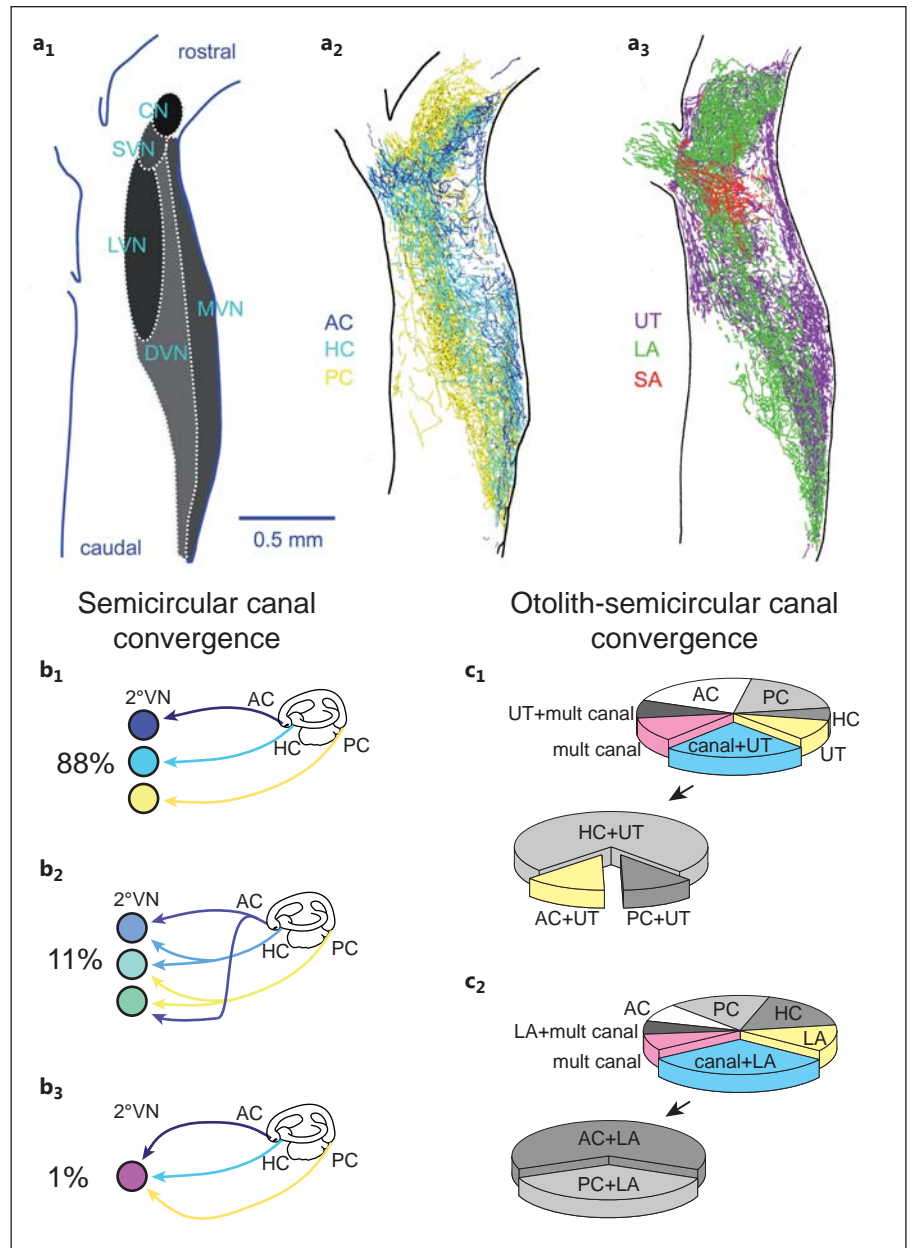
ent vestibular subnuclei (fig. 1a₁). However, despite a differential distribution and moderate spatial segregation, afferent terminations from the different vestibular end organs overlap considerably in all vertebrates (fig. 1a₂, a₃) [Maklad and Fritzsch, 2002]. This overlap enables a considerable convergence of information about angular acceleration, concomitant centrifugal force and positional changes in the gravitational field onto individual vestibular neurons [Straka and Dieringer, 2004].

Although afferent projections to the vestibular nuclei [Straka and Dieringer, 2004] and associated polysynaptic

circuits [Pflieger and Dubuc, 2004] channeling vestibular information to motoneurons appear to be the principal components of vestibular function in extant vertebrates, there is evidence for a simpler, two-neuron connection within the VOR. In the cat [Uchino et al., 1994, 1996], frog and goldfish [Straka, unpubl. results], a monosynaptic connection from utricular afferents onto ipsilateral abducens motoneurons has been identified anatomically and physiologically. In lampreys, the unusually positioned trochlear motoneurons in the cerebellum [Fritzsch et al., 1990] are very likely to receive direct vestibular afferent

input [Fritzsch, 1998]. Given the early evolutionary appearance of graviceptive vestibular end organs, this two-neuron reflex arc likely represents the ancestral condition, or a vestige thereof, whereby vestibular afferent information was channeled directly to extraocular motoneurons.

