Progress in Neurobiology 93 (2011) 488–508

Contents lists available at ScienceDirect

Progress in Neurobiology

journal homepage: www.elsevier.com/locate/pneurobio

Connecting the ear to the brain: Molecular mechanisms of auditory circuit assembly

Jessica M. Appler [a,b], Lisa V. Goodrich [a,*]

[a] Department of Neurobiology, Harvard Medical School, Boston, MA, United States
[b] Program in Neuroscience, Harvard Medical School, Boston, MA, United States

ARTICLE INFO

Article history:
Received 2 September 2010
Received in revised form 9 December 2010
Accepted 3 January 2011
Available online 11 January 2011

Keywords:
Auditory system
Inner ear
Neural development
Circuit assembly
Spiral ganglion neuron

ABSTRACT

Our sense of hearing depends on precisely organized circuits that allow us to sense, perceive, and respond to complex sounds in our environment, from music and language to simple warning signals. Auditory processing begins in the cochlea of the inner ear, where sounds are detected by sensory hair cells and then transmitted to the central nervous system by spiral ganglion neurons, which faithfully preserve the frequency, intensity, and timing of each stimulus. During the assembly of auditory circuits, spiral ganglion neurons establish precise connections that link hair cells in the cochlea to target neurons in the auditory brainstem, develop specific firing properties, and elaborate unusual synapses both in the periphery and in the CNS. Understanding how spiral ganglion neurons acquire these unique properties is a key goal in auditory neuroscience, as these neurons represent the sole input of auditory information to the brain. In addition, the best currently available treatment for many forms of deafness is the cochlear implant, which compensates for lost hair cell function by directly stimulating the auditory nerve. Historically, studies of the auditory system have lagged behind other sensory systems due to the small size and inaccessibility of the inner ear. With the advent of new molecular genetic tools, this gap is narrowing. Here, we summarize recent insights into the cellular and molecular cues that guide the development of spiral ganglion neurons, from their origin in the proneurosensory domain of the otic vesicle to the formation of specialized synapses that ensure rapid and reliable transmission of sound information from the ear to the brain.

Contents

Abbreviations: IHC, inner hair cell; OHC, outer hair cell; SR, spontaneous firing rate; CVG, cochlear-vestibular ganglion; bHLH, basic helix-loop-helix; Lfng, Lunatic fringe; Ngn1, Neurogenin 1; Dll1, Delta-like 1; Ntn1, Netrin1; BDNF, Brain-derived neurotrophic factor; NT3, Neurotrophin 3; FGF, Fibroblast growth factor; BMP, Bone morphogenetic protein; TMRD, tetramethylrhodamine-conjugated dextran; DCN, dorsal cochlear nucleus; VCN, ventral cochlear nucleus.

* Corresponding author. Tel.: +1 617 432 2951; fax: +1 617 432 2949.
E-mail address: Lisa_Goodrich@hms.harvard.edu (L.V. Goodrich).

0301-0082/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pneurobio.2011.01.004

1. Preface

Sensory information is communicated from the outside world to the brain through precisely organized neural circuits. Each sensory modality is processed by distinct populations of neurons with specialized properties that are unique to the demands of that system. While excellent progress has been made towards understanding the development and function of sensory systems such as the eye and the nose, much less is known about the circuits that underlie the perception of sound. This is a major gap in our knowledge, as defects in the auditory system are unusually common in the human population. For instance, the National Institute on Deafness and Other Communication Disorders estimates that hearing loss affects roughly 17% of adults in the United States, with millions more Americans suffering from central processing defects such as tinnitus (N.I.D.C.D.). Many forms of hearing impairment are caused by defects in two types of cells located in the cochlea of the inner ear: the hair cells and the spiral ganglion neurons. Currently, the best available treatment for deafness due to loss of hair cells is the cochlear implant, which works by directly stimulating spiral ganglion neurons. In this review, we provide a general overview of the circuits that communicate sound information from the ear to the brain, followed by a summary of recent studies that have shed light on the molecular cues guiding the development of spiral ganglion neurons, with a focus on the mammalian system.

2. The logic of auditory circuits

The sense of hearing originates in the cochlea of the inner ear. After traveling through the external canal and middle ear, sound waves cause movement of the fluids inside the cochlea and hence stimulation of hair cells housed in the organ of Corti. Each hair cell detects a narrow range of sound frequencies, dictated by its location along the length of the cochlear duct. Information about the timing, frequency and intensity of sounds is then transmitted via highly efficient ribbon synapses to the spiral ganglion neurons (Fig. 1A″). The primary receptor cells are the inner hair cells (IHC), which lie in a single row closest to the spiral ganglion neuron cell bodies (Fig. 1B). There are also three rows of outer hair cells (OHC), which improve the sensitivity of sound detection. Spiral ganglion neurons fall into two broad categories, Type I and Type II, which are defined based on whether they project to inner or outer hair cells. In the mouse, each IHC connects with 10–20 Type I spiral ganglion neurons (Meyer et al., 2009), while each Type I neuron receives input from only one hair cell (Liberman, 1980). Axons of Type I spiral ganglion neurons project into the hindbrain, bifurcate and make connections with multiple cell types in the cochlear nucleus, both in the magnocellular core and in the small cell cap (Fekete et al., 1984; Ryugo, 2008) (Fig. 1B). Type II neurons make up less that 10% of spiral ganglion neurons (Romand and Romand, 1987). Type II neurons extend past the single row of IHC, turn towards the base, and extend projections to contact multiple OHC (Fig. 1B). In the mature cochlea, the cell bodies of Type II neurons are often located on the edge of the ganglion nearest to the hair cells (Fig. 1B) (Hafidi, 1998; Huang et al., 2007). In the central nervous system, Type II axons terminate within the small cell cap of the cochlear nucleus (Fig. 1B) (Brown et al., 1988). Type II neurons clearly receive synaptic input from OHC, but their function *in vivo* is poorly understood (Weisz et al., 2009).

The sounds we hear in the environment vary in intensity, frequency, and timing, allowing us to distinguish melodies and language even against a backdrop of low level noise. Spiral ganglion neurons are charged with the difficult task of accurately capturing and encoding the features of each complex sound stimulus. How this is accomplished at the level of the circuit remains a mystery. At the most basic level, hair cells and neurons in the cochlea are topographically organized according to sound frequency along the spiral of the cochlea. Hair cells located at the wider basal portion of the cochlea detect high frequency sounds, while those located at the apex of the cochlea detect low frequency sounds. Similarly, the position of spiral ganglion neurons along the tonotopic axis of the cochlea correlates with the frequency of input each neuron receives, though occasional projections off this axis can occur (reviewed in Rubel and Fritzsch, 2002). The tonotopic order in the cochlea is preserved in the organization of central projections into the hindbrain, with apical (low frequency) neurons projecting to ventral regions of each division of the cochlea nucleus relative to basal (high frequency) neurons (Fig. 1B). This basic tonotopy is present at every level of auditory processing, from the brainstem up to the cortex (Kandler et al., 2009).

Although emphasis has been placed on tonotopic maps, auditory circuits display several other features that are equally critical for our ability to perceive the rich repertoire of sounds we encounter every day. Indeed, each IHC is contacted by multiple spiral ganglion neurons, offering a potential mechanism for encoding smooth gradations in sound intensity. For instance, in mice and gerbils, the most heavily innervated hair cells correspond to sound frequencies with more acute thresholds of detection (Ehret, 1979; Meyer et al., 2009), in agreement with reports of dense innervation in the middle of the cochlea in other species (Dannhof and Bruns, 1993; Liberman et al., 1990; Meyer and Moser, 2010). Therefore, the presence of multiple connections may simply increase the probability of detecting soft sounds. However, spiral ganglion neurons also exhibit intrinsic differences in firing properties, suggesting that they can serve as specialists for specific aspects of each sound stimulus. For example, basal neurons display quicker firing and faster accommodation than apical neurons (Adamson et al., 2002). Additionally, neurons exhibit a range of spontaneous firing rates (SR), with high SR (>18 spikes per second) neurons making up 60% of the neurons (Liberman, 1978). Neurons with a high SR have the lowest threshold of sound detection for any given frequency. In contrast, spiral ganglion neurons with a low SR can vary their response rates over a larger range of sound pressure levels, allowing them to better detect changes in sound volume. In the cat, the SR of a nerve fiber correlates with the location of its contact onto the hair cell (Liberman, 1982). Fibers with a low SR

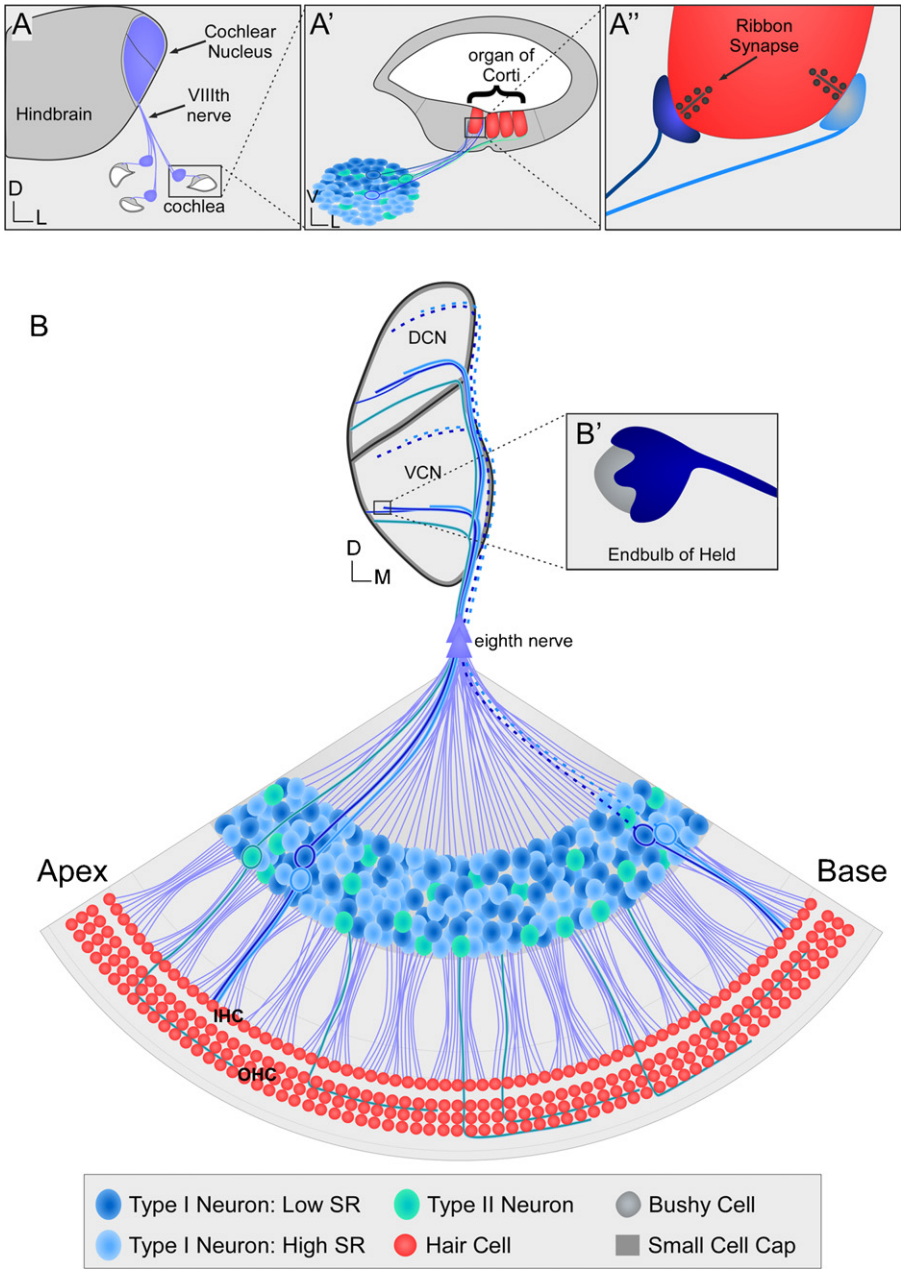

Fig. 1. The organization of connections from the ear to the brain. (A) Diagrammatic view of a cross-section through the hindbrain at the level of the cochlea. Dorsal is up; lateral is right. Spiral ganglion neurons (blue) convey auditory information from the cochlea via the eighth (VIII) nerve, which arborizes within the cochlear nucleus in the hindbrain. A single turn of the cochlea is boxed and shown at higher power in A′. (A′) By convention, the orientation is flipped such that ventral is now up. Projections from spiral ganglion neurons (light blue, dark blue and green) penetrate the cochlear duct to reach the organ of Corti, which houses the hair cells (red). In this cross-sectional view, spiral ganglion neurons with a low spontaneous rate (SR) (dark blue neurons) are located more ventrally than those with high SR (light blue neurons). The boxed region is shown at higher power in A″. (A″) Spiral ganglion neurons receive information from hair cells via ribbon synapses. Connections made by low SR neurons (dark blue synapse) are located on the neural (also called the modiolar) side of the hair cell, while those made by high SR neurons (light blue synapse) are located on the on the abneural side. In this diagram, neural is to the left and abneural is to the right. (B) Schematic view of a wedge from a flatmounted cochlea (bottom) and its connections with the cochlear nucleus complex (top). In the cochlea, peripheral projections are corralled in radial bundles that pass through the spiral lamina to the hair cells (red). Low SR (dark blue) and high SR (light blue) Type I neurons contact inner hair cells (IHC). Type II neurons (green) are positioned in the ganglia nearest to the hair cells, and extend a projection past the inner hair cells and turn towards the base, with each projection contacting multiple outer hair cells (OHC) along its length. Information is conveyed to the cochlear nucleus by central axons, which bundle together to form the eighth nerve (double arrowhead). Upon entering the brainstem, individual axons bifurcate. The ascending projections terminate with bouton endings in the dorsal cochlear nucleus (DCN), while the descending projections target the ventral cochlear nucleus (VCN), where they form boutons with a variety of post-synaptic target neurons as well as unusual endbulb of Held synapses with bushy cells (B′). Within each division of the cochlear nucleus, auditory axons are tonotopically organized, such that high frequency information from the base of the cochlea is processed dorsally (dotted lines) and low frequency information from the apex is processed more ventrally (solid lines). In addition, the central projections from neurons with low spontaneous firing rates project more laterally than those with high spontaneous firing rates. Type II neurons project to the small cell cap that surrounds the cochlear nucleus complex (dark gray), as do some arbors from low SR fibers.

contact the inner side of the IHC, while fibers with a high SR contact the outer side (Fig. 1A′). Additionally, although low and high SR neurons are both present along the length of the cochlea, low SR neurons are positioned more ventrally in the ganglion and project more laterally in cochlear nucleus than high SR neurons (Fig. 1A′ and B) (Kawase and Liberman, 1992; Leake and Snyder, 1989). In the mouse, the distribution of spiral ganglion neuron SR is less obviously bimodal than cats and other mammals, and there are a

larger proportion of low SR neurons (Taberner and Liberman, 2005). Additionally, synapses are present in a more uniform distribution around the circumference of the IHC (Meyer et al., 2009), leaving it unclear whether the location of synaptic endings correlates with SR in the mouse. Moreover, it is not known whether spiral ganglion neurons exhibit additional, as yet undescribed differences in connectivity or firing properties that influence how a sound stimulus is perceived. Hence, the division of spiral ganglion neurons into two basic categories is likely an oversimplification.

Due to their key role in the detection and transmission of sound stimuli, understanding how spiral ganglion neurons acquire their unique properties is an important goal in auditory neuroscience. From the point of view of the sense of hearing, analysis of auditory circuit assembly uncovers new molecular markers and generates tools that allow a more detailed understanding of how different types of spiral ganglion neurons respond to sound. This information will permit improved design of cochlear implants that more accurately reflect the normal activity of the spiral ganglion. With regard to new therapies, a thorough knowledge of auditory circuit assembly will provide a critical foundation for developing stem cell based therapies for deafness. Finally, while basic principles of neural development such as cell fate specification and axon guidance are well delineated, we know little about how these events are coordinated during the wiring of highly specific circuits. In mammals, significant progress has been made by focusing on accessible and orderly circuits in the spinal cord and retina (Dalla Torre di Sanguinetto et al., 2008; Mumm et al., 2005). Since spiral ganglion neurons establish well-defined connections that mediate a behavior that is easily tested in animal models, studies of the auditory system provide a useful parallel to investigations of circuit assembly in other systems. Here, we will summarize the cellular events of auditory circuit assembly, from the initial stages of neurogenesis to the development of synapses, as well as the underlying molecular mechanisms where known. Several excellent reviews on specific aspects of inner ear development and function are also available and should be referred to for more details (Abello and Alsina, 2007; Bok et al., 2007; Cramer, 2005; Driver and Kelley, 2009; Fekete and Campero, 2007; Fekete and Wu, 2002; Fritzsch et al., 2006; Fritzsch et al., 2010b; Kandler and Gillespie, 2005; Meyer and Moser, 2010; Rubel and Fritzsch, 2002; Rubel et al., 2004; Ryugo and Parks, 2003).

3. The production and differentiation of spiral ganglion neurons

The inner ear offers a remarkable example of organogenesis, as all of the hair cells and neurons for both hearing and balance, as well as many of the associated non-sensory tissues, arise from a single embryonic source, which is called the otic placode (Barald and Kelley, 2004). The otic placode is a patch of epithelial cells on the surface of an embryo adjacent to the developing neural tube. Early in embryogenesis, the otic placode invaginates and pinches off to form an otic vesicle, which is in close proximity to the hindbrain and notochord (Anniko and Wikstrom, 1984). As early as embryonic day 9 (E9) in the mouse, neuroblasts begin to delaminate from the otic vesicle and coalesce to form the cochlear-vestibular ganglion (CVG), long before the inner ear acquires its complex three-dimensional structure (Carney and Silver, 1983; Ma et al., 1998). As development progresses, the neurons of the CVG segregate to create the spiral ganglion, which coils along the length of the cochlear duct, and the vestibular ganglion, which sits on the surface of the inner ear and innervates the sensory epithelia of the vestibular structures. Recent studies suggest that spiral ganglion neurons are progressively specified by transcriptional networks that act within a common region of the otic vesicle that contains precursors for neurons, hair cells and support cells. Understanding these regulatory relationships is of prime importance, as any common precursor represents the ideal cell type for regenerating the sensory and neural components of the mature inner ear.

3.1. Division of the otic vesicle into neural, sensory, and non-sensory domains

The generation of neurons begins during the earliest stages of inner ear development, when the otic vesicle is patterned by combinations of fibroblast growth factor (FGF), Wnt, bone morphogenetic protein (BMP), and Hedgehog signals that are released from the neural tube, notochord, and surrounding mesenchyme (Bok et al., 2007). Together, these signaling pathways establish the anterior–posterior and dorsal–ventral axes and induce formation of the vestibular and auditory sensory organs: the three semicircular canals, the utricle, the saccule, and the cochlea. All six structures share the same basic epithelial-derived components: a sensory epithelium that is flanked by non-sensory tissues and innervated by neurons. As shown by *in vitro* culture of microdissected otic vesicles (Li et al., 1978) and gene expression patterns (Cole et al., 2000; Giraldez, 1998; Morsli et al., 1998; Raft et al., 2004), the neurons emerge from the anterior of the otic vesicle (Fig. 2A). Hence, the first step in neurogenesis is to establish the anterior–posterior axis. How this is coordinated with development of sensory and non-sensory structures is still under intensive investigation.

