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Talking back: development of the olivocochlear efferent system

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Abstract

Developing sensory systems must coordinate the growth of neural circuitry spanning from receptors in the peripheral nervous system (PNS) to multi-layered networks within the central nervous system (CNS). This breadth presents particular challenges, as nascent processes must navigate across the CNS-PNS boundary and coalesce into a tightly intermingled wiring pattern, thereby enabling reliable integration from the PNS to the CNS and back. In the auditory system, feedforward spiral ganglion neurons (SGNs) from the periphery collect sound information via tonotopically organized connections in the cochlea and transmit this information to the brainstem for processing via the VIIIth cranial nerve. In turn, feedback olivocochlear neurons (OCNs) housed in the auditory brainstem send projections into the periphery, also through the VIIIth nerve. OCNs are motor neuron-like efferent cells that influence auditory processing within the cochlea and protect against noise damage in adult animals. These aligned feedforward and feedback systems develop in parallel, with SGN central axons reaching the developing auditory brainstem around the same time that the OCN axons extend out towards the developing inner ear. Recent findings have begun to unravel the genetic and molecular mechanisms that guide OCN development, from their origins in a generic pool of motor neuron precursors to their specialized roles as modulators of cochlear activity. One recurrent theme is the importance of efferent-afferent interactions, as afferent SGNs guide OCNs to their final locations within the sensory epithelium, and efferent OCNs shape the activity of the developing auditory system.

Graphical abstract

Inner ear efferents (IEEs) provide feedback to the inner ear. Originating with facial branchial motor neurons (FBMNs) from a pool of MNv progenitors in the brainstem, IEE precursors produce olivocochlear neurons (OCNs) and vestibular efferent neurons (VENs). In the cochlea, OCN axons project along spiral ganglion neuron (SGN) afferent processes.

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Notes

Not applicable.

Introduction

The auditory system connects animals to their external environments in myriad ways: it allows them to locate and identify sounds, to communicate with other organisms, and to do all of this even amidst a jumble of background noises. To perform these complex tasks, animals rely on precisely wired and tuned auditory circuitry. As in other sensory systems, sound is detected by highly specialized receptor neurons in the peripheral nervous system (PNS) and then encoded in the activity of primary sensory neurons that project into the central nervous system (CNS). However, whereas visual signals are processed extensively in the retina before being transmitted to the thalamus, the bulk of early auditory processing occurs instead in the brainstem. Moreover, within the auditory brainstem is housed a small but crucial population of neurons that project back to the cochlea, establishing a route for efferent feedback that both protects sensory cells from damage and directly tunes afferent activity. The formation of these reciprocal connections between the CNS and the PNS presents some unusual developmental challenges, including specification and generation of diverse types of efferent neurons, navigation of efferent axons through and out of the brainstem, and alignment with the afferent connections forming in the cochlea. Although the understanding of auditory efferent development remains rudimentary, emerging evidence emphasizes the importance of afferent-efferent interactions, with afferents apparently guiding efferent axons into the inner ear and efferent neurons, in turn, influencing the maturation of sensory cells and their connections in the cochlea.

Overview of afferent and efferent organization

In mammals, incoming sounds are transduced by hair cells in the cochlea and transmitted to the brainstem via spiral ganglion neurons (SGNs) (Fig. 1). SGNs are divided into two primary groups: Type I SGNs, which receive synaptic signals from inner hair cells (IHCs), and Type II SGNs, which connect with outer hair cells (OHCs). The IHCs and Type I SGNs are almost certainly the primary carriers of perceptual auditory information, while the OHCs control cochlear gain. Although their precise function remains unclear, Type II SGNs are proposed to convey information about damage and pain^{1,2}. Hair cells and SGNs are organized tonotopically along the axis of the cochlea, from high characteristic frequencies in the base to low characteristic frequencies in the apex. All SGNs maintain this tonotopic organization as they extend their central axons into the VIIIth cranial nerve^{3–5}.

The central projections of auditory nerve fibers terminate within the cochlear nuclear complex (CNC), a collection of nuclei on the dorsal surface of the brainstem, tucked beneath the cerebellum (Fig. 1). The CNC contains a number of distinct cell types that differ in their morphology, responses to sound, projections, and functions⁶. Globular bushy cells provide a major source of output from the CNC, crossing the midline and terminating within the contralateral medial nucleus of the trapezoid body (MNTB). Spherical bushy cells, on the other hand, innervate the lateral and medial superior olives (LSO, MSO), which contain principal neurons that integrate information from both ears, enabling sound localization in the horizontal plane^{6–8}. These nuclei reside in the superior olivary complex (SOC), a large collection of nuclei devoted to auditory processing that sits just dorsal to the trapezoid body in the pons. Additionally, some neurons of the CNC project directly to the inferior colliculus (IC), where their information is integrated with ascending projections from the SOC⁶. At each level, nuclei are tonotopically organized³.

In addition to this feedforward, afferent pathway, normal hearing also relies on feedback circuits present at every level of auditory processing. At the lowest level, a small population of efferent neurons project from the auditory brainstem back into the cochlea to modulate both hair cells and SGNs⁹. The cell bodies of these olivocochlear neurons (OCNs) reside in the SOC, amidst circuitry required for early auditory processing and sound localization^{10,11}. Collectively, the OCNs help to protect the ear against acoustic trauma^{12–14}, separate speech sounds from background noise^{15,16}, and may balance inter-aural differences^{17–21}.

Like the hair cells and SGNs, mammalian OCNs are divided into two main subtypes, a medial and a lateral component, based on the positions of their somata in the SOC^{22,23}. Medial olivocochlear neurons (MOCs) send both ipsilateral and contralateral projections to OHCs, while lateral olivocochlear neurons (LOCs) project predominantly ipsilaterally to innervate Type I SGN terminals near the base of IHCs²². This general architecture is widely preserved across the mammalian class, although the precise location of efferent somata, the overall number of efferents, and the ratio of MOC to LOC neurons vary across species^{24,25}. In mice, MOC cell bodies reside mainly in the ventral nucleus of the trapezoid body (VNTB), whereas LOC neurons are found in and around the lateral superior olive (LSO). Both MOC and LOC neurons are intermingled with neurons that mediate afferent responses²⁵. Like the other auditory brainstem nuclei, LOCs are tonotopically organized in LSO, with high frequencies represented medially and low frequencies more laterally^{26–29}.