Regionalizing Vestibular Projection Neurons to Define the Flow of Vestibular Information from the Sensory Periphery to the Spinal Cord and to Extraocular Motor Nuclei

Vertebrate hindbrain development is characterized by segmental patterning at both cellular and genetic levels [Vaage, 1969; Nolte and Krumlauf, 2006]. The hindbrain neuroepithelium in all vertebrates is organized at the gross morphological level into a series of segments, the rhombomeres, which arise by formation of borders within and between earlier prorhombomeric subdivisions [Vaage, 1969; Gilland and Baker, 1993]. Unique combinations of genes encoding transcription factors and cell signaling molecules are expressed in each rhombomeric domain [Nolte and Krumlauf, 2006]. Perpendicular to this rostrocaudal segmentation, the dorsoventral axis becomes subdivided into unique, serial domains of transcription factor expression [Briscoe and Ericson, 2001; Fritzsch and Glover, 2007]. In combination, this patterning of gene expression appears to define the identities and histogenic fates of cells within each rhombomere [Prince et al., 1998; Dasen, 2013; Di Bonito et al., 2013]. Central vestibular neurons that develop within this genetically defined framework become specified into spatially coherent subpopulations with distinct axon projection trajectories and targets [Glover, 2003; Straka, 2010]. Projections from these central vestibular neurons and associated neuronal groups are anatomically and functionally diverse. The main targets are spinal (fig. 2a, c) and extraocular motor nuclei (fig. 2b, c, 3), the cerebellum and hippocampal and thalamic areas [Angelaki and Cullen, 2008].

The diverse sets of vestibular projection neurons are to a large extent spatially parcellated within the segmental and dorsoventral hindbrain scaffold into separate, coherent groups with defined targets (fig. 2). Vestibulospinal projection neurons in fish, frog [Straka et al., 2001], chicken [Glover and Petursdottir, 1991; Díaz et al., 2003] and mouse [Pasqualetti et al., 2007] are known to be nearly completely distinct from those giving rise to vestibulo-ocular or vestibulocerebellar projections, with only a small subgroup projecting to both spinal and oculomotor targets (fig. 2c) [Straka et al., 2001; Díaz et al., 2003; Díaz

and Puelles, 2003]. The specific and remarkably well-conserved segmental and dorsoventral locations of these general groups of vestibular projection neurons suggest a genetic blueprint that was established early in the vertebrate lineage [Glover, 1994; Baker, 1998; Díaz et al., 1998; Glover, 2000; Díaz and Glover, 2002; Straka and Baker, 2013] with possibly minor modifications in lampreys [Fritzsch, 1998].

With regard to distinct axonal trajectories and target regions, vestibulospinal projections derive from 3 separate, coherent groups, one that gives rise to the lateral vestibulospinal tract and 2 that give rise to separate ipsilateral and contralateral components of the medial vestibulospinal tract, respectively (fig. 2a), a pattern that is conserved from frogs to mammals [Glover and Petursdottir, 1991; Suwa et al., 1996; Glover, 2000; Straka et al., 2001; Pasqualetti et al., 2007]. Vestibulo-ocular projections derive from a larger number of groups (fig. $2b_1$, b_2), each of which can nevertheless be related to specific sets of extraocular motoneuron pools (fig. 3) [Glover, 2000, 2003; Straka et al., 2002a]. Although not absolute, the remarkable degree of spatial segregation exhibited by these coherent projection- and target-defined neuronal groups led to the concept of a 'hodological mosaic' within the vestibular nuclei, a clear indication that the location within the segmental and dorsoventral framework of the

Fig. 2. Regional patterning of vestibular projection neurons according to axon trajectory – the hodological mosaic. **a** Vestibulospinal projections derive from 3 different neuron populations, the lateral (LVST), the ipsilateral medial (iMVST) and the contralateral medial vestibulospinal tract (cMVST), each originating from a specific segmental and dorsoventral domain, shown here in the chicken embryo (**a_1–a_3**). **b** Vestibulo-ocular projections derive from at least 4 different neuron populations, the ipsilateral rostral (iR-VO), contralateral rostral (cR-VO), ipsilateral caudal (iC-VO) and the contralateral caudal vestibulo-ocular (cC-VO) groups, each originating from a specific hindbrain segmental and dorsoventral domain, shown here in the chicken embryo (**b_1, b_2**). **c** Segmental and dorsoventral domains of the vestibulospinal, vestibulo-ocular and other vestibular projection neuron types generate a hodological mosaic within the hindbrain with little actual overlap between vestibulo-ocular and vestibulospinal neuron groups in three dimensions. **d** Comparing the classical vestibular nuclei defined by cytoarchitectonics, the hodological organization of vestibular projections appears as a separate patterning scheme, with a clearer relationship to functional roles, as shown here schematically in the chicken embryo. ang = Nucleus angularis; d = descending vestibular nucleus; Dd = dorsal Deiters' nucleus; Dv = ventral Deiters' nucleus; lam = nucleus laminaris; m = medial vestibular nucleus; mag = nucleus magnocellularis; r1–r11 = rhombomeres 1–11; s = superior vestibular nucleus; t = tangential vestibular nucleus. **a, b, d** Illustrations are from Díaz et al. [1998]. **c** Adapted from Glover [1994, 2003].