Gene expression data and fate mapping studies suggest that the early otic vesicle exhibits broad competence, with signals such as Wnts progressively restricting production of neurons and sensory organs to more discrete areas (Ohyama et al., 2006; Freyer and Morrow, 2010). Subsequently, the anteroventral quadrant begins to produce all the neurons and many hair cells and support cells. This region is then further subdivided (Fig. 2B), with a central neurogenic domain that also contains sensory precursors for the utricle and saccule, and is flanked by prosensory domains for the semicircular canals and cochlea, as evidenced by the expression of genes required for neural and sensory fates (Morsli et al., 1998; Raft et al., 2004; Zou et al., 2004), as well as lineage tracing (Raft et al., 2004; Satoh and Fekete, 2005). We therefore refer to this region as the "proneurosensory domain." This domain may encompass the entire otic cup initially, based both on the broad expression of certain genes associated with neurosensory development (i.e. *Eya1*, *Six1*, *Sox2*) and the fact that sensory precursors are also present in posterior regions (i.e. the posterior cristae). By otic vesicle stages, however, the proneurosensory domain is restricted to the anterior half of the otic vesicle and is marked by the presence of *Lunatic fringe* (*Lfng*), *Sox2*, and *Eya1* (Cole et al., 2000; Kiernan et al., 2005b; Mak et al., 2009; Morsli et al., 1998; Zou et al., 2004). Although the function of *Lfng* remains unclear, mutations in *Sox2* (Kiernan et al., 2005b) and *Eya1* (Zou et al., 2004) cause massive disruptions in both the neurons and the sensory organs, underscoring the common origin of these two tissue types in the early otic vesicle. In parallel, *Tbx1* (Raft et al., 2004; Vitelli et al., 2003) and *Lmx1a* (Koo et al., 2009; Nichols et al., 2008) are excluded from the proneurosensory domain and play a critical role in development of non-sensory cells of the inner ear. Hence, neurogenesis is intimately linked to the development of the non-sensory and sensory tissues.

The subdivision of the otic vesicle into sensory, non-sensory, and neurogenic components appears to begin with the decision to develop as proneurosensory or non-sensory tissue. For instance, rotation of the chick otic vesicle at different stages of development showed that the anterior–posterior axis is fixed very early and before the dorsal–ventral axis (Wu et al., 1998). In addition, *Tbx1* expression is restricted to the posterior half of the otic placode, and

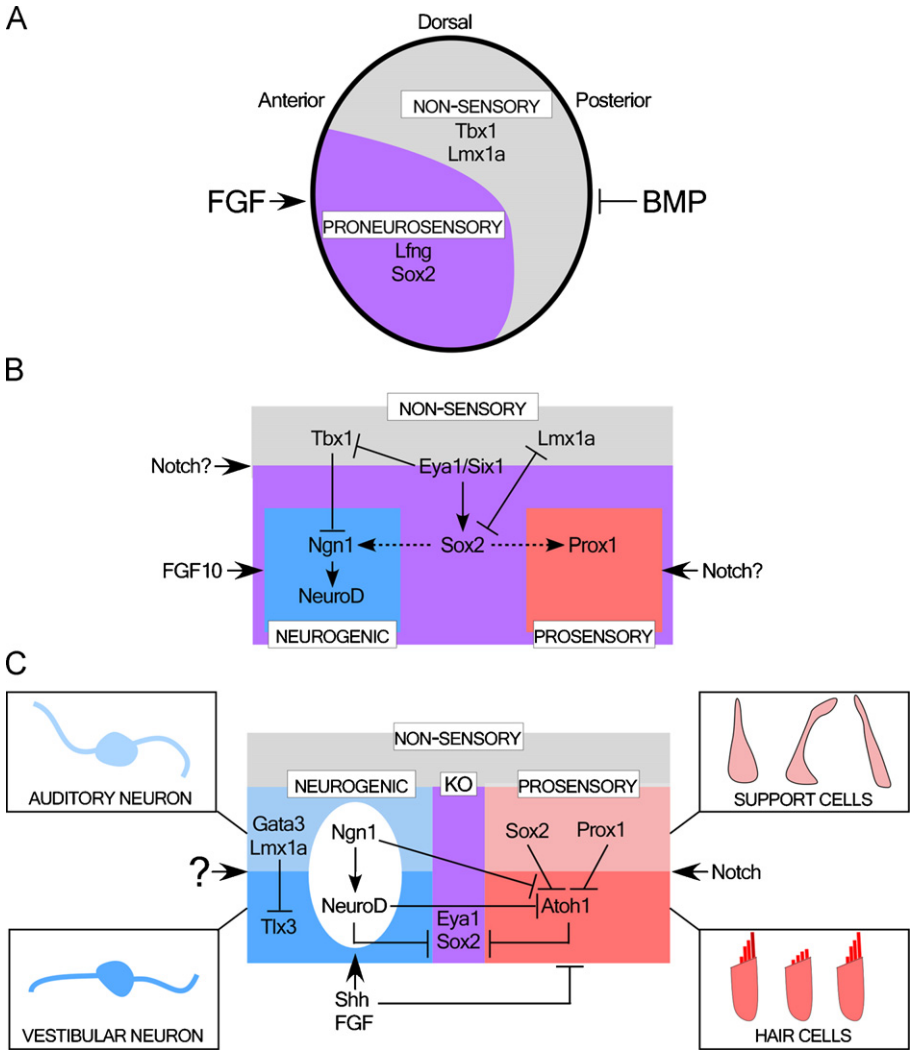

Fig. 2. Parcellation of the otic vesicle (A) Diagram of the early otic vesicle, as viewed laterally. The sensory epithelial cells and neurons develop within a proneurosensory domain (purple) in the anteroventral quadrant of the otic vesicle. This domain is marked by expression of *Lfng* and *Sox2*, while the complementary non-sensory domain expresses *Tbx1* and *Lmx1a*. This initial patterning event may be controlled by FGF and BMP signals. (B) Summary of the transcriptional interactions that divide the non-sensory (gray), neurogenic (blue) and prosensory (red) lineages. Signals that are thought to mediate specific fate decisions are indicated outside the boxes. Possible interactions that require further confirmation are noted with dashed lines. Note that this diagram is highly schematic and meant to illustrate events that may occur even within a single cell as it selects its final fate. In reality, the neurogenic and prosensory domains are contained within the Sox2-positive proneurosensory domain, with a neurogenic domain in the center and multiple prosensory domains that are either contained within the neurogenic domain (i.e. for the utricle and saccule) or are on the periphery of the proneurosensory domain (i.e. for the cristae and cochlea). (C) The neurogenic domain ultimately produces either auditory or vestibular neurons, while cell–cell interactions within the prosensory domain create a mosaic of hair cells surrounded by support cells. Kolliker's organ (KO) retains multipotent progenitors that share features with the initial progenitors in the proneurosensory domain. Shh, Sonic hedgehog.

is followed shortly by expression of the neurogenic transcription factor, Neurogenin1 (Ngn1), which initiates in the anterior just as the placode begins to invaginate (Raft et al., 2004). Presumably, this region is defined by morphogens that promote expression of *Lfng*, *Sox2*, and *Ngn1* at the expense of *Tbx1*. Particularly intriguing candidate molecules include the FGFs, which have been implicated both in otic induction and neurogenesis (Alsina et al., 2004; Pauley et al., 2003; Wright and Mansour, 2003a; Wright and Mansour, 2003b). For instance, in chicks, FGF8 is asymmetrically expressed in the anterior half of the otic placode in the same region that will eventually give rise to neurons and can cause expanded production of neurons when ectopically expressed (Abello et al., 2010). This FGF signal may be balanced by an opposing gradient of BMP, which is proposed to promote expression of non-sensory genes (Fig. 2A) (Abello et al., 2010).

Although the precise contribution of FGF and BMP signals remains unclear, the size of the proneurosensory domain is tied to the development of non-sensory regions. Initially, the otic vesicle is broadly divided, with *Lfng*, *Sox2*, and *Ngn1* in the anterior and *Lmx1a* and *Tbx1* in the posterior; *Eya1* is still expressed throughout at this stage. Tbx1 appears to refine this boundary and solidify early fate decisions. In mice lacking *Tbx1*, the proneurosensory region becomes larger, as marked by expanded expression of *Lfng*, *NeuroD1* and *Ngn1* (Raft et al., 2004). This results in more CVG neurons. In contrast, increased expression of *Tbx1* shrinks the proneurosensory domain and results in fewer CVG neurons. These observations suggest that cells at the border of *Tbx1* and *Ngn1* expression domains likely express both transcription factors transiently before robustly activating only one of the two genes. Consistent with this idea, fate mapping using Ngn1-CreER^{T2} labels a small number of non-sensory cells in the cristae and the cochlear duct (Koundakjian et al., 2007; Raft et al., 2007), tissues that were suggested by independent studies to arise at the boundary of the proneurosensory domain (Morsli et al., 1998; Raft et al., 2004). Conversely, fate mapping using the *Tbx1*^{mcm} allele labels cells in the CVG (Xu et al., 2007). Importantly, loss of *Tbx1* also affects the

sensory components of the inner ear (Moraes et al., 2005; Raft et al., 2004; Xu et al., 2007), consistent with the idea that these interactions serve to define the non-sensory vs. proneurosensory domains, rather than to promote the neuronal fate specifically. If the proneurosensory domain does indeed encompass the entire otic placode at early stages, then the initiation of *Tbx1* expression here may serve to redirect cells in the posterior towards non-sensory fates, while simultaneously restricting subsequent neurosensory development to the anterior. This decision may be reinforced by a parallel transcriptional network. The transcription factor Lmx1a acts in the non-sensory tissue to inhibit expression of *Sox2* and the neurogenic genes, as revealed by changes in gene expression accompanied by development of a larger CVG and ectopic hair cells in *Lmx1a* mutant mice (Koo et al., 2009; Nichols et al., 2008). Conversely, overexpression of Sox3 inhibits *Lmx1b* in the chick otic vesicle (Abello et al., 2010); chick *Sox3* and *Lmx1b* are closely related to the murine *Sox2* and *Lmx1a* and therefore likely to play similar functions. Together with additional genetic fate mapping studies, additional analysis of the earliest detectable defects in relevant mouse mutants is still needed to understand how the non-sensory/proneurosensory boundary is set.

3.2. Regulation of cell fate determination within the proneurosensory domain

The proneurosensory domain consists of a common pool of precursors that ultimately produces both neurons and sensory epithelial cells. Current evidence suggests that this domain is quickly partitioned into molecularly and physically distinct neurogenic and prosensory regions (Fig. 2B). The neurogenic domain is contained within the *Lfng* domain and is marked by the expression of *Ngn1* and *NeuroD1* (Raft et al., 2007), while the prosensory patches develop within *BMP4*-positive streaks on the edge of the *Lfng* domain (Morsli et al., 1998; Raft et al., 2007). Genetic fate mapping of the *Lfng* domain has not been performed, but would be predicted to reveal broad contributions to neurons and hair cells in the other sensory organs. Finally, the prosensory domains themselves undergo an additional round of cell fate decisions that determine whether precursors develop as support cells or hair cells, a process that has been well described in recent reviews (Alsina et al., 2009; Driver and Kelley, 2009; Puligilla and Kelley, 2009).

Within the proneurosensory domain, individual precursors are faced with two options: to remain as an uncommitted precursor or to differentiate as a specific cell type. The transcription factor Sox2 seems to play a dominant role in maintaining a pool of precursors, while Ngn1 promotes expansion of the neurogenic precursors. Subsequently, networks of basic helix-loop-helix (bHLH) transcription factors induce neuronal differentiation, inhibit non-neuronal cell fates, and impart subsets of neurons with auditory or vestibular-specific properties.

3.2.1. Sox2 and the maintenance of precursors in the inner ear

In order to produce enough cells for all six sensory organs of the inner ear, it is essential to maintain a pool of multipotent progenitors, a function that appears to be the jurisdiction of Sox2. Indeed, this is the role that has been ascribed to Sox2 and other members of the SoxB1 family (Sox1 and Sox3) in other regions of the nervous system (Wegner and Stolt, 2005). In the otic vesicle, both the neurogenic and the prosensory domains are contained within the Sox2-positive region, suggesting that Sox2 is a definitive marker of the proneurosensory domain (Abello et al., 2010; Kiernan et al., 2005b; Mak et al., 2009). At all stages of development, *Sox2* is consistently expressed in precursors: first in the proneurosensory domain, then in Kolliker's organ – which is a transient population of multipotent epithelial cells adjacent to the organ of Corti – and also in support cells in the developing organ of Corti (Dabdoub et al., 2008; Mak et al., 2009). Both Kolliker's organ cells and support cells retain precursor cell-like characteristics during late embryogenesis and early postnatal stages, as revealed by the fact that Kolliker's organ cells can differentiate as neurons, support cells, and hair cells under certain conditions (Puligilla et al., 2010; Woods et al., 2004), and isolated support cells can develop as hair cells *in vitro* (Doetzlhofer et al., 2006; White et al., 2006). Hence, the changes in *Sox2* expression mirror the progressive restriction of precursors in the developing inner ear. Consistent with a broad function in neurosensory progenitors, mutations in *Sox2* affect both neural and sensory tissues in the inner ear. For instance, *Light coat and circling* (*Lcc*) mutant mice never express *Sox2* in the inner ear and exhibit a loss of both neurons (Puligilla et al., 2010) and sensory organs (Kiernan et al., 2005b), though when and how these phenotypes arise is not fully understood. Sox2 has also been proposed to induce production of neurons (Puligilla et al. 2010); however, this conclusion is based on overexpression of Sox2 in Kolliker's organ cells, which may not retain the same properties as precursors in the initial proneurosensory domain. What seems more likely is that Sox2 promotes all neurosensory fates simply by ensuring that precursors are available.

Sox factors go on to mediate their effects in the proneurosensory domain in part by interfacing with the Eya1/Six1 transcriptional network. *Sox2*, *Eya1*, and *Six1* are all expressed in the early ventral otic vesicle, and like *Sox2*, the expression of both *Eya1* and *Six1* is initially throughout the entire otic vesicle and then restricted to the proneurosensory domain prior to neurogenesis (Kalatzis et al., 1998; Zheng et al., 2003; Zou et al., 2004). Loss of *Six1* results in a loss of *Lfng* expression in the proneurosensory domain and a lack of neurogenesis (Ozaki et al., 2004; Zheng et al., 2003). Similarly, in *Eya1* mutant mice, *Ngn1* and *NeuroD1* expression fails to be maintained, leading to decreased proliferation and increased death of neural precursors (Friedman et al., 2005; Zou et al., 2004; Zou et al., 2006). These changes are accompanied by reduced *Sox2* expression, and since Eya1 can bind to Sox2 *in vitro*, one possibility is that Eya1 cooperates directly with Sox2 during early stages of neurosensory development (Zou et al., 2008). Although expression of *Tbx1* expands when *Eya1* is lost, Eya1 and Tbx1 may act independently since reduced *Tbx1* expression is not always associated with increased neurogenesis (Freyer and Morrow, 2010; Ohyama et al., 2006). Hence, the transcriptional networks that initiate neurosensory development are complex, and more work is needed to understand when and how these networks interact with each other.

3.2.2. Neurogenesis

Within the neurogenic domain, Ngn1 functions at the top of a transcriptional hierarchy that sets in motion the production of both auditory and vestibular neurons. This series of events is accompanied by changes in cell position, as neurons are initially specified within the otic epithelium, delaminate from the otic vesicle, and then aggregate to form the spiral ganglion medially and the vestibular ganglion laterally. Hence, *Ngn1* expression is primarily found in early precursors within the otic epithelium (Ma et al., 1998), with limited expression in post-delaminated neuroblasts, which instead robustly express the neural differentiation bHLH gene *NeuroD1* (Koundakjian et al., 2007; Raft et al., 2007). Consistent with these expression patterns, loss of *Ngn1* leads to a complete absence of inner ear neurons (Ma et al., 2000; Ma et al., 1998), while in *NeuroD1* mutant mice, neurons are lost soon after delamination, with nearly no auditory neurons left at E13.5 (Jahan et al., 2010a; Kim et al., 2001; Liu et al., 2000). However, it remains unclear whether Ngn1 and NeuroD1 act in a simple linear relationship. *In vitro*, the presence of either Ngn1 or

NeuroD1 is sufficient to induce neuronal gene expression and morphology in non-sensory cells in the cochlea (Puligilla et al., 2010). However, in these experiments, ectopic Ngn1 did not induce ectopic *NeuroD1* expression, suggesting that these proteins may act independently.

After a period of proliferation, *Ngn1*-positive neurons undergo their final cell divisions and begin to differentiate in a basal to apical progression along the length of the cochlea. The sequential birth of spiral ganglion neurons in the mouse has been assessed independently using three different experimental techniques: BrdU incorporation, Ngn1-CreER^{T2} labeling and H^3-thymidine incorporation (Koundakjian et al., 2007; Matei et al., 2005; Ruben, 1967). While all three methods agree about the order of neuronal birth, recent examination suggests that the onset and conclusion of neurogenesis may be earlier than previously reported. Spiral ganglion neurons exit the cell cycle between E9.5 and E13.5, as judged by BrdU incorporation (Matei et al., 2005), 2 days earlier than previously reported (Ruben, 1967). A limited number of neurons in the base and middle of the cochlea undergo terminal differentiation between E9.5 and E10.5. The majority of spiral ganglion neurons exit the cell cycle around E11.5, for neurons in the base and middle of the cochlea, and at E12.5, for neurons located in the apex. Curiously, hair cells are born in an opposing gradient, but like neurons, differentiate from base to apex. The purpose of a waiting period between hair cell birth and differentiation is not known. As development progresses, *NeuroD1* expression initiates and cells become post-mitotic and begin to differentiate (Fig. 2C) (Kim et al., 2001; Lawoko-Kerali et al., 2004; Liu et al., 2000).

There is some evidence that neurotrophin-mediated survival acts downstream of the early acting transcriptional networks that pattern the proneurosensory domain and stimulate neurogenesis. For instance, *Eya1*$^{bor/bor}$ mice, which experience reduced Eya1 function, initially produce a limited number of CVG neurons (Friedman et al., 2005). However, adult *Eya1*$^{bor/bor}$ mice completely lack any spiral ganglion neurons (Johnson et al., 1999). While the CVG neurons seen in *Eya1*$^{bor/bor}$ mice maintain expression of *TrkB* and *TrkC*, the adjacent otic epithelium displays a profound lack of *neurotrophin-3* (*NT3*) and *brain-derived neurotrophic factor* (*BDNF*) expression (Friedman et al., 2005). Similarly, mice lacking *NeuroD1* produce CVG neurons in similar numbers as control animals at E10.5, but show a significant loss of neurons beginning at E11.5, with very few neurons present in the cochlea at birth (Kim et al., 2001). Unlike the CVG in *Eya1*$^{bor/bor}$ mice, *NeuroD1* null CVG neurons completely lack *TrkC*, and show very little *TrkB* expression early in development, while the otic expression of *NT3* and *BDNF* is unaltered. In both cases, it appears that a nearly complete absence of early trophic support from the adjacent otic epithelium leads to the loss of spiral ganglion neurons. Hence signaling between recently delaminated neurons and the nearby otocyst may keep the right number of neurons alive at the beginning of circuit assembly.