MOC neurons represent a relatively uniform population of neurons that act primarily to dampen cochlear activity via inhibitory, cholinergic synapses onto OHCs. OHCs receive this cholinergic input through nicotinic acetylcholine receptors (nAChRs) comprised of $\alpha 9$ and $\alpha 10$ subunits^{30–32}. In mature hair cells, efferents terminate opposite a postsynaptic cistern containing calcium stores that is closely apposed by calcium-gated SK2 channels. The $\alpha 9/\alpha 10$ nAChR is permeable to divalent cations, and calcium entry through this channel is amplified by calcium-induced calcium release from the nearby postsynaptic cistern. In turn, localized release of calcium triggers potassium efflux through local SK2 channels, thus resulting in OHC hyperpolarization in response to acetylcholine released from the MOC efferents^{20,32}. Like other cholinergic neurons, at least some MOCs also express GABA, although a definitive role for that neurotransmitter at the OHC synapse has yet to be

established^{33,34}. It has been proposed that Type II SGNs participate in the MOC reflex, but strong evidence for this hypothesis is lacking^{35,36}.

In contrast to the relatively uniform MOC population, LOC neurons exhibit a puzzling array of differences in their morphological and signaling properties. These neurons express a diverse range of neurotransmitters and neuropeptides, including acetylcholine, CGRP, GABA, enkephalins, dynorphins, and dopamine^{37,38}. Immunohistochemical studies suggest that many of these signaling molecules are found only transiently during development or in a subset of LOC neurons, in some cases along a tonotopic gradient^{39–41}. It is also possible that individual neurons express different transmitters at different times^{42,43}. LOC neurons also show obvious differences in the morphology and extent of their cochlear projections, but it is unknown how these anatomical differences correlate with variability in neurotransmitter expression or other aspects of LOC signaling⁴⁴. Several researchers have noted, however, that LOC shell neurons found adjacent to the LSO vary from LOC neurons housed within the LSO itself with respect to their morphologies, projection patterns, and expression of signalling molecules^{25,40,44,45}. At a functional level, stimulation studies indicate that LOC neurons can provide both excitatory and inhibitory signals to auditory nerve fibers⁴⁶. Thus, although all LOC neurons appear to target auditory nerve fibers, there is poorly understood heterogeneity at the level of both anatomy and function that could influence exactly how LOC activity alters the encoding of sound information in the cochlea.

In addition to the cochlear efferent pathway, similar efferent projections target hair cells of the vestibular and lateral line systems. Indeed, efferent neurons have been identified in virtually every species with a hair cell sensory system—including cephalopods²⁴. In non-mammalian species, efferent neurons to the vestibular, auditory, and lateral line (if present) systems all reside in the same brainstem nucleus²⁴. Even in mammals, where the vestibular and auditory efferents reside in distinct brain regions, the two efferent populations develop together^{47,48}. Although birds and mammals are separated by 300 million years in evolution, efferent innervation of the sensory epithelium in birds (the basilar papilla) is analogous to what is observed in mammals, with differential innervation of tall (IHC-like) and short (OHC-like) hair cells^{49,50}. Likewise, the developing inner ear efferents share a common developmental history in chickens and mice, with some differences^{48,51,52}. The ubiquity of these feedback systems across widely divergent species, along with their common developmental origins, suggest that the efferent neurons of these various sensory modalities may share a common evolutionary origin, and may even have co-evolved with hair cells themselves⁵³.

Across species, these inner ear efferents (IEEs) also share many properties of branchial cranial motor neurons, both functionally and developmentally. Like the pre-ganglionic motor neurons of the autonomic nervous system, IEEs represent a subset of motor neurons, despite the fact that they do not form connections with muscles. IEEs originate in a motor neuron progenitor zone, express many markers of motor neuron identity, including *Chat*, *Tbx20*, and *Isl1*, and develop alongside the facial branchial motor neurons (FBMNs)^{53,54}. FBMNs provide the motor division of the VIIth nerve, which innervates the face and is closely affiliated with the VIIIth nerve as they project together in the internal auditory meatus. Indeed, in all vertebrates, IEE and FBMN axons project together for at least some

distance^{24,53}. Given the many similarities between these two neuronal populations, it is hypothesized that IEEs are evolutionarily derived from FBMNs⁵³.

Origin, specification, and migration of OCNs within the brainstem

During development, OCNs are progressively specified, starting with the induction of generic IEE precursors and ending with the formation of functionally distinct populations of LOC and MOC neurons that form anatomically distinct connections with SGNs and OHCs, respectively. As in other regions of the nervous system, this gradual diversification of cell fates depends on combinatorially acting transcription factors, including factors that coordinate the migration of each efferent subtype to its proper location in the SOC.

The earliest stages of IEE specification depend on the overall patterning of the axes of the hindbrain, with IEEs developing from neural progenitors with a specific rostral-caudal and dorsal-ventral address (Fig. 2) (reviewed in ref. 54). During development, the brainstem is divided into discrete segments along the rostral-caudal axis called rhombomeres^{55–60}. Each rhombomere is defined by combinations of transcriptional regulators that control the patterning of neuronal populations developing within that segment, with homeobox (Hox) transcription factors playing a leading role⁶¹. Distinct neural progenitor domains also exist along the dorsal-ventral axis of each rhombomere. In the ventral neural tube, the formation of these domains is guided by a gradient of the morphogen Sonic hedgehog (SHH) and by suites of homeodomain proteins induced by varying levels of SHH^{59,62,63}. Thus, the identity of developing hindbrain neurons, including the IEEs, is determined by a combination of their extracellular milieu and the particular slate of transcription factors expressed within individual cells.

Within the brainstem, OCNs develop in parallel with many other related neurons essential for auditory processing. Indeed, the majority of the auditory brainstem is derived from rhombomeres 2–5^{54,64}, with most neurons exiting the cell cycle between E9 and E14 in mouse^{64–66}. Within this region, some neurons, including the OCNs, develop from progenitors in the ventricular zone, whereas others arise from the rhombic lip, a highly migratory pool of progenitors lining the roof of the fourth ventricle (Fig. 2). Moreover, many nuclei are comprised of neurons from multiple sources. For instance, neurons of the cochlear nucleus complex (CNC) arise in r2–5, primarily from *Wnt1*-expressing cells in the rhombic lip, but also from *Wnt1*-negative precursors ventral to the rhombic lip, which instead are marked by expression of *Ptf1a* and go on to produce GABAergic neurons in the CNC^{54,64,67,68}. Distinct CNC cell types originate in different rhombomeres and in different progenitor domains along the dorsal-ventral axis^{54,68}.