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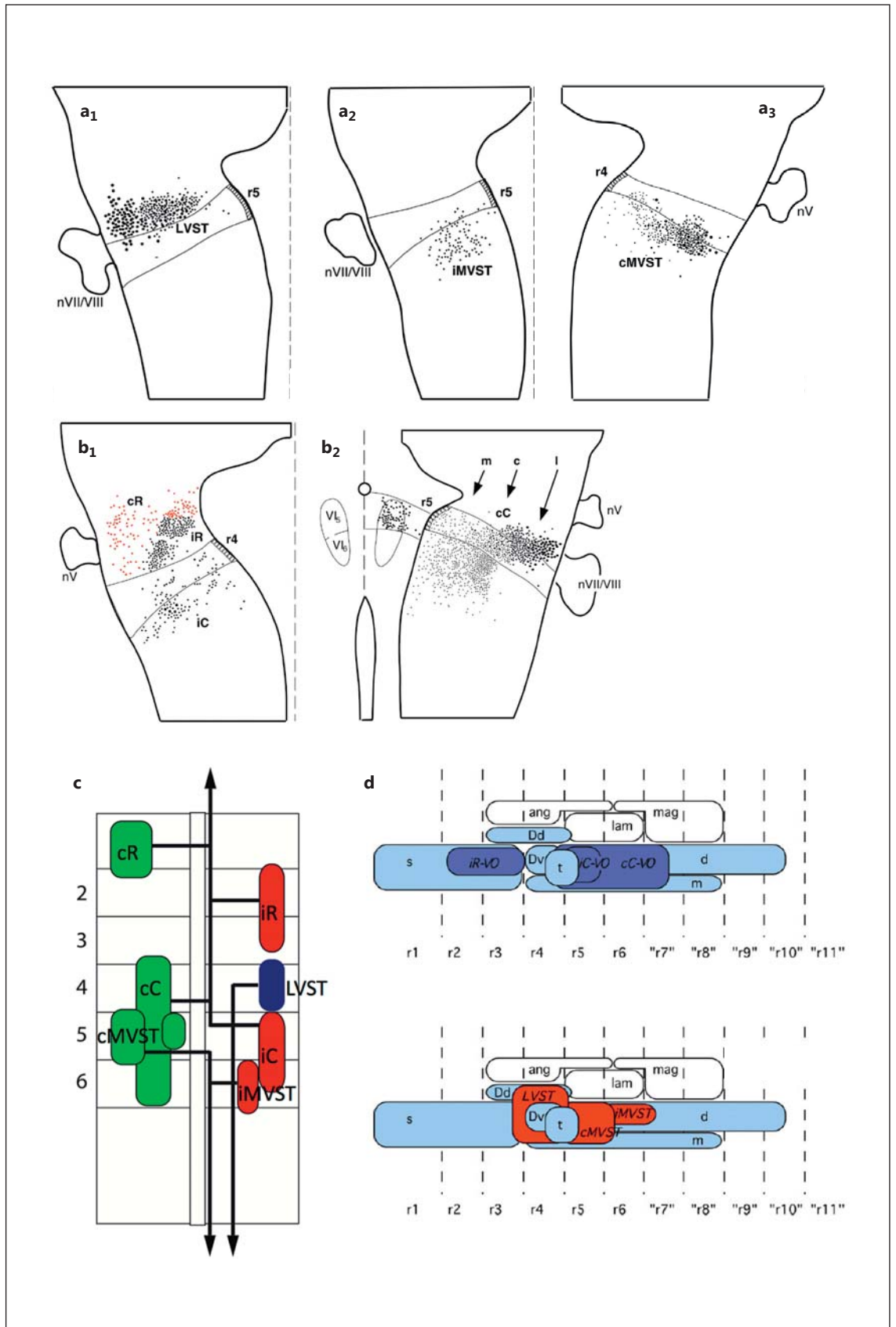
hindbrain is directly linked to the specification of functional phenotypes including synaptic connectivity [Glover, 1994, 2000; Baker, 1998].

A direct comparison of the hodologically defined vestibular projection neuron groups with the classic cytoarchitectonically defined vestibular nuclei (superior, medial, lateral/tangential and descending) in the chicken embryo and frog larva drives home an important point: functional connectivity is more tightly linked to segmental/dorsoventral domains than to cytoarchitectonic domains [Díaz et al., 1998; Cambronero and Puelles, 2000; Straka et al., 2001; Díaz et al., 2003]. Thus, although vestibulo-ocular projections in the classical sense derive from portions of the superior, medial, lateral and descending vestibular nuclei (fig. $1a_1$, 2d), this classification scheme poorly defines the picture because each of these nuclei is a melange of functional subgroups [Díaz et al., 2003; Straka et al., 2005]. The hodological definitions, on the other hand, provide an organizational scheme that not only directly denotes connectivity but also links connectivity to the genetic programs from which it, and likely other functional characters, derive. Thus, studies linking gene expression patterns to connectivity patterns are leading to a reclassification of vestibular anatomy that more clearly reflects the developmental and evolutionary processes responsible for its formation and ultimate function. Indeed, on this hodological backdrop, species-specific variants can be seen that obviously represent selections for or against specific elements of the presumptive vertebrate blueprint [Díaz and Glover, 2002; Pasqualetti et al., 2007; Straka and Baker, 2013].