3.2.3. Regulation of cell fate decisions by bHLH transcriptional networks

Although clearly critical for neurogenesis, the mere presence of *Ngn1* is not sufficient to turn a cell into a neuron. In particular, a substantial number of Ngn1-positive cells also differentiate as hair cells in the utricle and saccule (Raft et al., 2007). This may reflect a more global phenomenon, as precursors within the proneurosensory domain initially have the potential to differentiate as neurons, hair cells, or support cells. Current evidence suggests that bHLH transcription factors interact with each other within precursor cells to drive a cell towards one specific fate at the exclusion of the others.

The decision to differentiate as a neuron or hair cell is mediated in part by the activation of two bHLH transcription factors: Ngn1 and Atoh1 (formerly known as Math1). Just as neurons are wholly dependent on *Ngn1*, hair cells require *Atoh1* for their specification and development (Bermingham et al., 1999). In other systems, Ngn1 and Atoh1 interact with each other in a mutually inhibitory fashion, thereby ensuring that expression of each transcription factor is restricted to a different population of cells (Bertrand et al., 2002). Whether this happens in the proneurosensory domain remains unresolved. *Ngn1* and *Atoh1* have been proposed to be transiently expressed in a common precursor for both hair cells and neurons (Fritzsch et al., 2002). Subsequent cross-repressive interactions may then lead to robust *Ngn1* expression only in neuroblasts and robust *Atoh1* expression only in hair cells (Raft et al., 2007). This would offer a simple mechanism for generating two very different types of cells within an initially homogeneous proneurosensory domain.

While there is good evidence in favor of this model in the vestibular system, additional processes likely modulate the neuron vs. hair cell fate decision in the cochlea. For instance, the model predicts that Atoh1 and Ngn1-positive progenitors should ultimately differentiate as both hair cells and neurons. Initial studies provided some evidence that Atoh1-positive cells can differentiate as neurons, but relied on an isolated enhancer element (Matei et al., 2005). More recent genetic fate mapping using Cre that has been knocked into the *Atoh1* locus revealed no evidence of a shared progenitor (Yang et al., 2010), suggesting that Atoh1 promotes the hair cell fate only after the neuronal lineage has been set aside. One explanation for these differing results is that the knock-in mice retain a repressive regulatory element that quickly turns off expression of Cre in early shared progenitors; this hypothetical element may not be present in the isolated *Atoh1* enhancer.

Evidence for a common Ngn1-expressing progenitor is also inconclusive. In the vestibular system, hair cells of the utricle and saccule are quite clearly derived from Ngn1-positive cells in the proneurosensory domain (Raft et al., 2007). Therefore, in the gravitational organs, cross-repression of Ngn1 and Atoh1 may indeed be mediated by direct interactions within individual cells. However, similar results are not observed in the cochlea or in the semicircular canals (Koundakjian et al., 2007; Raft et al., 2007). For example, even activation of Cre-ER in Ngn1-positive cells as early as E7.5 labels does not label cochlear hair cells (E.J. Koundakjian and LVG, unpublished observation). A similar conclusion was reached using viral fate mapping of the early otic cup, where shared progenitors for hair cells and neurons were detected in the utricle and saccule but not in the cochlea (Satoh and Fekete, 2005). Instead, the vast majority of clones contained only neurons, suggesting that the neuronal lineage is set aside quite early in development; infection at the otic placode stage may be required to catch any common progenitor for the auditory system.

Although a shared progenitor has not yet been captured by viral or genetic fate mapping, a number of observations highlight the interdependent nature of the differentiation of neurons, hair cells, and support cells. In fact, although the most salient phenotype in *Atoh1* mouse mutants is the lack of hair cells, these animals also have fewer spiral ganglion neurons in the cochlea (Fritzsch et al., 2005). Conversely, removing *Ngn1* causes a decrease in hair cells along with a lack of sensory neurons (Ma et al., 2000). Similarly, although is quite clear that both Ngn1 and NeuroD1 promote the neuronal fate (Kim et al., 2001; Koundakjian et al., 2007; Liu et al., 2000; Ma et al., 2000; Ma et al., 1998; Puligilla et al., 2010; Raft et al., 2007), mutations in both genes also have effects on hair cells. For instance, loss of *Ngn1* causes hair cells in the cochlea to exit the cell cycle prematurely (Matei et al., 2005), while hair cells lacking NeuroD1 differentiate prematurely and in the wrong order, as evidenced by early expression of *Atoh1* in the apex of the cochlea (Jahan et al., 2010b). Even more striking is the appearance of hair

cells within the residual ganglia of the *NeuroD1* conditional knock-outs. One of the barriers towards resolution is the fact that sensory neurons also depend on the sensory epithelia for trophic support; hence, in some cases, the absence of neurons may be secondary to loss of BDNF and/or NT3-positive hair cells (e.g. Fritzsch et al., 2005). This highlights the need to focus on the earliest phenotypes and to use an array of molecular markers to distinguish loss of gene expression from loss of an entire cell population.

A role for cross-repressive interactions is much clearer for the hair cell–support cell decision within the prosensory domain (Fig. 2C). In this case, lineage tracing confirms the presence of a common progenitor in chicks (Fekete et al., 1998), and *Atoh1*-positive progenitors can differentiate as both hair cells and support cells in mice (Yang et al., 2010). Notably, many fewer support cells are labeled in the genetic fate mapping studies, suggesting that only low levels of Atoh1 are ever present in support cells and lending further support to the idea that Atoh1 is rapidly turned off here. An important missing element of this model is any cell-type transcription factor that induces the support cell fate. Prox1 is commonly thought of as a support cell marker and may promote the support cell fate by preventing Atoh1 activity and hence blocking hair cell differentiation (Dabdoub et al., 2008). However, Prox1 is also expressed in neurons (Stone et al., 2003) and does not seem to be required for differentiation of either hair cells or support cells (Fritzsch et al., 2010a). In fact, support cells comprise a heterogeneous population with distinct morphologies and functions. Hence, it is possible that early "support cells" are still precursor-like, as evidenced by expression of Sox2, but then go on to express distinct, as yet unidentified transcription factors that promote differentiation of pillar cells, Deiter cells and so on. Regardless of the identity of the factors involved, it seems that both neurons and support cells actively suppress the hair cell fate by blocking expression and/or function of Atoh1. At the same time, factors such as Atoh1 promote cell-cycle exit and differentiation. The accompanying loss of the precursor state may be effected through inhibition of Sox2, but currently the only evidence in support of this idea is the fact that Atoh1 can block Sox2 activity upon overexpression in Kolliker's organ (Dabdoub et al., 2008).

Whether or not there is transient co-expression in any one cell, *Ngn1* and *Atoh1* are eventually expressed in restricted pools of precursors. Interactions between these different cell populations and uncommitted progenitors may further modulate the timing and extent of differentiation, a mechanism that has been proposed for the generation of diverse cell types in the retina (Livesey and Cepko, 2001). For instance, the temporal progression of differentiation may be influenced by signaling between *Ngn1*-dependent cells and a common hair cell-neuron progenitor that does not express *Ngn1*. However, there is no evidence that such a progenitor exists. Additionally, it is unclear how progenitor-progeny interactions might induce the switch from neurogenesis to hair cell production. One idea is that neurons secrete Sonic hedgehog, which inhibits development of hair cells in Kolliker's organ and thereby ensures formation of four rows of hair cells in the organ of Corti. Indeed, Sonic hedgehog is expressed in the developing neurons (Driver et al., 2008; Liu et al., 2010) and blocks hair cell development (Driver et al., 2008) (Fig. 2C). Similarly, hair cells can induce development of support cells (Woods et al., 2004), further underscoring the importance of interactions between committed and uncommitted precursors.

Many of the proposed cell–cell interactions may be controlled by the Notch signaling pathway, which plays a well-described role in lateral inhibition (Ehebauer et al., 2006). In brief, interactions between cells expressing the Notch receptor and cells expressing Delta ligands ensure that the Delta-positive cells assume one fate and that the Notch-positive cells do not. In flies, these interactions result in development of neurons from the Delta-positive cells and epithelial cells from the Notch-positive cells. One indication that the Notch pathway might influence the segregation of neurogenic and prosensory progenitors is the early expression of the Notch ligand *Jagged-1* (Pan et al., 2010) and *Lfng*, which is a modulator of the Notch signaling pathway (Morsli et al., 1998). Early neural precursors also express another Notch ligand Delta-like 1 (Dll1) (Adam et al., 1998) and this expression requires *Ngn1* function (Ma et al., 1998), consistent with the idea that Ngn1-positive precursors use Dll1 to prevent surrounding cells from developing as neurons. Moreover, when Notch signaling is blocked, as occurs in *Pofut1* mutant mice, *Ngn1* expression is expanded (Raft et al., 2007). Similar loss of Notch signaling occurs in *mindbomb* zebrafish mutants (Haddon et al., 1998) and in *Dll1* conditional knock-out mice (Brooker et al., 2006), both of which develop extra neurons. Strikingly, loss of *Dll1* also disrupts development of the utricular and saccular maculae, as would be predicted if prosensory progenitors are diverted to the neural lineage. However, exactly when and how Notch signaling influences neurogenesis remains unclear, since Notch acts during multiple stages of inner ear development and is stimulated by a variety of ligands, which can act synergistically (i.e. Dll1 and Jagged-2; Kiernan et al., 2005a) but also exhibit unique functions (i.e. Dll1 vs. Jagged-1; Brooker et al., 2006). For example, loss of the ligand Jagged-1 causes a reduction in expression of *Sox2* and *Lfng*, and consequently disrupted development of the organ of Corti as well as fewer neurons, suggesting an early role in proneurosensory development (Pan et al., 2010). However, a similar effect is also observed upon broad activation of Notch, which would be predicted to cause the opposite phenotype (Pan et al., 2010). Hence, while there is solid evidence that Notch signaling regulates the hair cell-support cell signaling, the observed complexity of Notch signaling highlights the need for more work to understand the role of Notch signaling during other aspects of inner ear development.

3.2.4. The auditory vs. vestibular fate decision

Another critical step during circuit assembly is the segregation of auditory and vestibular neurons. Similar to the hair cell vs. neuron decision, current evidence suggests that the auditory vs. vestibular cell fate decision occurs very early, with spiral and vestibular ganglion neurons originating from distinct regions of the proneurosensory domain. It has long been known that vestibular ganglion neurons are born prior to spiral ganglion neurons across the animal kingdom (Bell et al., 2008; Ruben, 1967; Sapede and Pujades, 2010). Consistent with this observation, fate mapping in mice indicates that vestibular ganglion neurons express *Ngn1* before the spiral ganglion neurons (Koundakjian et al., 2007). In addition, vestibular and spiral ganglion neurons also derive from separate *Ngn1*-expressing populations (Koundakjian et al., 2007). For instance, early *Ngn1*-positive progenitors differentiate nearly exclusively as vestibular ganglion neurons, indicating that neurons are already committed to the vestibular fate before or shortly after *Ngn1* expression initiates. Consistent with this idea, the neuroblasts giving rise to the spiral and vestibular ganglia appear to delaminate from spatially distinct regions of the otic vesicle (Lawoko-Kerali et al., 2004). Similarly, fate mapping of the chicken otic vesicle indicates that spiral ganglion neurons are derived from a more posterior-medial region of the proneurosensory domain than vestibular neurons (Bell et al., 2008).

The nature of the molecular cues that underlie the specification of auditory vs. vestibular neurons is poorly understood. No cues required specifically for vestibular ganglion neuron generation have been identified to date, although maintenance of NeuroD1 has been suggested as an early indicator of the vestibular fate decision (Lawoko-Kerali et al., 2004). However, the fact that spiral ganglion neurons lacking *NeuroD1* exhibit pathfinding defects

suggests a more complicated role for NeuroD1 (Jahan et al., 2010a; Kim et al., 2001). Vestibular neurons also express the transcription factor Tlx3 (also known as Rnx) (Koo et al., 2009) and fate mapping studies confirm that only vestibular and not auditory neurons derive from Tlx3-positive progenitors (JMA and LVG, unpublished observation). Although Tlx3 is required for selection of glutamatergic fates in the spinal cord (Cheng et al., 2004, 2005), a specific role for Tlx3 in the specification or differentiation of vestibular neurons has not been described.

With respect to the spiral ganglion, the transcription factor GATA3 has been associated with the auditory cell fate decision (Lawoko-Kerali et al., 2004). GATA3 is initially expressed broadly throughout the otic vesicle, followed by progressive restriction to spiral ganglion neurons (Karis et al., 2001). In addition, *GATA3* expression parallels the apparent spatial segregation of auditory progenitors even before delamination, both in mice (Lawoko-Kerali et al., 2004) and chicks (Jones and Warchol, 2009). Moreover, while loss of *GATA3* causes morphogenetic defects in the ear, neurons are still present, ruling out a more general role in early neurogenesis (Karis et al., 2001). It is possible that the neurons that remain in *GATA3* mouse mutants belong to the vestibular ganglion, but due to the absence of cell type specific markers, their identity is an open question. In addition, while GATA3 marks auditory neurons in both mice and chicks, important differences in the expression patterns also exist, suggesting that the role of GATA3 may be more complex than originally thought (Jones and Warchol, 2009). Hence, the role for GATA3 in the initial segregation of spiral and vestibular ganglion neurons remains to be confirmed.

What factors act upstream to restrict GATA3 to the "auditory" division and Tlx3 to the "vestibular" division of the proneurosensory domain? One exciting candidate is Lmx1a. In addition to its role in development of the non-sensory tissues of the inner ear, *Lmx1a* expression extends into the *GATA3*-positive region of the proneurosensory domain (Fig. 2C). Moreover, loss of *Lmx1a* leads to an expanded production of Tlx3-positive vestibular ganglion neurons, but has no effect on spiral ganglion neurons (Koo et al., 2009). How and why Lmx1a divides the auditory and vestibular populations is particularly interesting from the evolutionary point of view, as the cochlea has been proposed to arise as an outgrowth of the saccule (Fritzsch et al., 2002). Intriguingly, in zebrafish, which use the saccule for hearing, Sonic hedgehog signaling is required for development of the neurons that innervate the saccule but not the utricle, suggesting one possible mechanism for subdividing the ganglion prior to the evolution of the cochlea (Sapede and Pujades, 2010). Hence, the genes that create the auditory neurogenic domain during development may be the same genes that permitted evolution of a specialized auditory organ.

4. Peripheral innervation of cochlear hair cells

After spiral ganglion neurons are specified, the next step is the development of tonotopically wired connections in the cochlea. As with other neuronal populations, auditory processes navigate towards their final targets by using a large growth cone to sample the environment *en route* (Fekete and Campero, 2007). In the cochlea, this journey begins when each auditory neuron becomes bipolar and extends a single peripheral process across the nascent spiral lamina and into the developing sensory epithelium. As they cross the spiral lamina, processes from multiple neurons come together to form radial bundles. Within the sensory epithelium, Type I neurons stop growing and make contact with a single IHC, while Type II neurons grow further into the sensory epithelium and turn to spiral towards the base, contacting multiple OHCs. Recent studies indicate that these axon guidance events begin independent of hair cells, and rely on a familiar set of guidance cues and survival molecules produced by non-sensory cells along the pathway.

4.1. Extending processes into the cochlear duct

While hair cells serve as the final target for growing auditory fibers, many other cell populations are present and may govern the earliest guidance events. Indeed, the initial stages of auditory circuit assembly begin in the cochlea even before the appearance of differentiated hair cells. Shortly after undergoing their final cell divisions, spiral ganglion neurons become bipolar and direct peripheral neurites towards the developing cochlear epithelium (Farinas et al., 2001). This is a prolonged process, so at E12.5 in the mouse many spiral ganglion neurons extend branched peripheral neurites, but others have not yet developed any projections (Koundakjian et al., 2007). At this age, few projections have penetrated the cochlear epithelium, and the processes are largely located in the mesenchyme that separates the ganglion proper from the developing sensory epithelium (Fig. 3, neuron 1) (Radde-Gallwitz et al., 2004). Immature morphologies are also observed at E13.5, with more and more neurons projecting neurites towards the developing sensory epithelium, but no evidence for radial bundles (JMA and LVG, unpublished observations). These projections appear to be among the pseudostratified supporting cells in Kolliker's organ and not yet in the organ of Corti (see, for example, TuJ labeled fibers in Kim et al., 2001). Hence, during the earliest stages of outgrowth, spiral ganglion neurites may interact largely with cells in Kolliker's organ rather than with their ultimate post-synaptic partners, the hair cells.

The final push into the developing sensory epithelium of the cochlea occurs as hair cells begin to differentiate. The sensory epithelium begins as an undifferentiated patch of epithelial cells with no clear cellular organization. The process of hair cell differentiation is characterized by the migration of the cell body towards the surface of the sensory epithelium, a pronounced rounding of the apical surface of the cell, and the expression of Myosin VI and then Myosin VII (Doetzlhofer et al., 2004; Kelley, 2007; Xiang et al., 2003). This process begins at E14.5 in the mouse, starting in the base and progressing towards the apex, with IHCs developing before OHCs. At the same time, spiral ganglion projections in the base begin to organize into radial bundles, made up of single unbranched peripheral projections from spiral ganglion neurons (Fig. 3, neurons 2 and 3) (Bruce et al., 1997; Koundakjian et al., 2007). Individual neurons in the apical region of the cochlea still exhibit an immature branched morphology at this age. By E15.5, spiral ganglion neurites have robustly invaded the developing sensory epithelium, which now houses many differentiating hair cells (Fig. 3, neuron 4) (apparent in TuJ stained sections published in Deng et al., 2006). However, the localization of neurites to the non-proliferating prosensory domain is more pronounced in the mid-basal turn of the cochlea than in the apical turn (Murata et al., 2006). This could indicate that neurons transition from a period of exploration, with branched neurites widely distributed in the cochlear epithelium (Fig. 3, neuron 1), to a phase of directed growth, with single unbranched neurites localized to the prosensory domain of the cochlear epithelium (Fig. 3, neuron 5). This hypothesis remains untested, as the position of neurites in the cochlear epithelium relative to molecularly identified developing cell types in the sensory epithelium has not been rigorously examined along the length of the cochlea.