More ventrally, the various nuclei of the SOC, where OCNs eventually reside, develop primarily in r5^{54,69–72}. Like the CNC, the neurons that will eventually populate the SOC derive from multiple sources, including *Atoh1*- and *Wnt1*-positive precursors in the rhombic lip and *En1*-expressing precursors of the ventricular zone^{54,72–74}. For instance, the LSO contains glycinergic neurons derived from the ventricular zone and glutamatergic neurons derived from the rhombic lip⁵⁴. All IEEs, however, originate from a distinct pool of progenitors in the ventricular zone of r4^{47,48,52,71}. Notably, the CNC interneurons that

innervate MOCs are also r4-derived, suggesting that the rhombomeric origins of auditory brainstem neurons may inform their later circuit connectivity and functions⁷¹.

The various nodes for auditory processing in the brainstem become interconnected early in embryogenesis. Beginning around E9.5 in mouse, SGNs start to delaminate from the otic placode, which forms from epithelium adjacent to r5-6^{75–77}. Around E11.5, SGNs begin sending projections into the region where the CNC will develop, while both types of neurons are still being born^{77–80}. By E15, those early projections have begun to form functional synaptic contacts with target neurons in the ventral cochlear nucleus, and by E17, electrical stimulations in the nascent cochlea can elicit responses from neurons in the SOC, indicating the formation of functional contacts both between SGNs and CNC neurons, and between CNC neurons and their targets in the SOC⁸¹. Thus, neurons throughout the auditory brainstem—including OCNs and their precursors—navigate through a complex and changing environment that is physically connected to the cochlea from very early stages.

Olivocochlear efferents develop together with typical motor neurons

The earliest stages of IEE development are intertwined with those of FBMNs. In all vertebrate species examined thus far—including mice, chickens, and zebrafish—IEEs and FBMNs originate together in the progenitor domain of visceral motor neurons (pMNv) in the ventricular zone, just medial to the floor plate of r4^{47,48,51,52,71,82}. This NKX2-2+ domain, analogous to p3 in the spinal cord, produces branchial and visceral motor neurons, while somatic motor neurons are generated from a more dorsal PAX6+ progenitor domain^{83,84}. The overall identity of r4 is determined by the homeobox transcription factor HOXB1, whose expression is restricted to r4 by E8.5 in mice^{57,59,61,85–88}. Loss of *Hoxb1* has major consequences for r4, including loss of both IEEs and the facial motor nucleus, where FBMN cell bodies reside^{89–91}. However, ISL1-positive motor neurons are still present, suggesting that *Hoxb1* is not necessary for motor neuron development *per se*, but instead directs a subset of motor neuron precursors to assume “r4” identities^{71,89}.

IEEs, like FBMNs and other branchial motor neurons, also express a collection of transcription factors that together assign a motor neuron identity. These genes include *Isl1*^{92,93}, *Phox2a* and *Phox2b*^{94–96}, *Nkx* genes (most critically *Nkx2-2*, *Nkx2-9*, *Nkx6-1*, and *Nkx6-2*)^{59,62,97–100}, and *Tbx20*^{101–103}. Expression of these transcription factors is restricted to motor neuron progenitors as part of the broader SHH signalling network, with pMNv forming just ventral to the pMN domain, which produces somatic motor neurons^{63,83}.

Motor neuron progenitors are subsequently diversified to produce subtypes of motor neurons by integrating patterning information from the anterior-posterior and dorsal-ventral axes. For IEEs and FBMNs, this means assuming a branchiovisceral fate within r4. The branchiovisceral fate is controlled by the combined action of NKX2-2 and NKX2-9 in progenitors within the pMNv domain¹⁰⁰. A key effector is the homeodomain protein PHOX2B, which promotes expression of *Asc11* and production of branchial and visceral motor neurons while inhibiting an alternative serotonergic fate^{95,96,104–107}. Thus, *Phox2b* knock-out animals lack all branchial and visceral cranial motor nuclei¹⁰⁴. In these animals, NKX2-2+ progenitors are specified normally but progress atypically to the next stage of development¹⁰⁴. This general PHOX2B-driven MNv program is customized in r4 through

the combined action of HOXB1 and NKX family members. HOXB1 is required for *Phox2b* expression⁹⁰ and can directly bind to regulatory sequences in the *Phox2b* locus that are necessary and sufficient for expression in ventral r4, where the IEEs and FBMNs develop¹⁰⁸. Moreover, NKX2-2 synergizes with HOXB1 and other co-factors, such that expression of *Phox2b* is a direct readout of the intersection between the A/P (i.e. HOXB1) and D/V (i.e. NKX2) patterning systems. NKX6 proteins reinforce this system by maintaining expression of *Hoxb1* in r4⁹⁵. Hence, in the absence of NKX6 protein activity, *Hoxb1* expression decreases prematurely and very few IEEs are produced⁹⁵. As a result of this complex spatiotemporal network, MNv progenitors in r4 produce only branchial and visceral MNs, including both IEEs and FBMNs.

IEEs also depend on unique genetic programs to differentiate them from the FBMNs developing simultaneously in pMNv. In chicks and mammals—but not zebrafish^{109,110}—IEEs are distinguished from FBMNs early on by the expression of zinc-finger transcription factors GATA2 and GATA3^{111–114}. Although both GATA2 and GATA3 are expressed in the pMNv progenitor domain, analysis of *Gata3* mutants indicates that GATA3 is not expressed by postmitotic FBMNs^{96,111,112}. In mice lacking either GATA2 or GATA3, efferent neurons project aberrantly and fail to cross the floorplate, with axons instead joining the VIIth nerve^{111,112,114}. In r4, *Gata3* expression is downstream of *Gata2*^{111,114}. Thus, a GATA2/3 network may drive a binary IEE versus FBMN fate decision, similar to the role of GATA factors in the immune system¹¹⁵. This network, in turn, is downstream of both *Hoxb1* and *Phox2b*. Thus, misexpression of *Hoxb1* in r2 induces ectopic expression of both *Gata2* and *Gata3*, as well as aberrant midline crossings, suggesting an induction of IEEs^{86,111}. PHOX2B may serve as an intermediary for this effect, as HOXB1 can induce *Phox2b*¹⁰⁸, and PHOX2B in turn drives expression of *Gata3* in the ventral region of r4⁹⁶. Indeed, IEEs in *Phox2b* knock-out mice fail to differentiate normally, exhibit reduced levels of GATA3, and occasionally mis-specify as serotonin progenitors⁹⁶. *Phox2a*, on the other hand, is not required for IEE development⁹⁶. Thus, IEE differentiation depends on a general HOXB1/PHOX2B network, with further specificity arising through the action of GATA factors.