The highly mosaic topography that relates the different functional subgroups of VOR-related vestibular projection neurons to specific premotor interneuron and motoneuron targets (fig. 3) contrasts with the highly overlapping termination areas of vestibular afferent fibers from the different labyrinthine end organs (fig. $1a_2$, a_3). Although the absence of an end-organ-specific structured or layered afferent terminal organization in the vestibular nuclei [Birinyi et al., 2001; Straka et al., 2003] suggests a substantial central convergence of inputs from all labyrinthine end organs, this is at variance with the observed functional specificity of central vestibular neurons. In fact, the majority of central vestibular neurons, independent of their position within the hindbrain scaffold, receive monosynaptic afferent inputs from only one semicircular canal (fig. 1b) and/or only one otolith organ [Straka et al., 1997, 2002b]. This means that the vectorial decomposition of angular and linear/graviceptive motion vectors by the different vestibular end organs is essentially maintained at the level of the central vestibular neurons. Moreover, when convergence occurs, semicircular canal inputs combine monosynaptically with otolith afferent inputs in an end-organ-specific manner (fig. 1c) [Straka et al., 2002b]. Accordingly, horizontal semicircular canal signals preferentially converge with utricular signals (fig. $1c_1$) and vertical semicircular canal signals preferentially converge with signals from vertical otolith organs (fig. $1c_2$; saccule or lagena, depending on the vertebrate taxa) [Straka et al., 2002b].

The specific connections from vestibular afferents to central vestibular neurons might be genetically preprogrammed [Fritzsch et al., 2005; Maklad et al., 2010] or determined retrogradely by the prior established connectivity of vestibular projection neurons with extraocular motoneuron targets [Glover, 2003; Straka, 2010]. Indeed, developmental studies in the chicken embryo show that as soon as synaptic contacts between vestibulo-ocular neurons and extraocular motoneurons are made, there is functional specificity within the VOR circuit spanning from afferents to projection neurons to motoneurons [Glover, 2003; Glover et al., unpubl. data]. The specific semicircular canal and otolith signal convergence (fig. 1c), if not present initially, might be consolidated later using Hebbian plasticity by end organ coactivation during body motion.