Several lines of evidence suggest that differentiated hair cells are not required for spiral ganglion neurites to target the sensory epithelium of the cochlea. First, peripheral processes extend into the cochlear epithelium before any hair cells have differentiated. Moreover, in cochleae lacking any committed hair cell precursors due to the loss of *Atoh1*, the peripheral processes are still able to organize into radial bundles that terminate near where hair cells are normally located (Fritzsch et al., 2005). Similarly, spiral

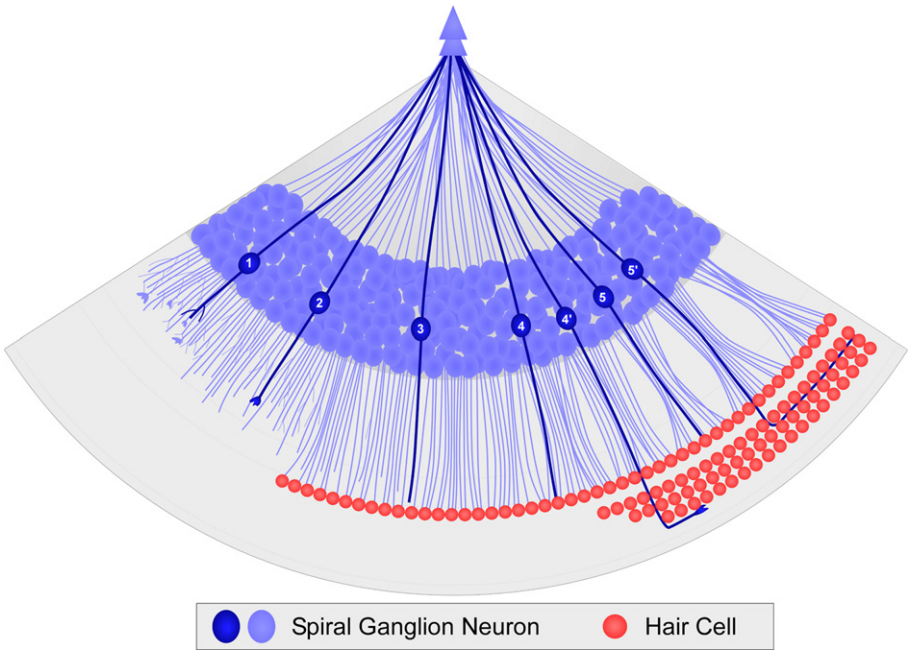

Fig. 3. The development of peripheral processes in the cochlea. Diagram of the cellular events that occur as spiral ganglion neurons (blue) develop in the cochlea, with immature cells shown on the left (1) and progressively maturing towards the right (5). Soon after becoming post-mitotic (1), the neurons become bipolar, with a central process extending into the eighth nerve and a peripheral process exploring the spiral lamina. Subsequently, neurons develop unbranched processes (2) that grow towards the edge of the organ of Corti, even before hair cells (red) have differentiated. Processes appear to wait while hair cell differentiation continues (3). Subsequently, Type I neurons (4) terminate beneath IHCs while Type II neurons (4′) develop large growth cones that navigate past the IHCs and into the rows of OHCs, where they turn towards the base, resulting in a mature pattern of innervation by birth (5, 5′).

ganglion neurons in cochleae with functionally immature hair cells still robustly innervate the sensory epithelium (Xiang et al., 1998, 2003). However, these spiral ganglion neurons progressively degenerate in the postnatal cochlea, possibly due to a lack of synaptic stimulation. Thus, while spiral ganglion-hair cell interactions are clearly required for later auditory circuit assembly events, differentiated hair cells do not appear to be necessary for the initial extension of processes towards the sensory epithelium. Nevertheless, it remains to be examined whether spiral ganglion neurons would still innervate a cochlear epithelium completely devoid of sensory epithelium. One hint that cells outside the sensory epithelium may be involved in the initial peripheral process extension comes from the vestibular system. Vestibular ganglion neurons are capable of initially sending the correct complement of projections into the developing inner ear despite the absence of one of the sensory epithelia (Pauley et al., 2003). Such guidance information might come from surrounding tissues, such as Kolliker's organ, the mesenchyme, or the glia. Indeed, several guidance molecules are expressed in non-sensory tissues of the ear, including the repulsive Slit and Ephrin ligands (Battisti and Fekete, 2008; Bianchi and Liu, 1999; Pickles et al., 2002), which may cordon stray neurites, as well as extracellular matrix components that may provide permissive substrates for axons as they pass through the spiral lamina (Davies, 2007; Rodgers et al., 2001; Whitlon et al., 1999).

Although the specific nature of cell–cell interactions during wiring of the cochlea is not known, these early guidance decisions are likely mediated by the same cues that set up connections in the rest of the nervous system. It has been suggested that the earliest auditory neurons send projections that essentially re-trace their steps back to their site of delamination from the otic vesicle (Bruce et al., 1997; Carney and Silver, 1983). However, while pioneering projections clearly exist and follow intriguing trajectories, active guidance mechanisms are also employed. Indeed, there is abundant evidence that auditory projections respond to cues in their environment. For instance, explanted rat spiral ganglia exhibit directed outgrowth towards ectopic patches of hair cells *in vitro* (Zheng and Gao, 2000) and endogenous chicken ganglion neurons can send projections towards ectopic sensory patches *in vivo* (Stevens et al., 2003). Moreover, experiments in the chicken reveal that the otocyst produces a secreted factor that encourages outgrowth from embryonic auditory ganglia *in vitro* (Hemond and Morest, 1992). The monocyte chemotactic protein 1, which is a chemokine, has been implicated as a component of this early acting signal (Bianchi et al., 2005). Work over the past two decades has revealed a host of molecules that provide instructions for wiring the nervous system, including traditional chemoattractants such as Netrin1 (Ntn1) and repellants such as Slits, Semaphorins and Ephrins (Dickson, 2002). As expected, many of these molecules can be detected in the bird and/or mammalian inner ear, including Semaphorins and their receptors (Chilton and Guthrie, 2003; Miyazaki et al., 1999; Murakami et al., 2001), Ephrins and the Eph receptors (Bianchi and Liu, 1999; Pickles et al., 2002), Ntn1 and its receptors (Abraira et al., 2008; Matilainen et al., 2007), Slits and their receptors (Battisti and Fekete, 2008), and Wnts and their receptors (Shah et al., 2009; Sienknecht and Fekete, 2008, 2009). Moreover, cultured neurons are clearly responsive to classic guidance cues *in vitro*, such as Ntn1 (Lee and Warchol, 2008) and EphrinB1 (Bianchi and Gray, 2002).

Despite intensive efforts to dissect guidance mechanisms in the inner ear, the specific functions for any one guidance molecule remain unclear. Perhaps the most effort has been directed towards neurotrophins such as BDNF and NT3. However, while there is good evidence that BDNF and NT3 are required for normal innervation of the cochlea, the neurotrophins and their receptors are broadly expressed in the developing inner ear, making it difficult to distinguish effects on overall outgrowth and survival vs effects on guidance (reviewed by Fritzsch et al., 2004). Similarly, FGFs can stimulate outgrowth from auditory neurons *in vitro*, but it is not known whether this represents a direct effect on the growth cone or an indirect effect on expression of other guidance receptors (Aletsee et al., 2003; Dazert et al., 1998; Hossain and Morest, 2000). Initial

studies suggest that new phenotypes remain to be discovered, since Semaphorin signaling is clearly involved in the innervation of the vestibular system but has not yet been investigated in the auditory system (Gu et al., 2003). Similarly, although *Ntn1* has been shown to be required for normal canal morphogenesis, the innervation of the cochlea has not been examined in *Ntn1* mutant mice (Salminen et al., 2000). Together with the fact that Ephrin signaling also has non-neuronal effects in the ear (Cowan et al., 2000), the *Ntn1* phenotype highlights the further challenge of distinguishing effects on morphogenesis from effects on guidance *per se*. Hence, the field awaits more detailed analysis of cochlear innervation in relevant mouse mutants, including tissue-specific mutants generated using Cre-lox technology.

4.2. Type I and Type II ganglion neuron differentiation

The *en masse* outgrowth of neuronal projections into the nascent organ of Corti occurs from the base to apex along the length of the cochlea, such that by the end of embryogenesis, radial fibers are present throughout the cochlea, and neurites are present under hair cells in the cochlear epithelium (Bruce et al., 1997). At this time in the mouse, rat and cat, both Type I and Type II spiral ganglion endings can be identified along the length of the cochlea (Bruce et al., 1997; Koundakjian et al., 2007; Perkins and Morest, 1975). How these two distinct types of connections are established is unknown, with contradictory observations from different species and in different regions of the cochlea fueling the confusion.

Type I and Type II spiral ganglion neurons have been proposed to develop through a process of overexuberant outgrowth followed by pruning, such that Type I neurons ultimately contact only IHCs and Type II neurons only contact OHCs (Echteler, 1992). In this model, the sensory epithelium is hypothesized to be a source of cues responsible for the selective retention of neurites contacting OHCs, resulting in Type I and Type II neurons. In support of this idea, mice lacking all OHCs do not appear to have morphologically identifiable Type II spiral ganglion projections (Fritzsch et al., 2005). Additionally, presumptive Type I spiral ganglion neurons have been observed contacting OHCs in gerbils, hamsters, and mice (Bruce et al., 1997; Echteler, 1992; Huang et al., 2007; Simmons, 1994; Wiechers et al., 1999). Moreover, individual Type II spiral ganglion neurons contacting both IHCs and OHCs have been observed occasionally in cats (<2% of Type II neurons labeled) (Ginzberg and Morest, 1983; Perkins and Morest, 1975) and gerbils (one Type II neuron at P6) (Echteler, 1992). This evidence has supported the suggestion that all neurons initially make contact with both IHCs and OHCs, with no neurons fitting this description observed in the mature cochlea (Berglund and Ryugo, 1987; Ginzberg and Morest, 1984). Since intermediate morphologies are only seen transiently, the loss of projections is proposed to occur as a result of activity-dependent remodeling, similar to circuit refining mechanisms seen in other regions of the nervous system (Guido, 2008; Kandler et al., 2009).

Other observations suggest that the prevailing model should be adjusted. Specifically, there is new evidence that Type II spiral ganglion neurons are already present at embryonic stages in the mouse. By E16.5, multiple rows of outer hair cells are evident in the sensory epithelium in the base of the cochlea. At the same time, a small number of spiral ganglion neurites in the base of the cochlea extend past the row of IHCs and turn towards the base, traveling under the rows of OHCs (Fig. 3, neuron 4′) (Bruce et al., 1997; Koundakjian et al., 2007). Moreover, individual Type II neurons can be seen extending past the row of IHCs without making enlarged endings onto IHCs (Koundakjian et al., 2007). This is similar to a small population of neurites in the apex of the gerbil cochlea that appear to exclusively contact OHCs, even before any neurite is observed turning towards the base of the cochlea (Echteler, 1992). Hence, most Type I and Type II spiral ganglion neurons may in fact be specified early in development and extend their projections directly towards the appropriate region of the developing sensory epithelium.

Independent evidence indicates that Type II spiral ganglion neurons are intrinsically different from Type I neurons, even at early stages of development. For example, during the first few days of postnatal development in the rat, expression of peripherin, which marks Type II neurons in the adult (Hafidi, 1998), is gradually restricted from all spiral ganglion neurons to a subset of spiral ganglion neurons similar to the proportion of Type II neurons found in the adult (Lallemend et al., 2007). Strong peripherin labeling in a subset of spiral ganglion neurons is seen at E18.5 and in the early postnatal mouse as well (Huang et al., 2007). These early postnatal peripherin positive cells do not take up tetramethylrhodamine-conjugated dextran (TMRD), a dye-labeling technique that favors the labeling of Type I neurons from P6 to P12. This indicates that by the end of embryogenesis in the mouse, the populations of Type I and Type II neurons are already molecularly and morphologically distinct; earlier stages have not been examined using this technique. Aside from their projection patterns, these two kinds of neurons exhibit additional intrinsic differences, since Type I neurons have larger cell bodies and thicker, myelinated processes, while Type II neurons are smaller, have a distinct cytoplasmic appearance, and extend thinner, unmyelinated processes. It has not been systematically examined when or how these intrinsic differences arise.

The apparently conflicting models for Type II spiral ganglion neuron specification may have arisen from the technical challenges of studying auditory circuit assembly. The various studies differ first and foremost in how each group labeled and then defined these two neuronal populations. Many studies use bulk dye labeling or antibody labeling to identify "Type I" and "Type II" projections. Since other axonal projections are also present in the cochlea, including axons from efferent neurons and sympathetic ganglion neurons, one possibility is that the perceived contact with both IHC and OHC is confounded by labeling of other neural populations (Bruce et al., 1997; Maklad and Fritzsch, 2003; Maklad et al., 2001; Rontal and Echteler, 2003; Shibamori et al., 1994). For instance, the TMRD-labeled projections described in the neonatal cochlea may not correspond to the mature TMRD-labeled Type I neurons, as no method is available to independently confirm the identity of the early population of neurons (Huang et al., 2007). Therefore, it is possible that the processes growing under OHCs may be olivocochlear projections, which begin to innervate OHCs at the end of embryogenesis (Bruce et al., 1997; Lallemend et al., 2007). Additionally, peripherin is initially expressed in all spiral ganglion neurons, and even expressed weakly in Type I neurons in the adult cochlea (Wang et al., 2006). Therefore, the origin of peripherin positive projections in the sensory epithelium cannot be unambiguously assigned to Type II neurons in early postnatal animals. In contrast, evidence for direct targeting of IHCs and OHCs came from individually labeled, genetically defined neurons (Koundakjian et al., 2007). In this case, Cre-lox technology was used to label early *Ngn1*-positive progenitors and follow the long-term fate of this specific cell population, which includes all of the neurons present in the ear. In addition, Type II neurons were further defined as neurons with a cell body in the spiral ganglion and a peripheral projection that turns towards the base and grows underneath developing OHCs. These criteria ensured that the analysis would not be confused by the presence of other labeled axons coursing through the eighth nerve, regardless of whether those other neurons are also *Ngn1* descendents. However, it remains possible that the GPI-linked enzyme used to label projections does not traffic efficiently to all processes, so the

presence of additional projections to OHCs cannot be ruled out with certainty. Nevertheless, the fact that processes with obvious Type II morphology appeared with the same frequency as in adult animals argues that Type I and Type II neurons are specified by this stage.

Technical issues have also forced most researchers to restrict their studies to the apex of the cochlea, which is the easiest region of the cochlea to access, dissect and mount at postnatal stages. Evidence for early direct targeting of OHCs by Type II neurons came from the mid to base regions of the cochlea and not from the apex, which remained immature with no signs of differentiated hair cells at the stages examined (Koundakjian et al., 2007). Studies in the gerbil indicate that the projections of Type I and Type II neurons in the most apical 10% of the cochlea are not completely segregated until P6; however, Type I and Type II neurons in the upper middle turn of the cochlea (25–37%, measuring from the apex) can be robustly identified at birth, and are morphologically similar to apical neurons at P6 (Echteler, 1992; Rontal and Echteler, 2003). While it is possible that development in the most apical section of the cochlea is significantly delayed in the gerbil relative to the mouse, it is more likely that these neurons are inherently different. For example, neurons in this region of the gerbil cochlea detect sound frequencies nearly an order of magnitude lower than any detected by the mouse (Meyer et al., 2009). Similarly, neurons in the most apical 10% and most basal 20% of the cat cochlea do not exhibit the characteristic distribution of SR seen throughout the rest of the cochlea (Liberman, 1978). Finally, neurons with a prominent transient projection contacting both IHCs and OHCs might make up a small population of spiral ganglion neurons that are more prevalent in the most apical section of the cochlea. This is supported by the observation of a potential third type of spiral ganglion process, the "giant fiber," which innervates hair cells only in the most apical region of the cat cochlea (Perkins and Morest, 1975).

Another major factor that may explain the differing observations is that few researchers have examined embryonic stages of development. Indeed, the two reports of spiral ganglion axon morphologies before birth both report the presence of projections with clear Type II morphology (Bruce et al., 1997; Koundakjian et al., 2007). Thus, pruning may be more prominent at postnatal stages, which have not been examined using a genetic labeling technique. If this is the case, then the remodeling process may be associated with target selection and synaptogenesis rather than specification. Most likely, both ideas are partially correct. For example, it seems likely that Type I and Type II spiral ganglion neurons are indeed specified early in development, but that some refinement of projections occurs either in a subset of neurons or during later stages of circuit assembly. Final resolution of these models awaits the discovery of reliable cell-type specific markers as well as more detailed examination of earlier and later stages using multiple methods. It will be of particular interest to identify early-acting transcription factors that define the Type I vs. Type II fate. One possible player is Prox1, since Prox1 is present in neurons prior to the expression of peripherin and has been proposed to influence the wiring of Type II fibers (Fritzsch et al., 2010a).

4.3. Developing and refining synaptic connections

A key milestone in the development of any neural circuit is the establishment of synapses. At this stage, the physical meshwork of connections is transformed into an active, functional network that can respond to and transmit electrical signals. As in other systems, this transition occurs in the auditory system before the animal has any direct experience of the world, but nevertheless relies on the use of activity-dependent mechanisms to ensure that the circuit is properly wired.

Although the precise onset of synaptogenesis has not been defined, it is clear that synaptic contacts between spiral ganglion neurons and hair cells are present prior to the onset of hearing. In mice, morphological synapses are present in the cochlea at birth, consisting of a ribbon structure in an IHC apposed to a postsynaptic density in a spiral ganglion neuron process (Shnerson et al., 1981; Sobkowicz et al., 1982). Similarly, in newborn rats, activation of hair cells triggers action potentials in all spiral ganglion neurons examined in the apex, the most immature region of the cochlea (Tritsch and Bergles, 2010). This evidence suggests that spiral ganglion neurons establish contact with hair cells during embryonic stages. However, the electrophysiological properties of synapses between hair cells and spiral ganglion neurons, including the amplitude and speed of excitatory postsynaptic currents and the proportion of monophasic and multiphasic events, mature after the onset of hearing, around P12 in the mouse and rat (Grant et al., 2010).

The initial formation of synapses between hair cells and spiral ganglion neurons appears to be largely activity-independent. For instance, mice lacking components of the synaptic exocytosis machinery in IHCs exhibit a loss of most synaptically driven activity in spiral ganglion neurons (Brandt et al., 2003; Roux et al., 2006). Nevertheless, mice with deficient IHC exocytosis still form appropriate numbers of ribbon synapses apposed to projections from spiral ganglion neurons, presumably forming functional synapses (Nemzou et al., 2006; Roux et al., 2006). Spiral ganglion neurons also do not require a large amount of synaptic activity to survive initially in this mutant background, as neurons do not degenerate until after the onset of hearing, and this progressive degeneration occurs over weeks to months. Therefore, minimal synaptic activation from IHCs is sufficient to support the early formation of synapses and survival of many spiral ganglion neurons.

Nevertheless, synaptic transmission clearly plays an important role in the refinement of auditory networks, even in pre-hearing animals. As in other systems, the formation of specifically matched connections between hair cells and spiral ganglion neurons involves pruning of both synapses and processes. Some Type I spiral ganglion neurons extend projections to a few neighboring hair cells during early postnatal development (Echteler, 1992; Perkins and Morest, 1975). These projections are modified within the first week of life to result in the critical one-to-one relationship between Type I spiral ganglion neurons and IHCs. The extent of pruning is still an open question, and could range from local rearrangement of connections to a handful of IHCs to widespread pruning of projections that contact both IHCs and OHCs (Huang et al., 2007).