The segregation of IEEs and FBMNs is reflected in their migration to distinct nuclei

Shortly after undergoing their final mitotic division, both FBMN and IEE cell bodies begin to migrate away from their shared point of origin (Fig. 2). In mice, these movements are initiated at about E10 and conclude around E15.5^{48,89,116–119}. During this time, IEEs and FBMNs separate from each other, as FBMNs travel caudally into their final position in r5 and r6¹²⁰. The IEEs populate r4-5, and are subsequently subdivided into the vestibular efferent neurons (VENs), which migrate dorsally, and the OCNs, which migrate ventrally and eventually give rise to MOCs (residing mainly in VNTB) and LOCs (located in and around LSO)^{48,96}. Importantly, FBMNs and IEEs extend axons together from their common origin and into the periphery as early as E10.5, so the trajectory of axons within and out of the brainstem reflects their migratory history⁴⁷.

Whereas FBMNs project ipsilaterally to innervate muscles on the same side of the body, many IEEs project to the contralateral cochlea. In particular, MOCs tend to project contralaterally and LOCs tend to project ipsilaterally, although the proportions of ipsilateral

and contralateral projections vary across species²⁵. In mouse, about 66% of MOCs (but only 1% of LOCs), project contralaterally, with some neurons even projecting to both ears^{25,121,122}. The contralateral projections, which form the olivocochlear bundle (OCB), appear as early as E10^{111,112}, and the OCB is fully formed by E13.5⁴⁸, thereby overlapping substantially with the period of IEE migration. Indeed, in chickens, contralateral projections are achieved by a translocation of the IEE cell bodies across the floor plate, thereby leaving a crossed projection in their wake^{51,52}. In mice, the means of midline crossing are less clear, as extensive cell body translocations have not been detected^{47,48,123}. However, neurons positive for the IEE marker GATA3 have been observed in the midline of E10.5 mice, suggesting that at least some IEE soma may move from one side to the other^{96,112}. In mammals, the formation of a contralateral projection may involve the axon guidance receptor EPHB2. In mice, EPHB2 is expressed by IEEs, FBMNs, and the floor plate¹²⁴. In the absence of *EphB2*, IEE axons meander aberrantly across the midline before achieving anatomically normal projections, albeit several days later than wild-type controls¹²⁴.

The mechanisms that entice IEEs across the midline—and prevent FBMNs from following—may also involve the Slit-Robo pathway. In *Slit1*, *Slit2* double knock-out mice, the total number of fibers crossing the midline in r4 is decreased by about half compared to wild-type controls, with some axons turning and running longitudinally along the floor plate rather than crossing directly¹²⁵. In contrast, *Robo2* knock-out animals show a marked increase in the number of projections crossing the floor plate, as well as aberrant migration of ISL1-positive cell bodies onto the floor plate^{125,126}. These findings suggest that ROBO2 may ordinarily provide a repulsive cue that keeps FBMNs away from the midline. One can speculate that a similar mechanism might be at play in guiding the ipsilateral projections of IEEs.

Some IEE migratory decisions appear to depend on transcriptional networks active in all motor neurons (Fig. 3). For instance, both IEEs and FBMNs require the pan-motor neuron marker ISL1 for proper differentiation. For FBMNs, ISL1 is crucial for their caudal migration, as reflected by an accumulation of FBMNs in r4-5 rather than r6 in mice with reduced ISL1 levels¹²⁷. Although no major IEE migratory phenotypes were described, the IEEs developed truncated and disorganized projections out of the brainstem, with the few remaining axons barely reaching the ear by E11.5. Both types of defects may be due to altered expression of *Slit2*, as *Slit2* levels are decreased in *Isl1*-deficient motor neurons¹²⁷.

Like ISL1, TBX20 is expressed by both IEEs and FBMNs, but has distinct effects on the migration of each population¹⁰¹. Caudal migration of FBMNs is controlled by the planar cell polarity (PCP) pathway^{110,128–140}, and *Tbx20* mutant mice exhibit defects in caudal FBMN migration as well as a downregulation of PCP genes in FBMN somata¹⁰¹. *Tbx20* expression is also required for midline crossing of IEEs, but it is unclear whether this phenotype is also due to changes in PCP gene expression¹⁰¹. Nevertheless, IEEs exhibit aberrant midline crossing in mice lacking the PCP component PRICKLE1, with reduced contralateral projections and some axons turning to wander along the floorplate longitudinally¹⁴⁰. In zebrafish, the caudal migration of octavolateralis efferents is also stalled in animals lacking the PCP proteins Scrib or Celsr2^{136,137}. More work is needed to understand what role—if any—the PCP pathway plays in the migration of mammalian IEEs. Thus, despite a common

reliance on TBX20 and possibly PCP genes, there appear to be significant differences in how these genes act in IEEs and FBMNs.

Shortly before the IEEs and FBMNs have fully separated, IEEs begin to further diversify and separate from each other, with VENs migrating dorsally, MOC neurons moving ventrally, and LOC neurons settling in a more ventrolateral position⁴⁸. In mice, this migration happens between E12.5 and E14.5, with VENs and OCNs separating first, followed by a separation of MOC and LOC neurons⁴⁸. This process requires the transcription factor ASCL1 (formerly referred to as MASH1), as the VEN nucleus does not form in *Ascl1* mutants⁹⁶. ASCL1 is also expressed in OCNs and FBMNs^{96,104,141}. However, although *Ascl1* deletion delays the caudal migration of FBMNs, it has no effect on the movement of OCNs^{96,141}. Unfortunately, the identity of additional cell-type specific transcription factors that might direct subsets of OCNs to their final destinations remains unclear.

The final separation of VENs from OCNs relies in part on a ventral migration of OCNs, with both OCNs and FBMNs undergoing similar migrations away from the ventricular zone to arrive close to the ventral pial surface of the hindbrain^{117,119} (Fig. 2). This process of radial migration depends on the *Reln* gene in both OCNs and FBMNs, although LOC neurons only appeared to be partially affected in *Reln* mutant mice, suggesting that additional mechanisms may be at work¹¹⁷. In FBMNs, *Reln* interacts with *Dab1* and *Cdk5*. It is unknown whether similar mechanisms mediate its effects in OCNs^{117,142} (see Table 1 for a summary of genes involved in OCN specification, migration, and guidance).