Whereas the developmental mechanisms that establish topographically specific VOR connections are beginning to be deciphered, it is less clear how central vestibular neurons with different functional dynamics [Straka et al., 2005] are specified and appropriately inserted into the respective circuits. Compatible with a distinction into frequency-tuned labyrinthine afferent pathways (fig. 4a) [Straka et al., 2009], central vestibular neurons are also subdivided into two major, dynamically different subclasses (fig. $4b_1$, b_2) with either low-pass or band-pass filter properties, ideally suited for coding either tonic/low-dynamic or fast, transient head movements (fig. $4b_3$) [Straka et al., 2005; Beraneck et al., 2007]. The presence of these dynamically distinct central vestibular neurons with specific intrinsic membrane properties and synaptic response characteristics (fig. 4b) suggests that different head motion components are processed by separate central vestibular networks, as they are by separate peripheral vestibular afferents (fig. 4a) [Straka et al., 2009]. The different dynamic vestibular neuron subclasses do not exhibit obvious preferential regional or segmental distributions, however [Straka et al., 2004]. The developmental mechanisms that link dynamically matched sensory afferents and central projection neurons are unknown, and could involve cell recognition based on intrinsic genetic
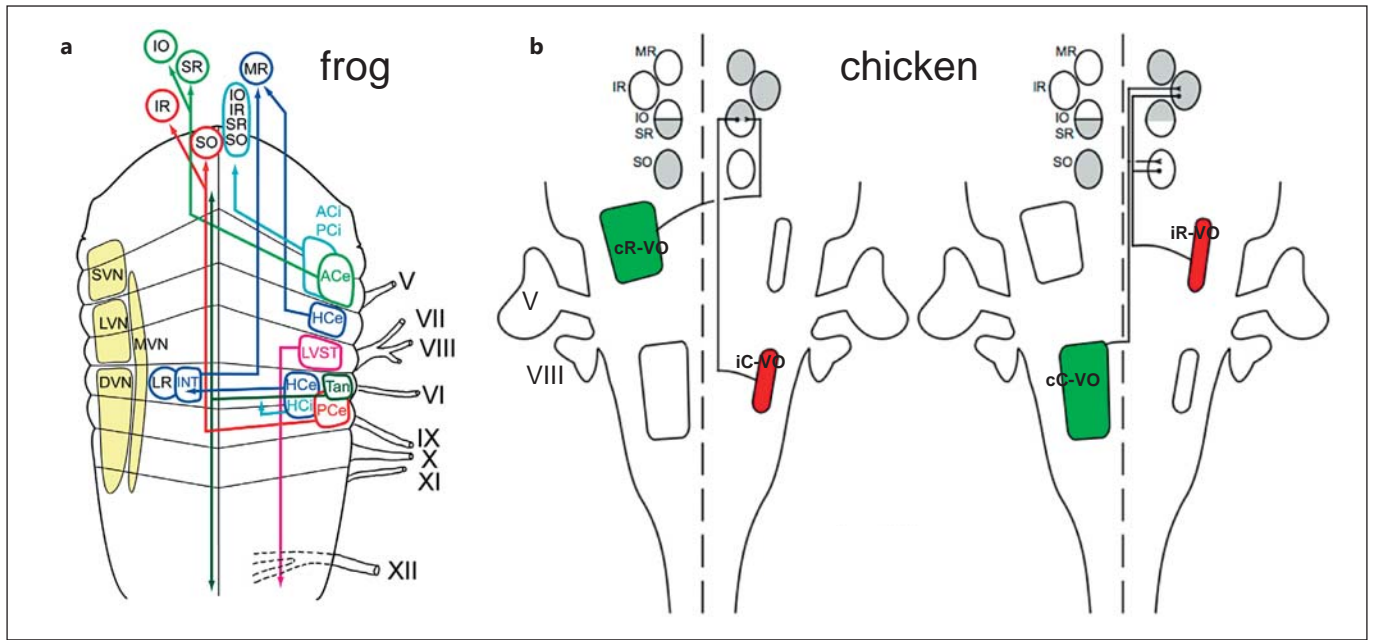
Fig. 3. Hodologically defined VOR projection neurons innervate specific motoneuron pools in a pattern largely conserved from frogs to birds and mammals. **a** In frogs, VOR projection neurons receiving inputs from specific vestibular sensory end organs project to functionally appropriate extraocular motoneuron pools. Each projection is color coded and labeled according to the peripheral sensory organ and functional effect (e.g. ACi = anterior canal, inhibitory; HCe = horizontal canal, excitatory; PCe = posterior canal, excitatory). DVN/LVN/MVN/SVN = Descending, lateral, medial, superior vestibular nucleus; LR/MR/IR/SR = lateral, medial, inferior, superior rectus motoneurons; IO/SO = inferior, superior oblique motoneurons; INT = abducens interneurons. **b** In the chicken, VOR projection neuron groups exhibit a similar connectivity pattern. Each group is color coded according to whether it mediates excitatory (green) or inhibitory (red) effects. The cR-VO group excites contralateral IO and SR motoneurons, much like the ACe group in the frog. The iC-VO group inhibits ipsilateral IO and SR motoneurons (a similar group has not been described in the frog). The iR-VO group inhibits ipsilateral IR and SO motoneurons, much like the ACi/PCi group in frogs. The cC-VO group excites contralateral IR and SO motoneurons, much like the PCe group in frogs. The cC-VO group also includes abducens interneurons (INT), which excite contralateral MR motoneurons (not shown for the chicken, but indicated by INT in the frog in **a**). **a** Modified from Straka et al. [2002a]. **b** Adapted from Glover [1994, 2003].

specification, or use-dependent plasticity, whereby dynamically inappropriate synapses are inactivated during development.