One possibility is that activity influences both pruning and neuronal survival. Indeed, although connections can form in some synaptic transmission mutants, independent studies suggest that activity is indeed required for survival. For instance, during early postnatal development in the gerbil cochlea, there is a modest increase in spiral ganglion neuron death (Echteler et al., 2005) that coincides with the refinement of projections, suggesting neurons that fail to make proper connections subsequently undergo programmed cell death. In addition, ongoing synaptic transmission appears to promote the long-term survival of spiral ganglion neurons. Hair cells lacking the vesicular glutamate transporter 3 are unable to stimulate spiral ganglion neurons synaptically (Seal et al., 2008). In these mutant animals, spiral ganglion neurons begin to degenerate throughout the length of the cochlea prior to the onset of hearing, such that fewer than half of the neurons remain by the third postnatal week. The mechanisms regulating spiral ganglion synaptic formation and refinement are largely unknown, and will be an exciting area of future investigation.

Neurotrophins offer obvious candidates to modulate neuronal survival during these stages of auditory circuit assembly, and

indeed spiral ganglion neurons are lost from *TrkB*, *TrkC*, and *NT3* mutants (Fritzsch et al., 1998; Silos-Santiago et al., 1997). Although a clear link to neuronal activity has not been described, there is the growing evidence that neurotrophins can modulate the nature of synaptic transmission during postnatal stages of development (Davis, 2003). Neurons from the base of the cochlea only fire one or a few action potentials during a strong depolarization, while neurons from the apex of the cochlea fire many more in response to the same stimulus (Adamson et al., 2002). The application of BDNF to apical neurons decreases the number of action potentials fired, causing them to fire like basal neurons. Conversely, application of NT3 to basal neurons transforms their firing pattern to one characteristic of apical neurons. The expression of a number of voltage gated and calcium activated potassium channels changes in spiral ganglion neurons after the application of BDNF or NT3, usually resulting in an increase in expression with BDNF and a decrease with NT3. Similarly, presynaptic machinery is present at a higher level in apical spiral ganglion neurons than basal spiral ganglion neurons, and this can also be altered by treatment with neurotrophins (Flores-Otero et al., 2007). Hence, exposure to gradients of NT3 and BDNF in the postnatal cochlea may tune the complement of channels and hence the mature firing properties of auditory neurons. Whether such functions are related to activity-dependent pruning or neuronal survival is not known.

4.4. Modulation of peripheral innervation by non-sensory cells in the cochlea

Although mature synapses consist of contacts between one hair cell and one spiral ganglion neuron, spiral ganglion neurons interact with multiple populations of non-sensory cells even before hair cells are present. For instance, spiral ganglion neurons are closely associated with Schwann cells in the ganglion, pass by mesenchymal cells in the spiral lamina as well as epithelial cells in Kolliker's organ, and then contact supporting cells once they reach the sensory epithelium itself (Fig. 4). Recent evidence indicates that all three populations of cells may play a role in the development or maintenance of auditory circuits.

4.4.1. Schwann cells and supporting cells

Schwann cells are the glia that myelinate both the peripheral and central projections of spiral ganglion neurons. Schwann cells develop from the neural crest, a highly motile population of progenitors that arises at the boundary of the neural plate and the non-neural ectoderm early in embryogenesis (Jessen and Mirsky, 2005). Hence, Schwann cells are one of the only cell populations in the inner ear that does not originate in the otic placode. Schwann cells migrate into the otic vesicle and arrive at the CVG by E10.5 in the mouse (Davies, 2007). Supporting cells are placodally derived, but share many characteristics with the Schwann cells, including expression of "glial" markers such as PLP and GFAP (Morris et al., 2006; Rio et al., 2002). Thus, many manipulations that influence the development of spiral ganglion neurons target both Schwann cells and supporting cells, making it difficult to pinpoint the specific contributions of either population.

Schwann cells and supporting cells influence multiple aspects of spiral ganglion development, acting first to guide peripheral wiring and then to encourage neuronal survival. Both functions are best illustrated by studies of the Neuregulin signaling pathway in the cochlea. Neuregulin signaling through ErbB receptors influences many diverse developmental processes, including the neuron-glia interactions that underlie gliogenesis and myelination (Birchmeier and Nave, 2008). Cochleae lacking the ErbB2 receptor exhibit two obvious defects (Morris et al., 2006). First, the spiral ganglion cell bodies are displaced towards the modiolus of the cochlea, resulting in longer radial fibers. Second, the fibers

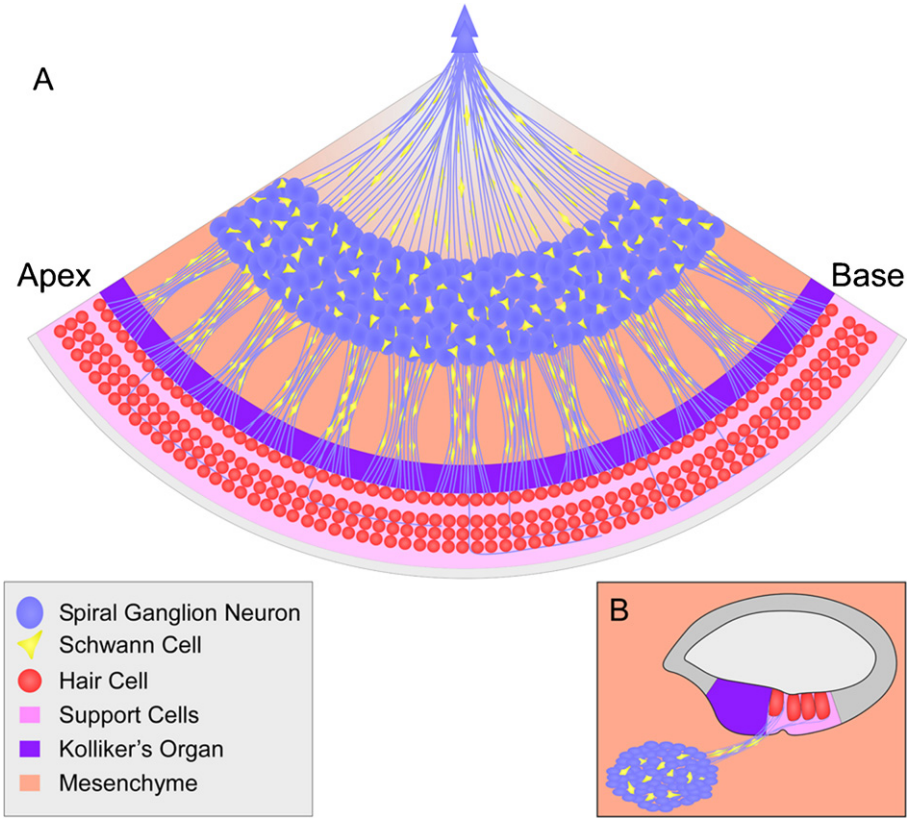

Fig. 4. The heterogeneous environment of the cochlea. A summary of the diverse cell types encountered by spiral ganglion neurons within the developing and mature cochlea, which is shown both as a flatmount (A) and in transverse section (B).

themselves are disorganized, forming a meshwork of projections rather than neatly organized radial bundles. The origin of this phenotype is unclear, as ErbB2 is required not only for proper gene expression in supporting cells but also for the production of Schwann cells, which are nearly completely absent by E16.5. Starting in the second postnatal week, disruption of ErbB signaling in Schwann cells and supporting cells causes a 60–80% loss of spiral ganglion neurons (Stankovic et al., 2004). Both manipulations of ErbB signaling in the cochlea result in a dramatic loss of *NT3* expression, indicating that a lack of trophic support could contribute to the defects observed.

Although the relative contributions of Schwann cells and supporting cells for spiral ganglion neuron development and survival have not been defined, several studies point to specific functions for these two cell populations. For instance, targeted disruption of FGF signaling in Schwann cells causes progressive hearing loss after 3 months of age (Wang et al., 2009). By 7 months of age, over 50% of spiral ganglion neurons have degenerated. On the other hand, disruption of FGF signaling in supporting cells and OHCs leads to a misorganization of fibers projecting under the OHC (Puligilla et al., 2007). With mounting evidence that multiple cell populations do in fact influence the final wiring of the cochlea, the exact roles for support cells in the sensory epithelium and Schwann cells in the cochlea clearly warrant further investigation.

4.4.2. Kolliker's organ

The epithelial cells of Kolliker's organ lie immediately adjacent to the developing sensory epithelium, thereby in close proximity to auditory projections as they pass by to reach the hair cells (Fig. 4B). Kolliker's organ cells remain poorly defined, but represent an intriguing and apparently plastic cell population, given their ability to generate hair cells or neurons upon introduction of *Atoh1* (Woods et al., 2004; Zheng and Gao, 2000) or *Ngn1* (Puligilla et al., 2010). Moreover, in mouse mutants with disrupted Hedgehog signaling, vestibular-like hair cells differentiate ectopically within Kolliker's organ (Driver et al., 2008). While these observations suggest that Kolliker's organ cells can serve as multipotent progenitors, almost nothing is known about their normal functions in the developing cochlea.

Recent studies suggest that Kolliker's organ cells play a previously unappreciated role in synaptic development. In the early postnatal rat cochlea, Kolliker's organ cells are electrically active and release ATP to stimulate IHCs to induce synaptic vesicle release onto terminals of spiral ganglion neurons (Tritsch et al., 2007). Because this only occurs in the immature cochlea and disappears around the onset of hearing, one hypothesis is that such activity regulates the refinement of tonotopic maps during pre-hearing stages. However, while ATP-gated ion channels, including $P2X_1$, $P2X_3$ and $P2X_7$ receptor, are robustly expressed by embryonic spiral ganglion neurons, expression is downregulated after birth (Huang et al., 2005, 2006; Nikolic et al., 2001, 2003). Additionally, in the postnatal rat, electrical activity in Kolliker's organ is highest at birth, with the frequency of activity decreasing with age (Tritsch and Bergles, 2010). These results suggest that electrical activity peaks embryonically, indicating additional functions.

The role of electrical activity in embryonic spiral ganglion neurons is entirely unknown. While direct stimulation with ATP does not cause postnatal spiral ganglion neurons to fire an action potential, it can depolarize these neurons (Tritsch et al., 2007). This depolarization could be directly involved in the guidance of spiral ganglion neurites. For example, the *in vitro* guidance of frog spinal cord neurons by diffusible chemoattractive and chemorepulsive signals is modulated by electrical activity in the neuron (Ming et al., 2001; Zheng and Poo, 2007). Similarly, in spiral ganglion neurons *in vitro*, activation of ATP-gated ion channels reduced neurite outgrowth in response to BDNF (Greenwood et al., 2007). ATP mediated electrical activity may act in a similar manner in spiral ganglion neurons *in vivo*. Experimental manipulations to silence Kolliker's organ may shed light on the interdependence between spontaneous activity, axon guidance, and synaptic refinement in spiral ganglion neurons.

4.4.3. Mesenchyme

The first cells that auditory axons encounter on their way to the sensory epithelium are the mesenchymal cells that will eventually comprise the spiral lamina (Fig. 4A and B). It is in this space that the radial bundles ultimately form, representing the first sign of organization in the peripheral wiring of the cochlea. Most studies of inner ear mesenchyme have focused on the development of the bony capsule of the inner ear (Braunstein et al., 2008; Liu et al., 2002, 2003), though *in vitro* assays suggest that the periotic mesenchyme also promotes hair cell differentiation (Doetzlhofer et al., 2004; Montcouquiol and Kelley, 2003). In contrast, little is known about any specific function for the nascent spiral lamina during auditory axon guidance. Visualization of developing spiral ganglion neurons shows branching and apparent exploration where the ganglion meets the spiral lamina (Koundakjian et al., 2007). Moreover, in the E14 mouse, the extracellular matrix molecule tenascin-C is expressed in stripes within the spiral lamina that presage the appearance of radial bundles, suggesting the presence of a permissive growth corridor here (Whitlon et al., 1999). Consistent with this idea, spiral ganglion processes respond differentially to laminin and fibronectin *in vitro* (Evans et al., 2007). One hint that the spiral lamina may also be instructive is the fact that the guidance molecule EphA4 is expressed here (Pickles et al., 2002). However, Ephrin-Eph signaling also has important functions in non-neuronal tissues in the ear (Cowan et al., 2000), so the presence of EphA4 may not be directly relevant for axon guidance. The role of the spiral lamina during cochlear wiring represents an area that will require more detailed exploration in the future.

5. Central innervation of the cochlear nucleus

Since the function of spiral ganglion neurons is to transmit information from the cochlea to the brain, wiring of the periphery must be perfectly correlated with wiring of the central nervous system. To make appropriate central connections, each spiral ganglion neuron sends its central projection towards the hindbrain, where it bifurcates to form two branches, one that innervates the dorsal division of the cochlear nucleus and one that innervates the ventral division. Within each division, each branch independently seeks tonotopically appropriate post-synaptic targets and elaborates synapses appropriate to that target, forming the large endbulbs of Held in the ventral cochlear nucleus (VCN) and boutons in the dorsal cochlear nucleus (DCN). Although each of these stages has been documented at the cellular level, our knowledge of the underlying mechanisms remains rudimentary.

5.1. Projecting from the ear to the brain

Development of central projections occurs early and as such, is unlikely to depend on proper peripheral innervation. The central projections of spiral ganglion neurons first reach the hindbrain around E11.5 in the mouse, a time when new spiral ganglion neurons are still being born and before peripheral projections have grown beyond the boundaries of the ganglion. At this stage, the central projections are loosely gathered at a single entry point at the level of rhombomeres 4/5 (C. Lu and LVG, unpublished observations). One day later, the axons have branched and are growing towards the presumptive DCN and VCN (Karis et al., 2001). At this stage, DCN is caudal to VCN; the developing hindbrain flexes slightly later in development, bringing DCN to rest

on top of VCN. Hence, spiral ganglion neurons bifurcate in the same basic place as dorsal root ganglion neurons, which send ascending and descending projections along the spinal cord. In dorsal root ganglion neurons, proper bifurcation relies on Ntn/Slit signaling (Ma and Tessier-Lavigne, 2007) as well as a Protein Kinase G pathway (Schmidt et al., 2002, 2007, 2009; Zhao et al., 2009). Whether or not spiral ganglion neurons respond to similar cues as they bifurcate has not yet been examined. Studies in the rat suggest that neurons project into the cochlear nucleus in a basal to apical progression (Angulo et al., 1990), parallel to the gradient of development in the cochlea. In the mouse, the precise location of the neurons branching in the hindbrain at E12.5 is unclear; however, these early branching projections are unlikely to belong to apical neurons, which are just exiting the cell cycle at this age. While it is possible for spiral ganglion neurons to project into the hindbrain prior to delamination (Bruce et al., 1997), it is unclear how frequently this occurs. Projections from neurons at all positions along the cochlea are present in the cochlear nucleus by E15.5 (Koundakjian et al., 2007).

The precise order in which spiral ganglion axons and cochlear nucleus neurons meet in the hindbrain is not known. Both populations of neurons are born during mid-embryogenesis, largely between E10.5 and E15.5 in the mouse (Martin and Rickets, 1981; Matei et al., 2005; Ruben, 1967). However, whether spiral ganglion axons guide cochlear nucleus neurons or *vice versa* is still an open question. Development of the eighth nerve *per se* can occur even when the cochlear nucleus is severely reduced. In conditional *Atoh1* knock out animals, the majority of neurons in the VCN are never formed. Despite the absence of most of their central target neurons, spiral ganglion neurons still project into the hindbrain and bifurcate (Maricich et al., 2009). Therefore, the presence of target neurons does not appear to be required for the initial extension of projections towards the area that the VCN occupies. Nevertheless, projections reaching this area do appear to be disorganized in P0 animals, likely due to the missing target neurons. While this study shows that auditory axons can target a hindbrain largely lacking target neurons, the inhibitory target neurons in the cochlear nucleus are presumably intact. To date, no published study has examined the targeting of spiral ganglion neurons into a hindbrain completely devoid of cochlear nucleus neurons, but the work by Maricich and colleagues suggests that the initial projection should be present. Nothing is known about the cues that guide auditory axons to a specific level of the hindbrain, nor how auditory axons are kept separate from vestibular axons within the eighth nerve. However, it is interesting to note that some guidance molecules, including Eph receptors, are restricted to auditory or vestibular projections and may play a role in the initial formation of connections between the ear and the brain (Siddiqui and Cramer, 2005). In addition, *NeuroD1* conditional mutant mice exhibit mixing of auditory and vestibular projections within the eighth nerve, as well as growth into inappropriate target areas (Jahan et al., 2010a). Identification of genes downstream of NeuroD1 could help to elucidate this aspect of auditory system wiring.

5.2. Development of tonotopic maps

The key organizing principle of auditory maps is tonotopy, i.e. the smooth organization of connections from low to high frequencies within each division of the auditory pathway, beginning at the cochlear nucleus. Although many of the specifics remain to be defined, the development of tonotopic maps adheres to many of the same rules that govern development of topographic maps in other sensory systems.

Tonotopic maps emerge early and with remarkable precision. For example, as early as E15.5 in the mouse, labeled projections from apical and basal spiral ganglion neurons are segregated within the developing cochlear nucleus, with no evidence for gross overshooting of apical projections into "basal" territory (Koundakjian et al., 2007). Auditory projections are similarly well organized prior to the onset of hearing in the chick embryo (Molea and Rubel, 2003). Moreover, basic tonotopic organization is preserved within both branches of the eighth nerve even when the VCN is essentially absent, suggesting that this order develops independent of interactions between axons and their target (Maricich et al., 2009). This contrasts with the visual system, where retinal ganglion neurons initially project widely into the tectum, and then later refine their arbors by over 90% to result in finely organized visual maps (Simon and O'Leary, 1992). While auditory projections do not exhibit a large-scale reorganization of projections in the hindbrain, some refinement in tonotopic organization is likely to occur. The precision of the earliest tonotopic maps has not been assessed (Koundakjian et al., 2007; Molea and Rubel, 2003). However, in kittens, the width of spiral ganglion innervation in the cochlear nucleus is reported to decrease by 1/4 to 1/3 during the early postnatal period (Leake et al., 2002). While the kitten projections occupy a proportionally larger region of the cochlear nucleus than adult projections, the cross-sectional area of the cochlear nucleus more than triples over this same period of time. Therefore, the refinement of projections observed might also reflect a different rate of projection expansion compared to cochlear nucleus expansion as the kitten matures. To date, the development of individual spiral ganglion neuron arbors has not been examined in the cochlear nucleus to define the true extent of refinement.