AFFERENT-EFFERENT INTERACTIONS IN THE COCHLEA

Efferents grow along existing nerves to find their synaptic targets

As they are migrating and settling in their final locations, OCNs begin to extend axons out of the brainstem and into the cochlea, ultimately terminating on post-synaptic targets in the organ of Corti (Fig. 4). These initial guidance events occur after the VIIIth nerve has begun to form, with SGN axons reaching the brainstem by E11.5⁷⁹, days before the IEEs have scattered to their distinct locations across the brainstem. The formation of efferent projections coincides with the formation of connections within the auditory brainstem, with bushy cell neurons in the CNC beginning to extend axons towards the midline around E13 and reaching the contralateral SOC by E17¹⁴⁸.

Consistent with the close relationship with the FBMNs, OCN axons initially follow along the facial genu in the brainstem but bypass the VIIth nerve and instead exit to join the nascent VIIIth nerve⁴⁸. Almost nothing is known about the mechanisms that direct OCN axons to grow into the VIIIth nerve, rather than continuing to follow the VIIth. One possibility is that the inner ear provides an attractive signal that could attract any motor neuron axons, but that OCN axons are in the best location to respond to the signal, an idea that has been supported by the behaviour of motor neuron axons presented with an ectopic otic vesicle^{149,150}. However, the inner ear is not necessary for growth into the periphery, as OCN axons mis-route into the VIIth nerve if the VIIIth nerve is absent¹⁵¹. IEEs also join the VIIth nerve inappropriately in *Gata3* mutant mice, but more work is needed to understand

whether this effect reflects a role for GATA3 in IEEs specifically, since GATA3 is also expressed in the developing cochlea and SGNs^{112,145}.

Upon reaching the VIIIth nerve exit point, OCN axons are thought to grow along a scaffold of SGN processes into the sensory epithelium of the inner ear^{152,153}. In support of this hypothesis, a wide array of mutations affecting SGN development and targeting also affect efferent innervation of the cochlea, with the phenotype of efferent neurons often matching the aberrant projections of SGNs^{145,146,151,154–156}. Lateral line efferents in zebrafish appear to rely on a similar mechanism to reach their targets¹⁵⁷. However, no molecular cues mediating fasciculation between afferents and efferents have been identified to date. In other peripheral nerves with both afferent and efferent components, this process depends on axon-axon interactions mediated by Eph signaling^{158,159}, but analogous roles for Eph receptors in the auditory system have not yet been described¹⁶⁰. Even more mysterious is the subsequent targeting of OHCs by MOC axons and of SGN terminals by LOC axons. However, it is thought that MOC outgrowth toward the OHCs may not rely on SGN scaffolding, as MOCs and Type II SGNs follow different trajectories between the IHC and OHC regions (schematized in Fig. 1), and MOCs still reach the OHCs in *BDNF* mutant mice that lack afferent innervation of OHCs^{161,162}.

Efferents reach the mammalian sensory epithelium during a period of circuit refinement

Efferents sit in a key node for auditory processing, both receiving sound information through the afferent pathway in the brainstem and then transmitting signals back to the afferents through synaptic contacts in the cochlea. It is therefore not surprising that this final pattern of connectivity depends on activity-dependent mechanisms that operate after the basic axon tracts have formed and synaptogenesis has begun, which occurs right around birth in mice¹⁶³. Indeed, the first two weeks of postnatal development in mice are a transformative epoch for the inner ear. Both SGNs and auditory brainstem neurons undergo extensive remodeling during this period to adopt distinct functional properties and converge on their final targets^{3,164}. As in other developing neural circuits, spontaneous activity plays a major role in the maturation and refinement of auditory circuitry¹⁶⁵. Before the onset of hearing (~P10 in mice¹⁶⁶), IHCs produce spontaneous calcium spikes, which propagate throughout the auditory system^{167–175}. In parallel, neurons in the auditory brainstem segregate into ever-more refined tonotopic arrangements, a process that is thought to depend on spontaneous activity from the inner ear¹⁷⁶.

During development, MOC axons are poised to influence the pattern of activity emanating from the cochlea, though the precise functions of this early cholinergic signaling remain unclear. In mice, tracing studies reveal that MOC neurons are the first to arrive in the sensory epithelium, appearing in the IHC region as early as E16.5 and in the OHC region around E18.5⁴⁸. Experiments in rats, hamsters, and gerbils identified a similar pattern, with dye-labeled MOCs first appearing near IHCs by P0, and extending to OHCs between P2 and P5, depending on the species^{177–180}. This time course also aligns with the appearance of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE)-positive terminals in the cochlea. Both enzymes, which regulate acetylcholine synthesis and degradation, have been identified in the inner ear at birth or shortly thereafter in mice, rats, and hamsters^{181–183}.

Consistent with dye-labeling studies, ChAT staining is first visible in the region of IHCs and can be seen around OHCs several days later¹⁸³. Like other aspects of cochlear development, OCNs arrive first in the base, and development progresses toward the apex¹⁷⁷.

Collectively, these findings suggest the existence of a “developmental waiting period,” in which MOC neurons transiently innervate the IHC region before synapsing onto their final targets, the OHCs^{152,179}. During this waiting period, OCNs also send projections to Kölliker’s organ^{48,178}, a transient structure believed to induce spontaneous activity in IHCs before the onset of hearing¹⁶⁷. It is not known whether OCNs form synapses with cells in this structure; however, subunits of acetylcholine receptors are expressed in Kölliker’s organ, raising the possibility of functional contacts^{184,185}. While the purpose of these temporary connections is unknown, transient innervations do influence the maturation of other developing circuits, such as thalamocortical neurons, which form short-lived connections in the subplate that prepare them for subsequent formation of synapses in layer four of the cortex^{186–188}.

MOC neurons affect prehearing synaptic activity

During this early postnatal waiting period, MOC terminals form synaptic contacts with IHCs, which transiently express SK2 channels and AChR subunits that mediate post-synaptic responses (Fig. 5)^{189,190}. Although little is known about how the MOC-to-IHC synapse forms, this transient connection does appear to be functional. Electrophysiological recordings from IHCs of rats during this time identified the presence of inhibitory postsynaptic currents (IPSCs) that disappeared with the application of the AChR antagonists strychnine and alpha-bungarotoxin, indicating that MOC neurons (the only cholinergic cells presynaptic to IHCs) inhibit IHCs^{189,190}. Similarly, both efferent stimulation and exogenous application of acetylcholine in cochlear explants reduce IHC firing rates, whereas blockade of cholinergic signaling with strychnine increases IHC firing rates^{190–193}. IHCs remain responsive to acetylcholine through at least the second postnatal week in both mice and rats^{194–196}. In rats, IHCs cease to respond to acetylcholine sometime between P16 and P21^{194,195}. IHCs in mice remain responsive to acetylcholine slightly longer than in rats, but they also stop responding to acetylcholine by the sixth postnatal week¹⁹⁶. By adulthood, the IHC membrane no longer responds to OCN stimulation^{196,197}.