Evolving Extraocular Muscles and Ocular Motoneurons

As is the case for the ear and the eye, extraocular muscles have to be considered a vertebrate novelty. Obviously, non-deuterostomes such as squids [Budelmann and Young, 1993] and copepods [Consi et al., 1987] have eye muscles. However, the most parsimonious interpretation of existing data is that eyes [Lamb, 2013] and extraocular muscles evolved in vertebrates anew among deuterostomes, building on a yet to be determined molecular toolkit of transcription factors. It is now clear that only one extant vertebrate, the hagfish, lacks all extraocular muscles and ocular motoneurons [Fritzsch, 1998], whereas all other vertebrates have six extraocular muscles innervated by three discrete cranial nerve nuclei: oculomotor, trochlear and abducens motoneurons. Only limited data exist on the development of extraocular muscles [Wahl et al., 1994], whereas more is known about extraocular motoneurons and their projections [Fritzsch et al., 1995; Ferrario et al., 2012]. Compared to other motoneurons, extraocular motoneurons have unusual properties in terms of susceptibility to disease [Kaminski et al., 2002; Bosley et al., 2006; Tischfield et al., 2010] and molecular specification, such as their unique developmental dependency on the expression of Phox2a and Phox2b [Brunet and Pattyn, 2002; Coppola et al., 2005]. This adds to the unusual characteristics of these motoneurons with respect to distribution, migration and axonal projections:
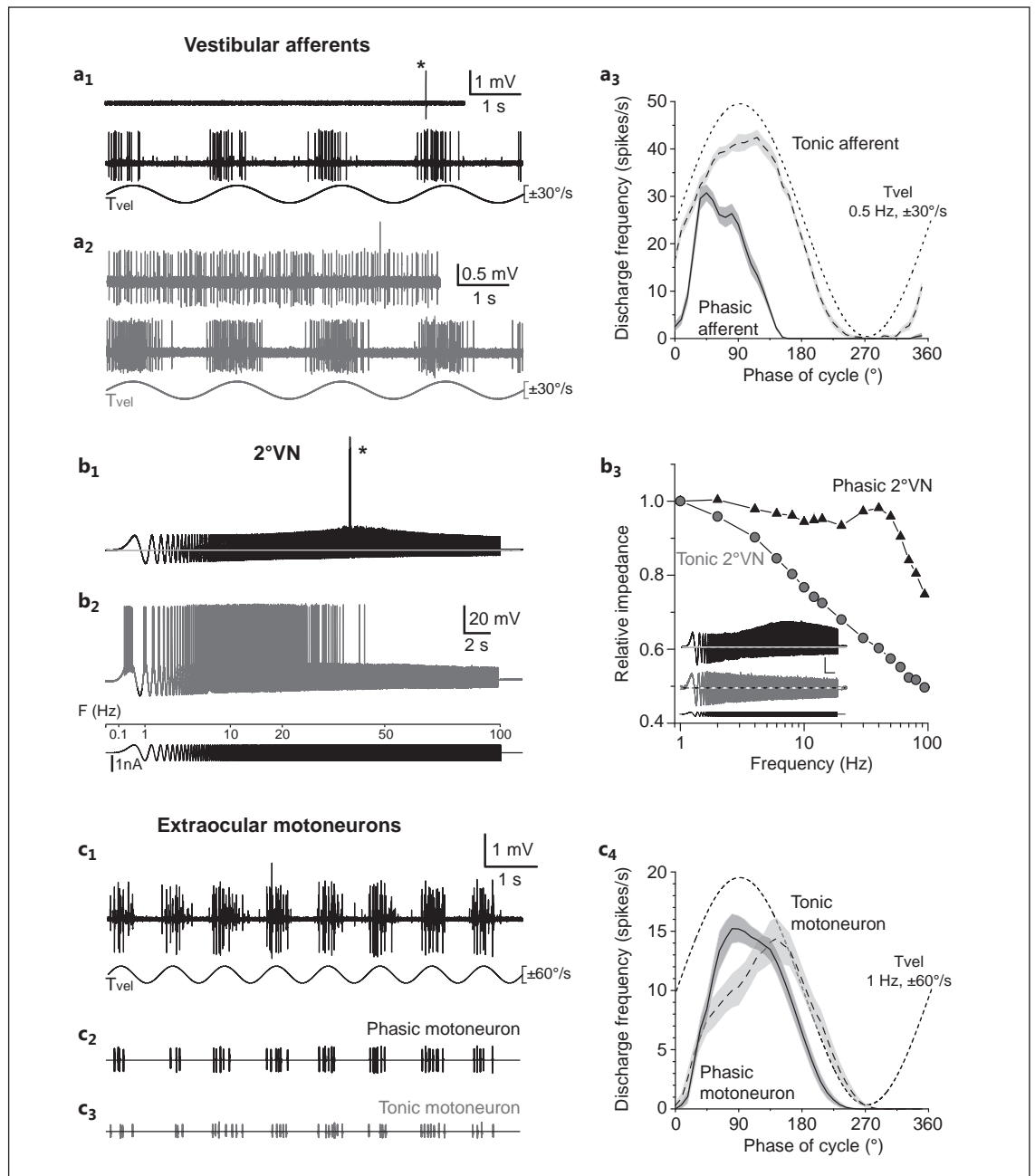
Fig. 4. Differential cellular characteristics of VOR neuronal components in frogs. **a** Extracellular single-unit recording of phasic (a_1) and tonic horizontal semicircular canal afferents (a_2); the classification is based on different resting (upper traces in a_1, a_2) and rotation-evoked discharge behavior (lower traces in a_1, a_2); note the low resting firing rate with a single spike in phasic afferents (* in a_1); peristimulus time histogram (a_3) of the discharge modulation over one cycle (0–360°) reveals a phase lead of responses in phasic (solid line) and a phase lag of responses in tonic afferents (dashed line) relative to table velocity (Tvel) [Gensberger and Straka, unpubl. data]. **b** Intracellular recordings of phasic (b_1) and tonic (b_2) central vestibular neurons (2°VN), identified by different response patterns to sinusoidal current injections; phasic 2°VN show single spike synchronization with sine wave current frequencies at 30–50 Hz (* in b_1); tonic 2°VN discharge at low frequencies and stop firing above 50 Hz; subthreshold impedance profiles of phasic (b_3, black trace in **inset**) and tonic 2°VN (b_3, gray trace in **inset**) corroborate respective functions as band-pass and low-pass filters; data adopted from Beraneck et al. [2007]. **c** Extracellular multiple-unit discharge recording of the abducens nerve during vertical-axis rotation (c_1); spike-shape analysis separated phasic (c_2) from tonic motoneurons (c_3); peristimulus time histogram (c_4) of the discharge modulation over one cycle (0–360°) reveals a phase lead of responses in phasic (solid line) and a phase lag of responses in tonic motoneurons (dashed line) relative to table velocity (Tvel) [Dietrich and Straka, unpubl. data].