Axon–axon interactions offer one potential mechanism for creating and maintaining tonotopic order. In other populations of sensory neurons, interactions with neighboring neurons guide the final pattern of innervation both centrally and peripherally. For example, single peripheral sensory neurons transplanted into zebrafish expand peripheral projections over a limited region of the head. However, a normal neuron transplanted into a zebrafish lacking other peripheral sensory neurons will project over nearly the entire head, perhaps attempting to compensate for the lack of other innervation (Sagasti et al., 2005). Similarly, in the developing visual system, removing half of the retina results in an expansion of the projections of the remaining neurons to cover the entire target field in the brain (Goodhill and Richards, 1999). Finally, there is ample evidence that olfactory maps depend largely on axon–axon interactions, which segregate projections from sensory neurons expressing the same olfactory receptors (Sakano, 2010). In this case, molecular cues serve to coarsely guide axons towards the appropriate region of the olfactory bulb, but axons are well organized even before reaching the target.

Although the issue has not yet been directly addressed, axon–axon interactions may also shape the central projections of spiral ganglion neurons. As in the olfactory system, auditory axons are roughly topographically organized within the eighth nerve, though whether this is the result of active signaling or simply secondary to the basal-apical gradient of development is unclear. Consequently, placing dye crystals in two small regions of the base and middle of the cochlea of newborn mice results in two cleanly separated bands of projections in the VCN (Farinas et al., 2001). In *NT3* null cochleae, which contain roughly 15% as many spiral ganglion neurons, the same labeling procedure reveals diffuse, overlapping bands of projections in the VCN. This suggests that neurons have expanded their innervation to fill in for the neurons that are missing from the cochlea. However, the topography of projections into the cochlear nucleus prior to spiral ganglion neuron death was not examined. In addition, interpretation of this result is complicated by the presence of *NT3* in the embryonic cochlear nucleus (Hossain et al., 2006). Thus, it is unclear whether the

expansion of spiral ganglion projections is due to the loss of neighboring spiral ganglion neurons or the loss of guidance cues in the cochlear nucleus. While this result suggests an intriguing parallel to the development of other sensory systems, more experiments are required to determine the exact role of axon–axon interactions in shaping the tonotopy of auditory projections.

A small number of molecular cues have been examined for their role in guiding the tonotopic organization of spiral ganglion neuron projections into the cochlear nucleus. Not surprisingly, Ephrin signaling is strongly implicated (Cramer, 2005). For instance, Ephrin B2 is expressed in neurons of both the spiral ganglion and cochlear nucleus in early postnatal mice (Miko et al., 2008; Miko et al., 2007). The tonotopic organization of projections in adult mice can be inferred from the pattern of neural activation in the cochlear nucleus after prolonged exposure to an isofrequency tone. Exposure to an 8 kHz tone results in a narrow band of c-fos positive cells activated in the normal mouse DCN. However, adult mice lacking one copy of *Ephrin B2* have a wider band of activated cells (Miko et al., 2007), suggesting a change in the tonotopic map of projections into the cochlear nucleus. This could be a result either of an overexuberant innervation of the cochlear nucleus or the lack of postnatal refinement of otherwise normal projections. Furthermore, if there are fewer spiral ganglion neurons in the cochlea, there could be an expansion of individual neuronal projections to provide full coverage of the cochlear nucleus, similar to *NT3* null mice. Examining the individual arborization patterns of spiral ganglion neurons could elucidate the mechanisms underlying the EphrinB2-dependent degradation of the tonotopic map. In addition, Ephrin signaling may cooperate with other topographic signaling systems, such as Wnt ligands, which influence retinotopic mapping via interactions with the Ryk receptor (Schmitt et al., 2006). Consistent with this idea, Wnt ligands and their receptors are broadly expressed in the developing chick inner ear (Sienknecht and Fekete, 2008, 2009). Although it is often assumed that topographic maps rely on common developmental mechanisms, each sensory system does in fact have unique features that must be accommodated. A more detailed analysis of putative topographic signaling molecules in the inner ear may reveal whether fundamental differences exist in different sensory systems.

5.3. Development of the endbulb of Held

Upon reaching a tonotopically correct target neuron, auditory axons develop synapses that vary in morphology depending on the identity of the post-synaptic cell. Most contacts consist of standard bouton endings. In addition, spiral ganglion neurons elaborate unusually large synapses called endbulbs of Held, which are essential for preserving the temporal features of sound stimuli. Endbulbs of Held are calyceal endings that are found between Type I spiral ganglion neurons and spherical bushy cells located in the most anterior portion of the VCN (Fig. 1B′); smaller, modified endbulbs can also be seen contacting globular bushy cells (Ryugo and Parks, 2003). Endbulbs develop at the end of the primary ascending branch of Type I spiral ganglion neurons, which contact and envelop the bushy cell soma. Endbulbs are ~10 times larger than the other boutons and have a highly complex morphology, consisting of multiple branches that tangle about the cell body and form multiple synaptic contacts (Ryugo et al., 2006). The unusual size and shape of the endbulb of Held is thought to ensure rapid and secure synaptic transmission that is critical for detecting timing differences in auditory stimuli.

We still have only a basic understanding of how the endbulb of Held acquires its claw-like morphology. Anatomical studies in chicks, mice, and cats have revealed an evolutionarily conserved sequence of events, though synaptic development appears to occur entirely postnatally in mice but initiates prenatally in cats (Limb and Ryugo, 2000; Ryugo and Fekete, 1982; Ryugo et al., 2006; Ryugo and Parks, 2003). During the earliest stages, spiral ganglion axons contact bushy cells via a large, growth cone-like structure. The nascent endbulb is spoon-shaped and relatively small. However, electron microscopy studies reveal extensive ruffling of the spiral ganglion axon membrane, which interdigitates with similar fenestrations in the bushy cell soma. At intervals, the apposing membranes form thickened sites of contact, possibly reflecting interactions between adhesion molecules on the bushy cell and the growth cone of the auditory axon (Ryugo et al., 2006). Indeed, adhesion between pre- and post-synaptic targets represents an early stage of synaptogenesis for other types of neurons (Colon-Ramos, 2009). After the initial contacts have formed, the ending gradually swells to become 10–15 times larger, with a small number of filopodia. At the same time, the bushy cell membrane gradually loses its irregular contour and there are fewer regions where the two membranes are closely apposed. This takes place during the first 2 weeks of postnatal development in mice and is therefore prior to the onset of hearing (Limb and Ryugo, 2000). Subsequently, the endbulbs become highly branched and eventually expand to encompass nearly half of the bushy cell surface. Although these descriptive studies suggest a role for cell–cell interactions, no relevant molecules have been defined. One attractive idea is that bushy cells display a unique cell surface marker that stimulates the auditory ending to develop an endbulb rather than a bouton. How such a pathway is limited to the ascending branch and not the many smaller branches that ramify within AVCN is a puzzle.

Although nearly nothing is known about the molecular basis of endbulb development, a number of studies have highlighted the importance of synaptic activity. At the most basic level, endbulb morphology correlates with the normal level of activity experienced by the auditory axon. For instance, spiral ganglion neurons with high SR exhibit larger swellings with fewer branches and smaller post-synaptic densities compared to low SR neurons (Ryugo and Parks, 2003). Moreover, endbulbs in congenitally deaf cats and mice develop simpler arbors with fewer branches, larger post-synaptic densities, fewer synaptic vesicles (Lee et al., 2003) and smaller volume (Youssoufian et al., 2008). The abnormal endbulbs observed in deaf animals do not appear to reflect a general developmental arrest (Limb and Ryugo, 2000). Remarkably, endbulb morphology is restored to normal in deaf cats that have been fitted with a cochlear implant (Ryugo et al., 2005). This finding cements the link between activity and development of the complex morphology of the mature endbulb of Held.

6. Stem cells

Since many forms of sensorineural hearing loss are caused by the physical loss of hair cells or neurons, there is a concerted effort to engineer stem cells that are able to integrate into existing systems and therefore restore function. Researchers are making active progress towards this vision, aided in large part by recent progress in understanding the normal development of the inner ear (Martinez-Monedero et al., 2007).

Stem cells for the treatment of deafness will ultimately derive either from endogenous or exogenous sources. Mobilization of an existing stem cell population is certainly the most attractive possibility, as this would be less invasive and closer to the physiological situation. The challenge, however, is that such a population of stem cells may be small or non-existent in the cochlea. To date, stem cells have been identified in the utricle (Li et al., 2003) and in the neonatal cochlea (Oshima et al., 2007). Moreover, support cells purified from the early postnatal cochlea can differentiate as hair cells *in vitro* (Doetzlhofer et al., 2006; White et al., 2006). However, no stem cells have been identified in

the auditory epithelium or spiral ganglia after this early postnatal period. Since endogenous stem cells may be rare in the cochlea, considerable effort has been focused instead on other easily obtained and well-characterized progenitors. For instance, embryonic stem cells from mouse (Corrales et al., 2006; Matsumoto et al., 2005, 2008; Parker et al., 2007) and human (Shi et al., 2007) can be grown and expanded *in vitro* and then induced to differentiate under certain culture conditions. Moreover, utricular stem cells may offer a more inner ear-like source, and indeed can be forced to differentiate as neurons that share many features with spiral ganglion neurons, including the expression of GATA3, TrkB and TrkC (Martinez-Monedero et al., 2008). These neurons are also electrophysiologically active.

A closely related issue is that any potential source of stem cells must be able to produce the required neurons with some efficiency. In the auditory system, this means generating neurons with all of the unique and highly specialized features of the spiral ganglion, including the firing properties and specialized synapses that preserve the frequency, intensity and timing of a complex sound stimulus. This will require not only identifying the factors that guide stem cells towards an auditory phenotype, but also being able to recognize a spiral ganglion neuron at the end of the process. This highlights the importance of identifying the specific factors that induce the proneurosensory domain in the otic vesicle, as such molecules offer the best hope of recapitulating the same developmental events in a dish. Currently, our ability to distinguish a spiral ganglion neuron from any other type of sensory neuron is poor. For instance, although stem cell-derived neurons may express *TrkB* and *TrkC*, additional markers are needed to distinguish between TrkB/C-positive dorsal root ganglia neurons vs. those in the inner ear. Towards this end, a molecular catalog of developing and mature spiral ganglion neurons would offer a critical resource for the field.

Stem cell-derived spiral ganglion neurons will only prove useful therapeutically if they are also able to re-create auditory circuits even in the adult. Several promising results suggest that this might be possible. For instance, upon co-culture with the auditory epithelia, both mouse (Corrales et al., 2006; Matsumoto et al., 2008) and human embryonic stem cells (Shi et al., 2007) can differentiate as neurons that send processes to contact IHCs. Moreover, upon injection into the cochlea, these stem cells can become neurons and grow along the natural route through the spiral lamina to reach the organ of Corti. Unfortunately, there is no evidence for recovered function, possibly due to the presence of scar tissue (Corrales et al., 2006). In addition, although some synaptic markers are present, ribbon synapses were not detected in the *in vitro* experiments, nor is there any electrophysiological evidence for synaptic transmission between hair cells and stem cell-derived neurons. Combined with the relative inefficiency of converting stem cells to neurons *in vitro* and *in vivo*, it is clear that many aspects of stem cell technology remain to be optimized.

7. Outstanding questions in the field

The past 10 years have witnessed tremendous progress in auditory research, mostly made possible by the introduction of new molecular tools that lessen the challenges of studying the small and delicate inner ear. In many cases, discoveries have raised new questions and controversies. At the most basic level, recent findings underscore the usefulness of defining the exact timing and sequence of auditory circuit assembly, particularly at the single cell level. Additionally, the molecular mechanisms underlying individual stages of spiral ganglion neuron development require further investigation. For example, while we know the general molecular requirements for neurogenesis in the ear, it is still unclear which genes are required specifically for spiral vs. vestibular ganglion fate specification or Type I vs. Type II neuronal development. GATA3 is commonly put forward as a potential auditory fate determinant, but is in fact broadly expressed throughout the otic epithelium and required for many aspects of inner ear development. Therefore, while GATA3 may indeed be necessary for production of spiral ganglion neurons, it may not be sufficient to promote spiral ganglion fate on its own.

Our knowledge of axon guidance in the auditory system is particularly unsatisfactory, especially when compared to studies of other sensory systems. Spiral ganglion neurons extend projections towards the cochlea and the cochlear nucleus well before the maturation of either structure. Recent experiments indicate that target cells, specifically hair cells and excitatory neurons in the cochlear nucleus, may not be required for the initial guidance of spiral ganglion neurites. The source and nature of the molecular factors guiding spiral ganglion neurites into both the cochlea and the brainstem remain a mystery. Identification of additional guidance cues, together with more detailed analysis of available mouse mutants, should provide some clarity.

While studies of neurogenesis and axon guidance have lagged behind in the inner ear relative to other systems, the auditory system offers an excellent system for uncovering basic principles of synaptogenesis. Understanding how neurons select specific targets and initiate development of the synapse is an area of active research, with most advances coming from studies of the large and accessible neuromuscular junction (Wu et al., 2010). The auditory system provides several similar advantages for studying synaptogenesis in the central nervous system. First, the auditory system houses the largest and fastest synapses in the central nervous system. Additionally, spiral ganglion neurons make specialized contacts both peripherally, in the form of the ribbon synapse, and centrally, in the form of the endbulb of Held. Moreover, many methods are available for probing synaptic function in mutant animals, including electrophysiological recordings and auditory brainstem response recordings. Finally, in contrast to other regions of the central nervous system, the logic of synaptic connectivity in auditory circuits is well defined and relatively simple. For instance, most Type I spiral ganglion neuron elaborate a single endbulb of Held with one bushy cell. This may make it simpler to find the cell adhesion molecules hypothesized to mediate cell-type specific target selection. Although the large number of outstanding questions is daunting, the field stands well-prepared and poised to launch new investigations into the molecular and morphological processes that enable spiral ganglion neurons to perform their essential role in the sense of hearing.

Acknowledgements

We thank members of the laboratory and colleagues in the field for many illuminating discussions over the years, as well as Donna Fekete, Doris Wu, and Karina Cramer for their keen insights and useful comments on the manuscript. Funding for our work on auditory circuit assembly was provided by N.I.D.C.D. (R01DC009223), the March of Dimes, the Edward R. and Anne G. Lefler Center, the Hellman Family Research Fund, the John and Virginia Kaneb Fellows Fund, and a Quan Fellowship to JMA.

References

Abello, G., Alsina, B., 2007. Establishment of a proneural field in the inner ear. Int. J. Dev. Biol. 51, 483–493.

Abello, G., Khatri, S., Radosevic, M., Scotting, P.J., Giraldez, F., Alsina, B., 2010. Independent regulation of Sox3 and Lmx1b by FGF and BMP signaling influences the neurogenic and non-neurogenic domains in the chick otic placode. Dev. Biol. 339, 166–178.

Abraira, V.E., Del Rio, T., Tucker, A.F., Slonimsky, J., Keirnes, H.L., Goodrich, L.V., 2008. Cross-repressive interactions between Lrig3 and netrin 1 shape the architecture of the inner ear. Development 135, 4091–4099.