Given the evidence that spontaneous activity originating in the cochlea ultimately propagates along the entire developing auditory neuraxis, it is tantalizing to suppose that efferent inhibition of IHCs during early postnatal stages might also shape auditory circuits in the CNS. Several studies have attempted to address this question by investigating auditory development in mice lacking the nAChR subunits $\alpha 9$ or $\alpha 10$, which are required for OCNs to form functional cholinergic synapses on hair cells^{198,199}. Indeed, tonotopic refinement of brainstem circuitry is disrupted in *nAChR $\alpha 9$* knockout mice, at least in the LSO¹⁷⁶. However, the primary defect appears to be a failure to prune synapses after the onset of hearing, suggesting a role independent of spontaneous activity. The IHCs also appear to have defects in vesicle fusion at ribbon synapses, indicating a possible role for early efferent innervation on hair cell maturation²⁰⁰, although other properties of IHCs seem to be normal in mice lacking cholinergic OCN innervation^{200,201}.

Following this period of temporary MOC-IHC contacts, MOC axons gradually transition into the OHC region, concomitant with the arrival of LOC neurons in the sensory epithelium during the first postnatal week, although distinguishing LOCs from MOCs by morphology at this stage remains a challenge. In rats, OHCs begin responding to acetylcholine at P6, shortly after the onset of SK2 expression^{202,203}. In the cat and rat, MOCs first form transient, axo-dendritic synapses on Type II SGNs before synapsing directly onto OHCs^{163,204,205}. At least some of the early contacts onto OHCs appear to be branched projections from MOCs innervating the IHC region^{180,206} (Fig. 5). The transition from IHCs to OHCs may involve BDNF, as MOC innervation of the OHC region is delayed in *BDNF* mutant mice¹⁶². However, the factors that ultimately guide MOCs toward the OHC region have yet to be identified.

The arrival of MOC neurons in the OHC region also coincides with a loss of Type II afferent synapses in several species and the onset of OHC electromotility (the physical contraction of OHCs in response to sound)^{205,207,208}. These findings led early researchers to speculate that the arrival of MOC neurons influences the final maturation of OHCs, including the formation of synapses with Type II SGNs^{209,210}. In support of this idea, in *Hoxb1* mutant mice, MOC neurons fail to develop and their absence is accompanied by a gradual degeneration of OHCs, and hence progressive hearing loss⁷¹. However, other essential auditory nuclei are also disrupted in these mutants, and the loss of OHCs is limited to the apical turn, leaving the specific contributions of MOCs unclear. Additionally, OHCs still become electromotile in the absence of functional MOC inputs^{211,212}. Other research suggests that MOCs are not required for normal synaptic connectivity between Type II SGNs and OHCs: in cats that have had all efferent connections to the cochlea severed neonatally—before MOC neurons typically reach the OHCs—SGN synapses are still pruned to a number similar to that seen in wild-type animals²¹³. Whether the removal of MOC contacts with IHCs makes room for the newly arrived LOC axons remains unknown.

Does the arrival of LOC neurons affect SGN subtype determination?

During this period of efferent rearrangement, SGNs acquire their own unique traits, raising the possibility that these two aspects of circuit maturation are causally linked. Type I SGNs are generally classified into three subtypes, based on their spontaneous firing rates and thresholds²¹⁴. In rats, diversification of firing rates occurs primarily between the second and third postnatal weeks, around the time OCNs are finalizing their own connections²¹⁵. Moreover, LOCs preferentially innervate the subclass of SGNs with the lowest spontaneous firing rate and highest firing threshold, raising the possibility that SGN subtype differentiation may involve cues from LOC neurons^{216,217}.

This hypothesis was tested directly in kittens by severing the OCB shortly after birth and measuring the firing rates of single auditory nerve fibers. Although firing rates overall were decreased in these animals, all three SGN subtypes remained present, as assessed by recording from fibers in the VIIIth nerve²¹⁸. LOC neurons do, however, seem to play a role in both establishing and maintaining morphological properties of the SGN-IHC synapse^{219,220}. Detailed anatomical studies suggest that low spontaneous firing rate (low-SR) SGNs form synapses on the modiolar side of the IHC (i.e. closer to the ganglion)

whereas high spontaneous firing rate (high-SR) SGNs synapse instead on the opposite side (i.e. the pillar side). SGN axon segregation is accompanied by striking differences in synaptic morphology: low-SR SGNs receive signals through small post-synaptic boutons apposed to large pre-synaptic ribbons, whereas high-SR SGNs develop larger boutons opposite to small ribbons²²¹. This distribution of synapses emerges gradually over the first month of life, with the mature pattern apparent right around the time that OCN axons exhibit fully mature electrophysiological properties. When the OCB was severed in adult mice, the gradient of synaptic morphology was disrupted²¹⁹, raising the possibility that LOC axons influence SGN firing rates and hence synaptic morphology. Unfortunately, since no genetic tools are available to manipulate LOC or MOC neurons prior to birth, the full extent of OCN influence on auditory maturation remains unclear.

Implications for human health

As in other species, human OCNs influence auditory function. Human OCNs appear to be especially important for protecting the cochlea against acoustic trauma and in separating speech sounds from background noise^{15,21,222}. Furthermore, studies in rodents suggest that animals raised in loud environments occasionally fail to develop normal temporal acuity, an effect that is exacerbated in animals lacking functional efferent synapses^{223,224}. OCNs may play a similar protective role in human development.

The OC efferent system has also been implicated in a number of human neurological disorders that involve altered auditory processing, including autism spectrum disorder (ASD), schizophrenia, and tinnitus. For patients with ASD, abnormalities in auditory processing are associated with a diminished SOC^{225,226} and a diminished or asymmetrical MOC reflex^{227,228}, suggesting that OCNs may play a role in the altered processing of auditory stimuli. Altered MOC symmetry has also been identified in patients with schizophrenia^{229,230}. However, differences between patients and normal-hearing controls tend to be small, and seem to vary both across patients and with experimental methods^{231,232}. More work is also needed to determine whether effects seen in these various disorders are specific to efferents, or reflect broader changes in brain structure and connectivity²³³.