- Oculomotor motoneurons are the only motoneuron population of which a subset migrates across the floor plate to the contralateral side, thus causing it to innervate the contralateral superior rectus muscle in all vertebrates [Puelles, 1978; Fritzsch and Northcutt, 1993b; Fritzsch et al., 1995].
- Trochlear motoneurons are the only motoneurons that have a dorsal exit to reach the contralateral superior oblique muscle [Fritzsch and Northcutt, 1993].
- Abducens motoneurons are the only hindbrain motoneurons that exhibit phyletic differences in the exit of their axons, ranging from sharing the trigeminal nerve root to having an independent ventral exit with variable segmental positions of the somata (r4 to r6 in lampreys [Fritzsch, 1998; Murakami et al., 2004]; r5 and r6 in teleosts, reptiles and birds; r6 in elasmobranchs [Gilland and Baker, 1993, 2005], and r5 in mammals and anurans [Fritzsch and Nichols, 1993; Straka et al., 2002a]).

Beyond these molecular and anatomical peculiarities, motoneuron innervation of the six extraocular muscles shows only two distinct patterns: the lamprey pattern and the jawed vertebrate pattern [Fritzsch et al., 1990]. The lamprey pattern is characterized by three extraocular muscles innervated by the oculomotor, one by the trochlear and two by the abducens nerve. In contrast, all jawed vertebrates have four extraocular muscles innervated by the oculomotor, one by the trochlear and one by the abducens nerve. Many vertebrates also have a seventh eye muscle that functions variably to retract the eye bulb or to close the eyelid. This extra muscle is innervated by motoneurons located in the proximity of lateral rectus (abducens) motoneurons with axons that exit the hindbrain with the abducens nerve [Gilland and Baker, 2005]. In principle, there are two possibilities to explain these differences. One is that the lamprey displays the original configuration, and jawed vertebrates express the derived condition, characterized by relegating one eye muscle innervated by the abducens nerve to function as a retractor and at least one oculomotor nerve-innervated muscle splitting to form an additional extraocular muscle [Fritzsch, 1998]. However, given that lampreys and hagfish are now considered to be sister taxa [Rota-Stabelli et al., 2011], the lack of extraocular muscles and their innervation in hagfish must be considered as derived. Assuming a secondary loss of extraocular muscles in hagfish, the other possibility is that the last common ancestor of lampreys, hagfish and jawed vertebrates had seven extraocular muscles innervated in a pattern that combines the conditions found in lampreys and jawed vertebrates [Puzdrowski, 1998]. In

contrast to this plausible construct, fossil data suggest that stem groups of jawed vertebrates had the lamprey eye muscle pattern [Young, 2008].

Regardless of the particular evolutionary scenario that led to the establishment of the VOR, all pools of extraocular motoneurons have to be contacted by spatially and dynamically adequate sets of VOR projection neurons to ensure appropriate reflex function [Lorente de Nó, 1933; Szentagothai, 1950; Straka and Dieringer, 2004]. Compatible with the idea of VOR pathways as frequency-tuned channels [Straka et al., 2009], the presence of dynamically different subtypes of vestibular afferents (fig. 4a) and VOR-related projection neurons (fig. 4b) is matched by a similar division of extraocular motoneurons into two subgroups with discharge behaviors suitable for coding low- (fig. $4c_1$–c_3) and high-dynamic signals (fig. $4c_1$, c_2), innervating muscle fibers with matching properties [Straka and Dieringer, 2004]. Developmental studies indicate that VOR projection neurons establish synaptic connections with the respective pools of extraocular motoneurons before the onset of sensory function [Glover, 2003; Straka, 2010]. This suggests a formation of appropriate connections between VOR projection neurons and extraocular motoneurons guided by molecular markers, potentially depending on prior connectivity between motoneurons and target eye muscles [Glover, 2003].

Evolving the VOR as an Integrative Transformation of Body Movements into Eye Movements

Based on the presumed phylogenetic precedence of otolith organ-derived to semicircular canal-derived vestibular reflexes, VOR circuits mediating low-dynamic head motion signals likely appeared before those mediating high-dynamic head motion signals. In both types of circuits, central vestibular neurons represent a center for integrating multimodal motion-related signals and a convenient substrate for plastic modification of the sensory-motor transformation [Straka and Dieringer, 2004]. The activation of a dynamic VOR during self-motion, however, is not sufficient for effective retinal image motion stabilization, in particular in animals that have a foveated retina. Visual feedback signals representing residual retinal image slip are also required. Accordingly, signals activated by large-field image motion and mediated by optokinetic circuits in the pretectum converge with vestibular signals immediately at the level of the extraocular motoneurons [Cochran et al., 1984] or are transmitted

indirectly through olivary and cerebellar pathways. The matching specificity of the directionally partitioned optokinetic system and the three semicircular canals [Simpson and Graf, 1985] is either a coincidence related to an optimal coding strategy for vectorial decomposition of 3D motion in space, or evidence for an organizational influence of an earlier evolving visual motion detection system on the spatial arrangement of a later evolving labyrinthine sensory system. Irrespective of the actual evolutionary interactions between the two systems, precise genetic regulation during ontogeny is necessary to generate an optimal visuovestibulo-motor circuitry for the stabilization of retinal image motion.