Adam, J., Myat, A., Le Roux, I., Eddison, M., Henrique, D., Ish-Horowicz, D., Lewis, J., 1998. Cell fate choices and the expression of Notch Delta and Serrate homologues in the chick inner ear: parallels with Drosophila sense-organ development. Development 125, 4645–4654.
Adamson, C.L., Reid, M.A., Davis, R.L., 2002. Opposite actions of brain-derived neurotrophic factor and neurotrophin-3 on firing features and ion channel composition of murine spiral ganglion neurons. J. Neurosci. 22, 1385–1396.
Aletsee, C., Brors, D., Mlynski, R., Ryan, A.F., Dazert, S., 2003. Branching of spiral ganglion neurites is induced by focal application of fibroblast growth factor-1. Laryngoscope 113, 791–796.
Alsina, B., Abello, G., Ulloa, E., Henrique, D., Pujades, C., Giraldez, F., 2004. FGF signaling is required for determination of otic neuroblasts in the chick embryo. Dev. Biol. 267, 119–134.
Alsina, B., Giraldez, F., Pujades, C., 2009. Patterning and cell fate in ear development. Int. J. Dev. Biol. 53, 1503–1513.
Angulo, A., Merchan, J.A., Merchan, M.A., 1990. Morphology of the rat cochlear primary afferents during prenatal development: a Cajal's reduced silver and rapid Golgi study. J. Anat. 168, 241–255.
Anniko, M., Wikstrom, S.O., 1984. Pattern formation of the otic placode and morphogenesis of the otocyst. Am. J. Otolaryngol. 5, 373–381.
Barald, K.F., Kelley, M.W., 2004. From placode to polarization: new tunes in inner ear development. Development 131, 4119–4130.
Battisti, A.C., Fekete, D.M., 2008. Slits and Robos in the developing chicken inner ear. Dev. Dyn. 237, 476–484.
Bell, D., Streit, A., Gorospe, I., Varela-Nieto, I., Alsina, B., Giraldez, F., 2008. Spatial and temporal segregation of auditory and vestibular neurons in the otic placode. Dev. Biol. 322, 109–120.
Berglund, A.M., Ryugo, D.K., 1987. Hair cell innervation by spiral ganglion neurons in the mouse. J. Comp. Neurol. 255, 560–570.
Bermingham, N.A., Hassan, B.A., Price, S.D., Vollrath, M.A., Ben-Arie, N., Eatock, R.A., Bellen, H.J., Lysakowski, A., Zoghbi, H.Y., 1999. Math1: an essential gene for the generation of inner ear hair cells. Science 284, 1837–1841.
Bertrand, N., Castro, D.S., Guillemot, F., 2002. Proneural genes and the specification of neural cell types. Nat. Rev. Neurosci. 3, 517–530.
Bianchi, L.M., Gray, N.A., 2002. EphB receptors influence growth of ephrin-B1-positive statoacoustic nerve fibers. Eur. J. Neurosci. 16, 1499–1506.
Bianchi, L.M., Liu, H., 1999. Comparison of ephrin-A ligand and EphA receptor distribution in the developing inner ear. Anat. Rec. 254, 127–134.
Bianchi, L.M., Daruwalla, Z., Roth, T.M., Attia, N.P., Lukacs, N.W., Richards, A.L., White, I.O., Allen, S.J., Barald, K.F., 2005. Immortalized mouse inner ear cell lines demonstrate a role for chemokines in promoting the growth of developing statoacoustic ganglion neurons. J. Assoc. Res. Otolaryngol. 6, 355–367.
Birchmeier, C., Nave, K.A., 2008. Neuregulin-1, a key axonal signal that drives Schwann cell growth and differentiation. Glia 56, 1491–1497.
Bok, J., Chang, W., Wu, D.K., 2007. Patterning and morphogenesis of the vertebrate inner ear. Int. J. Dev. Biol. 51, 521–533.
Brandt, A., Striessnig, J., Moser, T., 2003. CaV1.3 channels are essential for development and presynaptic activity of cochlear inner hair cells. J. Neurosci. 23, 10832–10840.
Braunstein, E.M., Crenshaw 3rd, E.B., Morrow, B.E., Adams, J.C., 2008. Cooperative function of Tbx1 and Brn4 in the periotic mesenchyme is necessary for cochlea formation. J. Assoc. Res. Otolaryngol. 9, 33–43.
Brooker, R., Hozumi, K., Lewis, J., 2006. Notch ligands with contrasting functions: Jagged1 and Delta1 in the mouse inner ear. Development 133, 1277–1286.
Brown, M.C., Berglund, A.M., Kiang, N.Y., Ryugo, D.K., 1988. Central trajectories of type II spiral ganglion neurons. J. Comp. Neurol. 278, 581–590.
Bruce, L.L., Kingsley, J., Nichols, D.H., Fritzsch, B., 1997. The development of vestibulocochlear efferents and cochlear afferents in mice. Int. J. Dev. Neurosci. 15, 671–692.
Carney, P.R., Silver, J., 1983. Studies on cell migration and axon guidance in the developing distal auditory system of the mouse. J. Comp. Neurol. 215, 359–369.
Cheng, L., Arata, A., Mizuguchi, R., Qian, Y., Karunaratne, A., Gray, P.A., Arata, S., Shirasawa, S., Bouchard, M., Luo, P., Chen, C.L., Busslinger, M., Goulding, M., Onimaru, H., Ma, Q., 2004. Tlx3 and Tlx1 are post-mitotic selector genes determining glutamatergic over GABAergic cell fates. Nat. Neurosci. 7, 510–517.
Cheng, L., Samad, O.A., Xu, Y., Mizuguchi, R., Luo, P., Shirasawa, S., Goulding, M., Ma, Q., 2005. Lbx1 and Tlx3 are opposing switches in determining GABAergic versus glutamatergic transmitter phenotypes. Nat. Neurosci. 8, 1510–1515.
Chilton, J.K., Guthrie, S., 2003. Cranial expression of class 3 secreted semaphorins and their neuropilin receptors. Dev. Dyn. 228, 726–733.
Cole, L.K., Le Roux, I., Nunes, F., Laufer, E., Lewis, J., Wu, D.K., 2000. Sensory organ generation in the chicken inner ear: contributions of bone morphogenetic protein 4, serrate1, and lunatic fringe. J. Comp. Neurol. 424, 509–520.
Colon-Ramos, D.A., 2009. Synapse formation in developing neural circuits. Curr. Top. Dev. Biol. 87, 53–79.
Corrales, C.E., Pan, L., Li, H., Liberman, M.C., Heller, S., Edge, A.S., 2006. Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of Corti. J. Neurobiol. 66, 1489–1500.
Cowan, C.A., Yokoyama, N., Bianchi, L.M., Henkemeyer, M., Fritzsch, B., 2000. EphB2 guides axons at the midline and is necessary for normal vestibular function. Neuron 26, 417–430.
Cramer, K.S., 2005. Eph proteins and the assembly of auditory circuits. Hear Res. 206, 42–51.
Dabdoub, A., Puligilla, C., Jones, J.M., Fritzsch, B., Cheah, K.S., Pevny, L.H., Kelley, M.W., 2008. Sox2 signaling in prosensory domain specification and subsequent hair cell differentiation in the developing cochlea. Proc. Natl. Acad. Sci. U.S.A. 105, 18396–18401.
Dalla Torre di Sanguinetto, S.A., Dasen, J.S., Arber, S., 2008. Transcriptional mechanisms controlling motor neuron diversity and connectivity. Curr. Opin. Neurobiol. 18, 36–43.
Dannhof, B.J., Bruns, V., 1993. The innervation of the organ of Corti in the rat. Hear Res. 66, 8–22.
Davies, D., 2007. Temporal and spatial regulation of alpha6 integrin expression during the development of the cochlear-vestibular ganglion. J. Comp. Neurol. 502, 673–682.
Davis, R.L., 2003. Gradients of neurotrophins, ion channels, and tuning in the cochlea. Neuroscientist 9, 311–316.
Dazert, S., Kim, D., Luo, L., Aletsee, C., Garfunkel, S., Maciag, T., Baird, A., Ryan, A.F., 1998. Focal delivery of fibroblast growth factor-1 by transfected cells induces spiral ganglion neurite targeting in vitro. J. Cell Physiol. 177, 123–129.
Deng, M., Pan, L., Xie, X., Gan, L., 2006. Differential expression of LIM domain-only (LMO) genes in the developing mouse inner ear. Gene Expr. Patterns 6, 857–863.
Dickson, B.J., 2002. Molecular mechanisms of axon guidance. Science 298, 1959–1964.
Doetzlhofer, A., White, P.M., Johnson, J.E., Segil, N., Groves, A.K., 2004. In vitro growth and differentiation of mammalian sensory hair cell progenitors: a requirement for EGF and periotic mesenchyme. Dev. Biol. 272, 432–447.
Doetzlhofer, A., White, P., Lee, Y.S., Groves, A., Segil, N., 2006. Prospective identification and purification of hair cell and supporting cell progenitors from the embryonic cochlea. Brain Res. 1091, 282–288.
Driver, E.C., Kelley, M.W., 2009. Specification of cell fate in the mammalian cochlea. Birth Defects Res. C: Embryo Today 87, 212–221.
Driver, E.C., Pryor, S.P., Hill, P., Turner, J., Ruther, U., Biesecker, L.G., Griffith, A.J., Kelley, M.W., 2008. Hedgehog signaling regulates sensory cell formation and auditory function in mice and humans. J. Neurosci. 28, 7350–7358.
Echteler, S.M., 1992. Developmental segregation in the afferent projections to mammalian auditory hair cells. Proc. Natl. Acad. Sci. U.S.A. 89, 6324–6327.
Echteler, S.M., Magardino, T., Rontal, M., 2005. Spatiotemporal patterns of neuronal programmed cell death during postnatal development of the gerbil cochlea. Brain Res. Dev. Brain Res. 157, 192–200.
Ehebauer, M., Hayward, P., Martinez-Arias, A., 2006. Notch signaling pathway. Sci. STKE 2006, cm7.
Ehret, G., 1979. Quantitative analysis of nerve fibre densities in the cochlea of the house mouse (*Mus musculus*). J. Comp. Neurol. 183, 73–88.
Evans, A.R., Euteneuer, S., Chavez, E., Mullen, L.M., Hui, E.E., Bhatia, S.N., Ryan, A.F., 2007. Laminin and fibronectin modulate inner ear spiral ganglion neurite outgrowth in an in vitro alternate choice assay. Dev. Neurobiol. 67, 1721–1730.
Farinas, I., Jones, K.R., Tessarollo, L., Vigers, A.J., Huang, E., Kirstein, M., de Caprona, D.C., Coppola, V., Backus, C., Reichardt, L.F., Fritzsch, B., 2001. Spatial shaping of cochlear innervation by temporally regulated neurotrophin expression. J. Neurosci. 21, 6170–6180.
Fekete, D.M., Campero, A.M., 2007. Axon guidance in the inner ear. Int. J. Dev. Biol. 51, 549–556.
Fekete, D.M., Wu, D.K., 2002. Revisiting cell fate specification in the inner ear. Curr. Opin. Neurobiol. 12, 35–42.
Fekete, D.M., Rouiller, E.M., Liberman, M.C., Ryugo, D.K., 1984. The central projections of intracellularly labeled auditory nerve fibers in cats. J. Comp. Neurol. 229, 432–450.
Fekete, D.M., Muthukumar, S., Karagogeos, D., 1998. Hair cells and supporting cells share a common progenitor in the avian inner ear. J. Neurosci. 18, 7811–7821.
Flores-Otero, J., Xue, H.Z., Davis, R.L., 2007. Reciprocal regulation of presynaptic and postsynaptic proteins in bipolar spiral ganglion neurons by neurotrophins. J. Neurosci. 27, 14023–14034.
Freyer, L., Morrow, B.E., 2010. Canonical Wnt signaling modulates Tbx1 Eya1, and Six1 expression, restricting neurogenesis in the otic vesicle. Dev. Dyn. 239, 1708–1722.
Friedman, R.A., Makmura, L., Biesiada, E., Wang, X., Keithley, E.M., 2005. Eya1 acts upstream of Tbx1, Neurogenin 1 NeuroD and the neurotrophins BDNF and NT-3 during inner ear development. Mech. Dev. 122, 625–634.
Fritzsch, B., Barbacid, M., Silos-Santiago, I., 1998. The combined effects of trkB and trkC mutations on the innervation of the inner ear. Int. J. Dev. Neurosci. 16, 493–505.
Fritzsch, B., Beisel, K.W., Jones, K., Farinas, I., Maklad, A., Lee, J., Reichardt, L.F., 2002. Development and evolution of inner ear sensory epithelia and their innervation. J. Neurobiol. 53, 143–156.
Fritzsch, B., Tessarollo, L., Coppola, E., Reichardt, L.F., 2004. Neurotrophins in the ear: their roles in sensory neuron survival and fiber guidance. Prog. Brain Res. 146, 265–278.
Fritzsch, B., Matei, V.A., Nichols, D.H., Bermingham, N., Jones, K., Beisel, K.W., Wang, V.Y., 2005. Atoh1 null mice show directed afferent fiber growth to undifferentiated ear sensory epithelia followed by incomplete fiber retention. Dev. Dyn. 233, 570–583.
Fritzsch, B., Beisel, K.W., Hansen, L.A., 2006. The molecular basis of neurosensory cell formation in ear development: a blueprint for hair cell and sensory neuron regeneration? Bioessays 28, 1181–1193.
Fritzsch, B., Dillard, M., Lavado, A., Harvey, N.L., Jahan, I., 2010. Canal cristae growth and fiber extension to the outer hair cells of the mouse ear require Prox1 activity. PLoS One 5, e9377.
Fritzsch, B., Eberl, D.F., Beisel, K.W., 2010. The role of bHLH genes in ear development and evolution: revisiting a 10-year-old hypothesis. Cell Mol. Life Sci. 67, 3089–3099.

Ginzberg, R.D., Morest, D.K., 1983. A study of cochlear innervation in the young cat with the Golgi method. Hear Res. 10, 227–246.
Ginzberg, R.D., Morest, D.K., 1984. Fine structure of cochlear innervation in the cat. Hear Res. 14, 109–127.
Giraldez, F., 1998. Regionalized organizing activity of the neural tube revealed by the regulation of lmx1 in the otic vesicle. Dev. Biol. 203, 189–200.
Goodhill, G.J., Richards, L.J., 1999. Retinotectal maps: molecules, models and misplaced data. Trends Neurosci. 22, 529–534.
Grant, L., Yi, E., Glowatzki, E., 2010. Two modes of release shape the postsynaptic response at the inner hair cell ribbon synapse. J. Neurosci. 30, 4210–4220.
Greenwood, D., Jagger, D.J., Huang, L.C., Hoya, N., Thorne, P.R., Wildman, S.S., King, B.F., Pak, K., Ryan, A.F., Housley, G.D., 2007. P2X receptor signaling inhibits BDNF-mediated spiral ganglion neuron development in the neonatal rat cochlea. Development 134, 1407–1417.
Gu, C., Rodriguez, E.R., Reimert, D.V., Shu, T., Fritzsch, B., Richards, L.J., Kolodkin, A.L., Ginty, D.D., 2003. Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. Dev. Cell 5, 45–57.
Guido, W., 2008. Refinement of the retinogeniculate pathway. J. Physiol. 586, 4357–4362.
Haddon, C., Jiang, Y.J., Smithers, L., Lewis, J., 1998. Delta-Notch signalling and the patterning of sensory cell differentiation in the zebrafish ear: evidence from the mind bomb mutant. Development 125, 4637–4644.
Hafidi, A., 1998. Peripherin-like immunoreactivity in type II spiral ganglion cell body and projections. Brain Res. 805, 181–190.
Hemond, S.G., Morest, D.K., 1992. Tropic effects of otic epithelium on cochleovestibular ganglion fiber growth in vitro. Anat. Rec. 232, 273–284.
Hossain, W.A., Morest, D.K., 2000. Fibroblast growth factors (FGF-1 FGF-2) promote migration and neurite growth of mouse cochlear ganglion cells in vitro: immunohistochemistry and antibody perturbation. J. Neurosci. Res. 62, 40–55.
Hossain, W.A., D'Sa, C., Morest, D.K., 2006. Site-specific interactions of neurotrophin-3 and fibroblast growth factor (FGF2) in the embryonic development of the mouse cochlear nucleus. J. Neurobiol. 66, 897–915.
Huang, L.C., Greenwood, D., Thorne, P.R., Housley, G.D., 2005. Developmental regulation of neuron-specific P2X3 receptor expression in the rat cochlea. J. Comp. Neurol. 484, 133–143.
Huang, L.C., Ryan, A.F., Cockayne, D.A., Housley, G.D., 2006. Developmentally regulated expression of the P2X3 receptor in the mouse cochlea. Histochem. Cell Biol. 125, 681–692.
Huang, L.C., Thorne, P.R., Housley, G.D., Montgomery, J.M., 2007. Spatiotemporal definition of neurite outgrowth, refinement and retraction in the developing mouse cochlea. Development 134, 2925–2933.
Jahan, I., Kersigo, J., Pan, N., Fritzsch, B., 2010a. Neurod1 regulates survival and formation of connections in mouse ear and brain. Cell Tissue Res. 341, 95–110.
Jahan, I., Pan, N., Kersigo, J., Fritzsch, B., 2010b. Neurod1 suppresses hair cell differentiation in ear ganglia and regulates hair cell subtype development in the cochlea. PLoS One 5, e11661.
Jessen, K.R., Mirsky, R., 2005. The origin and development of glial cells in peripheral nerves. Nat. Rev. Neurosci. 6, 671–682.
Johnson, K.R., Cook, S.A., Erway, L.C., Matthews, A.N., Sanford, L.P., Paradies, N.E., Friedman, R.A., 1999. Inner ear and kidney anomalies caused by IAP insertion in an intron of the Eya1 gene in a mouse model of BOR syndrome. Hum. Mol. Genet. 8, 645–653.
Jones, J.M., Warchol, M.E., 2009. Expression of the Gata3 transcription factor in the acoustic ganglion of the developing avian inner ear. J. Comp. Neurol. 516, 507–518.
Kalatzis, V., Sahly, I., El-Amraoui, A., Petit, C., 1998. Eya1 expression in the developing ear and kidney: towards the understanding of the pathogenesis of Branchio-Oto-Renal (BOR) syndrome. Dev. Dyn. 213, 486–499.
Kandler, K., Gillespie, D.C., 2005. Developmental refinement of inhibitory sound-localization circuits. Trends Neurosci. 28, 290–296.
Kandler, K., Clause, A., Noh, J., 2009. Tonotopic reorganization of developing auditory brainstem circuits. Nat. Neurosci. 12, 711–717.
Karis, A., Pata, I., van Doorninck, J.H., Grosveld, F., de Zeeuw, C.I., de Caprona, D., Fritzsch, B., 2001. Transcription factor GATA-3 alters pathway selection of olivocochlear neurons and affects morphogenesis of the ear. J. Comp. Neurol. 429, 615–630.
Kawase, T., Liberman, M.C., 1992. Spatial organization of the auditory nerve according to spontaneous discharge rate. J. Comp. Neurol. 319, 312–318.
Kelley, M.W., 2007. Cellular commitment and differentiation in the organ of Corti. Int. J. Dev. Biol. 51, 571–583.
Kiernan, A.E., Cordes, R., Kopan, R., Gossler, A., Gridley, T., 2005. The Notch ligands DLL1 and JAG2 act synergistically to regulate hair cell development in the mammalian inner ear. Development 132, 4353–4362.
Kiernan, A.E., Pelling, A.L., Leung, K.K., Tang, A.S., Bell, D.M., Tease, C., Lovell-Badge, R., Steel, K.P., Cheah, K.S., 2005. Sox2 is required for sensory organ development in the mammalian inner ear. Nature 434, 1031–1035.
Kim, W.Y., Fritzsch, B., Serls, A., Bakel, L.A., Huang, E.J., Reichardt, L.F., Barth, D.S., Lee, J.E., 2001. NeuroD-null mice are deaf due to a severe loss of the inner ear sensory neurons during development. Development 128, 417–426.
Koo, S.K., Hill, J.K., Hwang, C.H., Lin, Z.S., Millen, K.J., Wu, D.K., 2009. Lmx1a maintains proper neurogenic, sensory, and non-sensory domains in the mammalian inner ear. Dev. Biol. 333, 14–25.
Koundakjian, E.J., Appler, J.L., Goodrich, L.V., 2007. Auditory neurons make stereotyped wiring decisions before maturation of their targets. J. Neurosci. 27, 14078–14088.
Lallemend, F., Vandenbosch, R., Hadjab, S., Bodson, M., Breuskin, I., Moonen, G., Lefebvre, P.P., Malgrange, B., 2007. New insights into peripherin expression in cochlear neurons. Neuroscience 150, 212–222.
Lawoko-Kerali, G., Rivolta, M.N., Lawlor, P., Cacciabue-Rivolta, D.I., Langton-Hewer, C., van Doorninck, J.H., Holley, M.C., 2004. GATA3 and NeuroD distinguish auditory and vestibular neurons during development of the mammalian inner ear. Mech. Dev. 121, 287–299.
Leake, P.A., Snyder, R.L., 1989. Topographic organization of the central projections of the spiral ganglion in cats. J. Comp. Neurol. 281, 612–629.
Leake, P.A., Snyder, R.L., Hradek, G.T., 2002. Postnatal refinement of auditory nerve projections to the cochlear nucleus in cats. J. Comp. Neurol. 448, 6–27.
Lee, K.H., Warchol, M.E., 2008. Promotion of neurite outgrowth and axon guidance in spiral ganglion cells by netrin-1. Arch. Otolaryngol. Head Neck Surg. 134, 146–151.
Lee, D.J., Cahill, H.B., Ryugo, D.K., 2003. Effects of congenital deafness in the cochlear nuclei of Shaker-2 mice: an ultrastructural analysis of synapse morphology in the endbulbs of Held. J. Neurocytol. 32, 229–243.
Li, C., Van De Water, T., Ruben, R., 1978. The fate mapping of the eleventh and twelfth day mouse otocyst: an in vitro study of the sites of origin of the embryonic inner ear sensory structures. J. Morphol. 157, 249–267.
Li, H., Liu, H., Heller, S., 2003. Pluripotent stem cells from the adult mouse inner ear. Nat. Med. 9, 1293–1299.
Liberman, M.C., 1978. Auditory-nerve response from cats raised in a low-noise chamber. J. Acoust. Soc. Am. 63, 442–455.
Liberman, M.C., 1980. Morphological differences among radial afferent fibers in the cat cochlea: an electron-microscopic study of serial sections. Hear Res. 3, 45–63.
Liberman, M.C., 1982. Single-neuron labeling in the cat auditory nerve. Science 216, 1239–1241.
Liberman, M.C., Dodds, L.W., Pierce, S., 1990. Afferent and efferent innervation of the cat cochlea: quantitative analysis with light and electron microscopy. J. Comp. Neurol. 301, 443–460.
Limb, C.J., Ryugo, D.K., 2000. Development of primary axosomatic endings in the anteroventral cochlear nucleus of mice. J. Assoc. Res. Otolaryngol. 1, 103–119.
Liu, M., Pereira, F.A., Price, S.D., Chu, M.J., Shope, C., Himes, D., Eatock, R.A., Brownell, W.E., Lysakowski, A., Tsai, M.J., 2000. Essential role of BETA2/NeuroD1 in development of the vestibular and auditory systems. Genes Dev. 14, 2839–2854.
Liu, W., Li, G., Chien, J.S., Raft, S., Zhang, H., Chiang, C., Frenz, D.A., 2002. Sonic hedgehog regulates otic capsule chondrogenesis and inner ear development in the mouse embryo. Dev. Biol. 248, 240–250.
Liu, W., Oh, S.H., Kang Yk, Y., Li, G., Doan, T.M., Little, M., Li, L., Ahn, K., Crenshaw 3rd, E.B., Frenz, D.A., 2003. Bone morphogenetic protein 4 (BMP4): a regulator of capsule chondrogenesis in the developing mouse inner ear. Dev. Dyn. 226, 427–438.
Liu, Z., Owen, T., Zhang, L., Zuo, J., 2010. Dynamic expression pattern of Sonic hedgehog in developing cochlear spiral ganglion neurons. Dev. Dyn. 239, 1674–1683.
Livesey, F.J., Cepko, C.L., 2001. Vertebrate neural cell-fate determination: lessons from the retina. Nat. Rev. Neurosci. 2, 109–118.
Ma, L., Tessier-Lavigne, M., 2007. Dual branch-promoting and branch-repelling actions of Slit/Robo signaling on peripheral and central branches of developing sensory axons. J. Neurosci. 27, 6843–6851.
Ma, Q., Chen, Z., del Barco Barrantes, I., de la Pompa, J.L., Anderson, D.J., 1998. neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia. Neuron 20, 469–482.
Ma, Q., Anderson, D.J., Fritzsch, B., 2000. Neurogenin 1 null mutant ears develop fewer, morphologically normal hair cells in smaller sensory epithelia devoid of innervation. J. Assoc. Res. Otolaryngol. 1, 129–143.
Mak, A.C., Szeto, I.Y., Fritzsch, B., Cheah, K.S., 2009. Differential and overlapping expression pattern of SOX2 and SOX9 in inner ear development. Gene Expr. Patterns 9, 444–453.
Maklad, A., Fritzsch, B., 2003. Development of vestibular afferent projections into the hindbrain and their central targets. Brain Res. Bull. 60, 497–510.
Maklad, A., Quinn, T., Fritzsch, B., 2001. Intracranial distribution of the sympathetic system in mice: Dil tracing and immunocytochemical labeling. Anat. Rec. 263, 99–111.
Maricich, S.M., Xia, A., Mathes, E.L., Wang, V.Y., Oghalai, J.S., Fritzsch, B., Zoghbi, H.Y., 2009. Atoh1-lineal neurons are required for hearing and for the survival of neurons in the spiral ganglion and brainstem accessory auditory nuclei. J. Neurosci. 29, 11123–11133.
Martin, M.R., Rickets, C., 1981. Histogenesis of the cochlear nucleus of the mouse. J. Comp. Neurol. 197, 169–184.
Martinez-Monedero, R., Oshima, K., Heller, S., Edge, A.S., 2007. The potential role of endogenous stem cells in regeneration of the inner ear. Hear Res. 227, 48–52.
Martinez-Monedero, R., Yi, E., Oshima, K., Glowatzki, E., Edge, A.S., 2008. Differentiation of inner ear stem cells to functional sensory neurons. Dev. Neurobiol. 68, 669–684.
Matei, V., Pauley, S., Kaing, S., Rowitch, D., Beisel, K.W., Morris, K., Feng, F., Jones, K., Lee, J., Fritzsch, B., 2005. Smaller inner ear sensory epithelia in Neurog 1 null mice are related to earlier hair cell cycle exit. Dev. Dyn. 234, 633–650.
Matilainen, T., Haugas, M., Kreidberg, J.A., Salminen, M., 2007. Analysis of Netrin 1 receptors during inner ear development. Int. J. Dev. Biol. 51, 409–413.
Matsumoto, M., Nakagawa, T., Higashi, T., Kim, T.S., Kojima, K., Kita, T., Sakamoto, T., Ito, J., 2005. Innervation of stem cell-derived neurons into auditory epithelia of mice. Neuroreport 16, 787–790.