Efferent synapses are also altered in aging animals, and thus may contribute to the age-related defects in auditory processing shown in human studies. In particular, older mice lose MOC synapses onto OHCs²³⁴. MOC neurons also begin to re-form synapses onto IHCs later in life^{235,236}. The effects of this MOC-IHC contact are not understood, nor are the mechanisms that cause these alterations in MOC synapses. This change may, however, contribute to defects in auditory processing, especially in discriminating speech sounds. Animal models also suggest that the presence of OCNs can delay age-related hearing loss, as acutely de-efferented animals exhibit an accelerated decline in auditory thresholds with age²³⁷. Ultimately, developing successful therapies to treat any of these conditions will likely hinge on a robust understanding of both the development and function of OCN circuitry.

Conclusion: looking forward

Despite their important position at the intersection of the central and peripheral auditory systems, efferent neurons remain poorly understood at many levels. Unresolved issues include the range of diversity among OCNs in general and among LOC neurons especially, as well as the nature of cues that direct each subtype to its proper location in the developing brainstem. A related question is how each of these populations navigates to distinct synaptic targets in the cochlea, as well as subsequent effects of LOCs on the number, size, and positions of contacts between IHCs and SGNs. Efforts to tackle these fundamental developmental questions have been hindered by the lack of markers for subtypes of OCNs and SGNs, as well as the lack of genetic tools to access either population during early development. The recent explosion in the use of reliable single cell RNA-sequencing methods offers an exciting opportunity to break through this bottleneck. In the meantime, inroads may come from ongoing analysis of the few genes known to play important developmental roles, such as GATA3. Intriguingly, GATA3 is also a prominent regulator of SGN development, raising the possibility that one transcription factor may promote complementary developmental programs in the afferent and efferent neurons of the auditory system.

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Figure 1.

Organization of the mammalian olivocochlear efferent system. Auditory information transduced by inner (IHCs) and outer hair cells (OHCs) is conveyed to the auditory brainstem via Type I and Type II spiral ganglion neurons (SGNs), respectively. SGNs project into the brain via the VIIIth nerve and synapse onto neurons in the cochlear nucleus complex (CNC). The superior olivary complex (SOC, dashed circle) contains multiple nuclei involved in sound localization, including the medial superior olive (MSO), lateral superior olive (LSO), ventral nucleus of the trapezoid body (VNTB), lateral nucleus of the trapezoid body (LNTB), medial nucleus of the trapezoid body (MNTB), and the superior periolivary nucleus (SPN). Medial (MOCs) and lateral (LOCs) olivocochlear neurons reside primarily in VNTB and LSO, respectively, and project back to the sensory epithelium via the VIIIth nerve. Other types of neurons are also housed in VNTB and LSO, including the neurons that mediate afferent responses, but only MOCs and LOCs are indicated for simplicity. Within the cochlea, LOCs form synapses with Type I SGNs, whereas MOCs terminate on OHCs.

Figure 2.

Cell body movements of inner ear efferents (IEEs) and facial branchial motor neurons (FBMNs) during mouse embryogenesis. (A) As shown in a sagittal view, FBMNs and IEEs both derive from a common pool of visceral motor neuron (MNv) progenitors in rhombomere 4 (r4). During embryogenesis, FBMNs move caudally. IEEs gradually separate into distinct subtypes and migrate to their final positions in r4 and r5. Rostral is left and dorsal is up. LOC, lateral olivocochlear neuron; MOC, medial olivocochlear neuron; VEN, vestibular efferent neuron. (B) Starting around E9, IEEs and FBMNs begin to differentiate from a shared pool of cells in the progenitor domain of visceral motor neurons (pMNv), directly adjacent to the floor plate (FP), illustrated in a transverse view of r4. As they develop, immature neurons exit the cell cycle and migrate from the ventricular zone (VZ) to the mantle zone (MZ). By E10.5, IEEs and FBMNs have begun to separate: FBMN cell bodies migrate caudally (see A), while IEEs begin to send projections (or translocate) across the floor plate. Both FBMNs and IEEs also begin extending projections to the periphery via the VIIth and VIIIth nerves. By E13.5, FBMNs and IEEs have fully separated, and olivocochlear neurons (OCNs) have separated from VENs. OCNs migrate towards the pial surface whereas VENs migrate dorsally.

Figure 3.

Transcription factors driving the production of FBMNs and IEEs. FBMNs and IEEs arise in the fourth rhombomere (r4), which is specified by an autoregulatory *Hoxb1/b2* network. Within the pMNV domain of r4, SHH induces expression of a suite of transcription factors (grey box), including *Nkx2-2*, *Nkx2-9*, *Nkx6-1*, and *Nkx6-2*, that direct a branchiovisceral MN fate. The presence of *Hoxb1* and *Nkx* factors collectively induce *Phox2b*, which is subsequently maintained in branchiovisceral precursors, including those that become IEEs or FBMNs. Within r4, *Nkx6* factors are required for the maintenance (but not the onset) of *Hoxb1* expression (grey arrow). In branchiovisceral precursors, *Phox2b* drives expression of early markers of visceral and branchial MN identity, including *Phox2a*, *Isl1*, and *Chat*. In addition, *Nkx6-1* and *Ascl1* expression persists after the MNv progenitors exit the cell cycle and become branchiovisceral precursors. Within this pool of precursors, those that will become IEEs likely deviate from an FBMN fate due to the expression of *Gata2/3*. Together, *Phox2b* and *Hoxb1* drive expression of *Gata2*, which in turn drives expression of *Gata3*. *Ascl1* remains active in postmitotic IEEs (but not FBMNs) until about E12.5 and influences the level of *Gata3* expression. Many of these transcription factors impinge on each other and on common cassettes of gene expression, with some acting at multiple stages and in multiple ways. For simplicity, only the key transcriptional interactions that drive the specification and differentiation of IEEs are shown. See Table 1 for additional details regarding the specific contributions of each factor.

Figure 4.

Organization and growth of OCN and SGN projections in the cochlea. (A) Organization of the mature cochlea. MOC axons project along the intraganglionic spiral bundle (IGSB) before turning to branch among the OHC layers. LOCs also travel within the IGSB before turning to the IHC layer. LOCs either turn unilaterally or branch to spiral on either side of IHCs in the inner spiral bundle (ISB) and tunnel spiral bundle (TSB). MOC and LOC neuron traces inspired by Brown 2011 (ref.²⁵). (B) E17.5 mouse cochlea showing OCNs (red) and SGNs (white). OCNs appear to grow along SGN axons, as indicated by the close association between OCNs and SGNs and the way incoming OCN projections lag behind extending SGN peripheral axons. Image provided by N. Druckendbrod.

Figure 5.