Although transformation of visuovestibular sensory signals into extraocular motor commands has long been considered to be the exclusive mechanism for gaze stabilization [Angelaki and Cullen, 2008], spinal central pattern generator-derived efference copies have recently been shown to directly elicit compensatory eye movements during locomotion in *Xenopus* without involvement of sensory feedback [Combes et al., 2008; Lambert et al., 2012]. These nonsensory, intrinsic feed-forward commands, however, do not combine additively with vestibular signals but actively suppress the horizontal semicircular canal-derived angular VOR [Lambert et al., 2012]. The implementation of such a signaling pathway also in adult frogs with limb-based locomotion [von Uckermann et al., 2013] suggests that it is a general vertebrate property and independent of locomotor strategy. Furthermore, the role of locomotor efference copy signals in image motion stabilization might be representative of an ancestral condition in early vertebrates before the formation of specific inner ear organs for the detection and encoding of oscillatory horizontal head rotations during undulatory swimming. The ubiquitous availability of locomotor efference copy during rhythmic tail-based swimming already in early tadpole-like chordate ancestors [Fetcho, 1992; Wada, 1998] and its potential recruitment for other motor tasks, including eye movements, might have reduced the evolutionary selective pressure for developing a specific sensory organ in the inner ear for horizontal motion detection [Lambert et al., 2012]. Until the relatively late formation of horizontal semicircular canals through a recruitment of the *Foxg1* gene in jawless [Pauley et al., 2006] and the *Otx1* gene in jawed vertebrates [Mazan et al., 2000; Fritzsch et al., 2001], spatiodynamically appropriate, image-stabilizing eye movements could have been elicited exclusively by intrinsic locomotor efference copies during tail-based locomotion in aquatic vertebrate ancestors.

Increased locomotor speed and performance, development of more flexible necks and other morphological adaptations that significantly reduced the predictability of head motion based on locomotor profiles [Chagnaud et al., 2012] or the appearance of limbs with head-attached muscles that increased the occurrence of unpredictable motion of the head [Trinajstic et al., 2013] could have driven the development of a specific sensory feedback system to detect head motion in space independent of body motion. The presence of a retinal image motion-stabilizing mechanism that relies exclusively on intrinsic locomotor signals, independent of motion-sensing balance organs [Lambert et al., 2012], is compatible with the idea that eyes and extraocular muscles appeared before the horizontal duct system of the inner ear. Once an intrinsic efference copy-dependent mechanism for gaze stabilization had evolved, it was likely retained throughout vertebrate evolution. It is thus probable that efference copies also access gaze control centers in vertebrates that are confronted with more complex visual disturbances resulting from flexible necks and limb-based locomotor strategies.

Conclusions

During evolution, the inner ear was transformed from a simple torus with three distinct sensory epithelia into a labyrinth consisting of three canals and two/three otolith organs, each with a separate associated sensory epithelium. These peripheral sensors connect to the hindbrain through sensory afferents, which terminate on two types of neuronal targets, extraocular motoneurons and premotor vestibular projection neurons. They thus exhibit at least vestiges of the likely ancestral two-neuron reflex arc as well as the extant three-neuron VOR arc that targets the afferents, in a partially overlapping yet spatially specific pattern, onto subpopulations of vestibular projection neurons. This convergence onto an intermediate layer of interneurons allows an integration of signals encoding changes of head/body position in the gravitational field with those related to angular acceleration or to neck/limb proprioception, creating in addition the capacity for adaptive plasticity of visuovestibular signals to changing ecophysiological contexts. VOR projection neurons with different output connections originate from specific segmental and dorsoventral locations within the hindbrain rhombomeric scaffold. This relationship links gene expression directly to functional connectivity, thus generating the 'hodological mosaic' organization that represents

an evolutionarily conserved bauplan with species-specific variations. It also indicates the implementation of a topographic principle of central vestibular organization based on motor output rather than sensory input, and thus a particularly important role of prior extraocular motoneuron development on the specificity of VOR projections. The spatial specificity of these connections is complemented by a matching dynamic specificity at hierarchal levels, illustrating the functional principle of frequency-tuned neuronal channels.

Whereas extraocular motoneurons are relatively well conserved, the specific positions of eye muscles in the orbit and their innervation patterns differ taxonomically, and in jawed vertebrates they are associated with the evolution of the lateral/horizontal semicircular canal. The late evolutionary arrival of this canal is surprising given the large horizontal head movements that almost certainly occurred during swimming in vertebrate ancestors. A likely explanation is that compensatory lateral eye movements were originally activated by intrinsic locomotor efference copies in early aquatic vertebrates. Since vertebrates eventually evolved limbs and the ability to move the head independently of the body, an additional component of the sensory system was required to monitor more complex head movements to generate appropriate extraocular motor commands for retinal image stabilization.

Disclosure Statement

The authors report no conflicting interests.

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