Development and refinement of cochlear wiring in the mouse. (A) By P0, MOCs have arrived in the vicinity of IHCs. Both Type I and Type II SGNs have projected to IHCs and OHCs, respectively. Between P0 and P4, both MOC neurons and Type I SGNs form synapses with IHCs, while Type II SGNs form connections with OHCs. The MOC axons then grow on towards the OHC region. At least some of these fibers appear to be branches from MOC axons in the IHC region. Over the next two weeks, both SGNs and MOCs prune excess synapses. By adulthood, MOCs project exclusively to OHCs. (B) Development of IHC synapses. At P0, both Type I SGN and MOC projections are present at the IHC surface. Although synaptic machinery is present in IHCs, many components are not yet localized to the cell membrane. Synaptogenesis proceeds over the next several days, and by P4 all IHCs respond to MOC stimulation. The arrival of MOC terminals coincides with the expression of SK2 calcium-gated potassium channels and $\alpha 9/\alpha 10$ nicotinic acetylcholine receptors (nAChRs) in IHCs. Ribbon synapses (purple) presynaptic to Type I SGNs also mature over this time period. By adulthood, both MOC terminals and their postsynaptic receptors in IHCs have been eliminated. LOCs also innervate the cochlea during this period (not shown).

Table 1

Genetic factors influencing IEE identity and migration.

Gene	Protein type	Effect on IEE Development	References
<i>Hoxb1</i>	Homeodomain transcription factor	Critical for establishing an r4 identity. No IEEs or FBMNs in <i>Hoxb1</i> mutant mice; branchiovisceral MNs are mis-specified. Mutants fail to maintain <i>Phox2b</i> expression in progenitors and exhibit no <i>Gata3</i> expression in r4. Ectopic expression outside of r4 induces aberrant midline crossings and <i>Gata3</i> expression.	71,86,89–91,95,111
<i>Nkx2-2, Nkx2-9</i>	Homeodomain transcription factors	Both genes are key for interpreting graded SHH signals and assigning a dorsal-ventral identity. In <i>Nkx2-2, Nkx2-9</i> double knock-out mice, hindbrain branchiovisceral MNs lose <i>Phox2b</i> expression and mis-specify as somatic MNs. In the presence of <i>Hoxb1</i> , ectopic expression of <i>Nkx2-2</i> induces <i>Phox2b</i> expression and a branchiovisceral MN identity.	62,84,95,97,100,108
<i>Nkx6-1, Nkx6-2</i>	Homeodomain transcription factors	Both genes are key for establishing dorsal-ventral patterning and hence motor neuron development. Somatic MN identity is mis-specified in <i>Nkx6-1</i> mouse mutants. FBMN migration—but not initial specification—is also altered, likely through altered expression of cell surface receptors like RET and UNC5C. Effects on IEE migration are unknown. In <i>Nkx6-1, Nkx6-2</i> double knock-outs, some visceral MNs differentiate as serotonin neurons. After E10.5 in double knockouts, <i>Hoxb1</i> expression decreases in ventral r4.	62,95,98,99
<i>Phox2b</i>	Homeodomain transcription factor	Key regulator of branchiovisceral MN specification. <i>Phox2b</i> mutants lack all branchial cranial motor neuron nuclei, with no IEEs and no FBMNs. Visceral motor neurons are mis-specified. Sufficient to induce itself, <i>Isl1</i> , <i>Phox2a</i> , and <i>Chat</i> in branchiovisceral MN precursors. Can cooperate with <i>Nkx2-2</i> to induce <i>Asc11</i> and generic neuronal differentiation. Upstream of <i>Gata2/3</i> in IEEs.	96,104–107
<i>Asc11</i>	bHLH transcription factor	Promotes motor neuron development widely with specific effects branchiovisceral MN development. Can induce <i>Gata3</i> expression in chicks, and mouse mutants exhibit a reduced number of GATA3-positive progenitors in ventral r4. FBMN migration is delayed and the vestibular efferent nucleus fails to form.	96,141,143
<i>Isl1</i>	Homeodomain transcription factor	Promotes motor neuron development widely with specific effects on FBMN and IEE differentiation. In mouse mutants, spinal motor neurons fail to develop. In hypomorphs, IEE axons are disorganized and short, barely reaching the periphery. FBMN migration stalls in r5.	127,144
<i>Tbx20</i>	T-box transcription factor	Important for FBMN and IEE differentiation. In nervous-system-wide conditional knock-out mice, FBMNs do not migrate caudally and IEEs do not form contralateral projections. <i>Gata2</i> expression is diminished. In FBMNs, <i>Tbx20</i> recruits PCP machinery.	101
<i>Gata2</i>	Zinc-finger transcription factor	Important for IEE differentiation. <i>Gata2</i> mutants show no expression of <i>Gata3</i> . Mutants also show fewer fibers crossing the floor plate, with fewer cells in r4 and fewer FBMNs.	111,114
<i>Gata3</i>	Zinc-finger transcription factor	Important for IEE differentiation. IEE migration and targeting is aberrant in <i>Gata3</i> mutants: embryos have no contralateral IEE projections and IEE axons exit with the facial nerve instead of the VIII th , failing to reach the inner ear. SGN wiring is also disrupted. Autonomous function of <i>Gata3</i> in IEEs remains to be defined.	111,112,145–147
<i>Reln</i>	Extracellular matrix protein	Influences FBMN and IEE migration. Both FBMNs and OCNs fail to reach the pial surface of the hindbrain in <i>Reln</i> mouse mutants. VENs develop in a normal location.	117
<i>Prickle1</i>	Planar cell polarity protein	Influences FBMN and IEE migration. In <i>Prickle1</i> mutant mice, some IEE projections fail to cross the midline and appear to run the length of the floor plate instead of crossing it. Aberrant caudal and radial migration of FBMNs.	140

Gene	Protein type	Effect on IEE Development	References
<i>celsr2</i>	Cadherin	Influences branchiovisceral MN migration. In mutant zebrafish, octavolateralis efferents do not migrate caudally with FBMNs.	136
<i>scrib</i>	Planar cell polarity protein	Influences branchiovisceral MN migration. In mutant zebrafish, octavolateralis efferents do not migrate caudally with FBMNs.	137
<i>EphB2</i>	Eph receptor	Mouse mutants exhibit aberrant, delayed targeting of IEE axons.	124
<i>Robo2</i>	Transmembrane receptor	<i>Robo2</i> mouse mutants show increased and aberrant projections across the floor plate in r4, potentially from FBMN axons. Aberrant migration of ISL1+ cell bodies onto the midline.	125,126
<i>Slit1, Slit2</i>	Glycoprotein	<i>Slit1, Slit2</i> double knock-out mice have reduced midline crossing in r4.	125