Matsumoto, M., Nakagawa, T., Kojima, K., Sakamoto, T., Fujiyama, F., Ito, J., 2008. Potential of embryonic stem cell-derived neurons for synapse formation with auditory hair cells. J. Neurosci. Res. 86, 3075–3085.
Meyer, A.C., Moser, T., 2010. Structure and function of cochlear afferent innervation. Curr. Opin. Otolaryngol. Head Neck Surg. 18, 441–446.
Meyer, A.C., Frank, T., Khimich, D., Hoch, G., Riedel, D., Chapochnikov, N.M., Yarin, Y.M., Harke, B., Hell, S.W., Egner, A., Moser, T., 2009. Tuning of synapse number, structure and function in the cochlea. Nat. Neurosci. 12, 444–453.
Miko, I.J., Nakamura, P.A., Henkemeyer, M., Cramer, K.S., 2007. Auditory brainstem neural activation patterns are altered in EphA4- and ephrin-B2-deficient mice. J. Comp. Neurol. 505, 669–681.
Miko, I.J., Henkemeyer, M., Cramer, K.S., 2008. Auditory brainstem responses are impaired in EphA4 and ephrin-B2 deficient mice. Hear Res. 235, 39–46.
Ming, G., Henley, J., Tessier-Lavigne, M., Song, H., Poo, M., 2001. Electrical activity modulates growth cone guidance by diffusible factors. Neuron 29, 441–452.
Miyazaki, N., Furuyama, T., Takeda, N., Inoue, T., Kubo, T., Inagaki, S., 1999. Expression of mouse semaphorin H mRNA in the inner ear of mouse fetuses. Neurosci. Lett. 261, 127–129.
Molea, D., Rubel, E.W., 2003. Timing and topography of nucleus magnocellularis innervation by the cochlear ganglion. J. Comp. Neurol. 466, 577–591.
Montcouquiol, M., Kelley, M.W., 2003. Planar and vertical signals control cellular differentiation and patterning in the mammalian cochlea. J. Neurosci. 23, 9469–9478.
Moraes, F., Novoa, A., Jerome-Majewska, L.A., Papaioannou, V.E., Mallo, M., 2005. Tbx1 is required for proper neural crest migration and to stabilize spatial patterns during middle and inner ear development. Mech. Dev. 122, 199–212.
Morris, J.K., Maklad, A., Hansen, L.A., Feng, F., Sorensen, C., Lee, K.F., Macklin, W.B., Fritzsch, B., 2006. A disorganized innervation of the inner ear persists in the absence of ErbB2. Brain Res. 1091, 186–199.
Morsli, H., Choo, D., Ryan, A., Johnson, R., Wu, D.K., 1998. Development of the mouse inner ear and origin of its sensory organs. J. Neurosci. 18, 3327–3335.
Mumm, J.S., Godinho, L., Morgan, J.L., Oakley, D.M., Schroeter, E.H., Wong, R.O., 2005. Laminar circuit formation in the vertebrate retina. Prog. Brain Res. 147, 155–169.
Murakami, Y., Suto, F., Shimizu, M., Shinoda, T., Kameyama, T., Fujisawa, H., 2001. Differential expression of plexin-A subfamily members in the mouse nervous system. Dev. Dyn. 220, 246–258.
Murata, J., Tokunaga, A., Okano, H., Kubo, T., 2006. Mapping of notch activation during cochlear development in mice: implications for determination of prosensory domain and cell fate diversification. J. Comp. Neurol. 497, 502–518.
Nemzou, N.R., Bulankina, A.V., Khimich, D., Giese, A., Moser, T., 2006. Synaptic organization in cochlear inner hair cells deficient for the CaV1.3 (alpha1D) subunit of L-type Ca^{2+} channels. Neuroscience 141, 1849–1860.
Nichols, D.H., Pauley, S., Jahan, I., Beisel, K.W., Millen, K.J., Fritzsch, B., 2008. Lmx1a is required for segregation of sensory epithelia and normal ear histogenesis and morphogenesis. Cell Tissue Res. 334, 339–358.
N.I.D.C.D., http://www.nidcd.nih.gov/health/hearing/.
Nikolic, P., Housley, G.D., Luo, L., Ryan, A.F., Thorne, P.R., 2001. Transient expression of P2X(1) receptor subunits of ATP-gated ion channels in the developing rat cochlea. Brain Res. Dev. Brain Res. 126, 173–182.
Nikolic, P., Housley, G.D., Thorne, P.R., 2003. Expression of the P2X7 receptor subunit of the adenosine 5′-triphosphate-gated ion channel in the developing and adult rat cochlea. Audiol. Neurootol. 8, 28–37.
Ohyama, T., Mohamed, O.A., Taketo, M.M., Dufort, D., Groves, A.K., 2006. Wnt signals mediate a fate decision between otic placode and epidermis. Development 133, 865–875.
Oshima, K., Grimm, C.M., Corrales, C.E., Senn, P., Martinez Monedero, R., Geleoc, G.S., Edge, A., Holt, J.R., Heller, S., 2007. Differential distribution of stem cells in the auditory and vestibular organs of the inner ear. J. Assoc. Res. Otolaryngol. 8, 18–31.
Ozaki, H., Nakamura, K., Funahashi, J., Ikeda, K., Yamada, G., Tokano, H., Okamura, H.O., Kitamura, K., Muto, S., Kotaki, H., Sudo, K., Horai, R., Iwakura, Y., Kawakami, K., 2004. Six1 controls patterning of the mouse otic vesicle. Development 131, 551–562.
Pan, W., Jin, Y., Stanger, B., Kiernan, A.E., 2010. Notch signaling is required for the generation of hair cells and supporting cells in the mammalian inner ear. Proc. Natl. Acad. Sci. U.S.A. 107, 15798–15803.
Parker, M.A., Corliss, D.A., Gray, B., Anderson, J.K., Bobbin, R.P., Snyder, E.Y., Cotanche, D.A., 2007. Neural stem cells injected into the sound-damaged cochlea migrate throughout the cochlea and express markers of hair cells, supporting cells, and spiral ganglion cells. Hear Res. 232, 29–43.
Pauley, S., Wright, T.J., Pirvola, U., Ornitz, D., Beisel, K., Fritzsch, B., 2003. Expression and function of FGF10 in mammalian inner ear development. Dev. Dyn. 227, 203–215.
Perkins, R.E., Morest, D.K., 1975. A study of cochlear innervation patterns in cats and rats with the Golgi method and Nomarkski Optics. J. Comp. Neurol. 163, 129–158.
Pickles, J.O., Claxton, C., Van Heumen, W.R., 2002. Complementary and layered expression of Ephs and ephrins in developing mouse inner ear. J. Comp. Neurol. 449, 207–216.
Puligilla, C., Kelley, M.W., 2009. Building the world's best hearing aid; regulation of cell fate in the cochlea. Curr. Opin. Genet. Dev. 19, 368–373.
Puligilla, C., Feng, F., Ishikawa, K., Bertuzzi, S., Dabdoub, A., Griffith, A.J., Fritzsch, B., Kelley, M.W., 2007. Disruption of fibroblast growth factor receptor 3 signaling results in defects in cellular differentiation, neuronal patterning, and hearing impairment. Dev. Dyn. 236, 1905–1917.
Puligilla, C., Dabdoub, A., Brenowitz, S.D., Kelley, M.W., 2010. Sox2 induces neuronal formation in the developing mammalian cochlea. J. Neurosci. 30, 714–722.
Radde-Gallwitz, K., Pan, L., Gan, L., Lin, X., Segil, N., Chen, P., 2004. Expression of Islet1 marks the sensory and neuronal lineages in the mammalian inner ear. J. Comp. Neurol. 477, 412–421.
Raft, S., Nowotschin, S., Liao, J., Morrow, B.E., 2004. Suppression of neural fate and control of inner ear morphogenesis by Tbx1. Development 131, 1801–1812.
Raft, S., Koundakjian, E.J., Quinones, H., Jayasena, C.S., Goodrich, L.V., Johnson, J.E., Segil, N., Groves, A.K., 2007. Cross-regulation of Ngn1 and Math1 coordinates the production of neurons and sensory hair cells during inner ear development. Development 134, 4405–4415.
Rio, C., Dikkes, P., Liberman, M.C., Corfas, G., 2002. Glial fibrillary acidic protein expression and promoter activity in the inner ear of developing and adult mice. J. Comp. Neurol. 442, 156–162.
Rodgers, K.D., Barritt, L., Miner, J.H., Cosgrove, D., 2001. The laminins in the murine inner ear: developmental transitions and expression in cochlear basement membranes. Hear Res. 158, 39–50.
Romand, M.R., Romand, R., 1987. The ultrastructure of spiral ganglion cells in the mouse. Acta Otolaryngol. 104, 29–39.
Rontal, D.A., Echteler, S.M., 2003. Developmental segregation in the efferent projections to auditory hair cells in the gerbil. J. Comp. Neurol. 467, 509–520.
Roux, I., Safieddine, S., Nouvian, R., Grati, M., Simmler, M.C., Bahloul, A., Perfettini, I., Le Gall, M., Rostaing, P., Hamard, G., Triller, A., Avan, P., Moser, T., Petit, C., 2006. Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. Cell 127, 277–289.
Rubel, E.W., Fritzsch, B., 2002. Auditory system development: primary auditory neurons and their targets. Annu. Rev. Neurosci. 25, 51–101.
Rubel, E.W., Parks, T.N., Zirpel, L., 2004. Assembling, connecting and maintaining the cochlear nucleus. In: Parks, T.N., Rubel, E.W., Fay, R.R., Popper, A.N. (Eds.), Plasticity of the Auditory System. Springer-Verlag, New York.
Ruben, R.J., 1967. Development of the inner ear of the mouse: a radioautographic study of terminal mitoses. Acta Otolaryngol. 220 (Suppl.), 221–244.
Ryugo, D.K., 2008. Projections of low spontaneous rate, high threshold auditory nerve fibers to the small cell cap of the cochlear nucleus in cats. Neuroscience 154, 114–126.
Ryugo, D.K., Fekete, D.M., 1982. Morphology of primary axosomatic endings in the anteroventral cochlear nucleus of the cat: a study of the endbulbs of Held. J. Comp. Neurol. 210, 239–257.
Ryugo, D.K., Parks, T.N., 2003. Primary innervation of the avian and mammalian cochlear nucleus. Brain Res. Bull. 60, 435–456.
Ryugo, D.K., Kretzmer, E.A., Niparko, J.K., 2005. Restoration of auditory nerve synapses in cats by cochlear implants. Science 310, 1490–1492.
Ryugo, D.K., Montey, K.L., Wright, A.L., Bennett, M.L., Pongstaporn, T., 2006. Postnatal development of a large auditory nerve terminal: the endbulb of Held in cats. Hear Res. 216–217, 100–115.
Sagasti, A., Guido, M.R., Raible, D.W., Schier, A.F., 2005. Repulsive interactions shape the morphologies and functional arrangement of zebrafish peripheral sensory arbors. Curr. Biol. 15, 804–814.
Sakano, H., 2010. Neural map formation in the mouse olfactory system. Neuron 67, 530–542.
Salminen, M., Meyer, B.I., Bober, E., Gruss, P., 2000. Netrin 1 is required for semicircular canal formation in the mouse inner ear. Development 127, 13–22.
Sapede, D., Pujades, C., 2010. Hedgehog signaling governs the development of otic sensory epithelium and its associated innervation in zebrafish. J. Neurosci. 30, 3612–3623.
Satoh, T., Fekete, D.M., 2005. Clonal analysis of the relationships between mechanosensory cells and the neurons that innervate them in the chicken ear. Development 132, 1687–1697.
Schmidt, H., Werner, M., Heppenstall, P.A., Henning, M., More, M.I., Kuhbandner, S., Lewin, G.R., Hofmann, F., Feil, R., Rathjen, F.G., 2002. cGMP-mediated signaling via cGKIalpha is required for the guidance and connectivity of sensory axons. J. Cell Biol. 159, 489–498.
Schmidt, H., Stonkute, A., Juttner, R., Schaffer, S., Buttgereit, J., Feil, R., Hofmann, F., Rathjen, F.G., 2007. The receptor guanylyl cyclase Npr2 is essential for sensory axon bifurcation within the spinal cord. J. Cell Biol. 179, 331–340.
Schmidt, H., Stonkute, A., Juttner, R., Koesling, D., Friebe, A., Rathjen, F.G., 2009. C-type natriuretic peptide (CNP) is a bifurcation factor for sensory neurons. Proc. Natl. Acad. Sci. U.S.A. 106, 16847–16852.
Schmitt, A.M., Shi, J., Wolf, A.M., Lu, C.C., King, L.A., Zou, Y., 2006. Wnt-Ryk signalling mediates medial-lateral retinotectal topographic mapping. Nature 439, 31–37.
Seal, R.P., Akil, O., Yi, E., Weber, C.M., Grant, L., Yoo, J., Clause, A., Kandler, K., Noebels, J.L., Glowatzki, E., Lustig, L.R., Edwards, R.H., 2008. Sensorineural deafness and seizures in mice lacking vesicular glutamate transporter 3. Neuron 57, 263–275.
Shah, S.M., Kang, Y.J., Christensen, B.L., Feng, A.S., Kollmar, R., 2009. Expression of Wnt receptors in adult spiral ganglion neurons: frizzled 9 localization at growth cones of regenerating neurites. Neuroscience 164, 478–487.
Shi, F., Corrales, C.E., Liberman, M.C., Edge, A.S., 2007. BMP4 induction of sensory neurons from human embryonic stem cells and reinnervation of sensory epithelium. Eur. J. Neurosci. 26, 3016–3023.
Shibamori, Y., Tamamaki, N., Saito, H., Nojyo, Y., 1994. The trajectory of the sympathetic nerve fibers to the rat cochlea as revealed by anterograde and retrograde WGA-HRP tracing. Brain Res. 646, 223–229.
Shnerson, A., Devigne, C., Pujol, R., 1981. Age-related changes in the C57BL/6J mouse cochlea. II. Ultrastructural findings. Brain Res. 254, 77–88.

Siddiqui, S.A., Cramer, K.S., 2005. Differential expression of Eph receptors and ephrins in the cochlear ganglion and eighth cranial nerve of the chick embryo. J. Comp. Neurol. 482, 309–319.
Sienknecht, U.J., Fekete, D.M., 2008. Comprehensive Wnt-related gene expression during cochlear duct development in chicken. J. Comp. Neurol. 510, 378–395.
Sienknecht, U.J., Fekete, D.M., 2009. Mapping of Wnt, frizzled, and Wnt inhibitor gene expression domains in the avian otic primordium. J. Comp. Neurol. 517, 751–764.
Silos-Santiago, I., Fagan, A.M., Garber, M., Fritzsch, B., Barbacid, M., 1997. Severe sensory deficits but normal CNS development in newborn mice lacking TrkB and TrkC tyrosine protein kinase receptors. Eur. J. Neurosci. 9, 2045–2056.
Simmons, D.D., 1994. A transient afferent innervation of outer hair cells in the postnatal cochlea. Neuroreport 5, 1309–1312.
Simon, D.K., O'Leary, D.D., 1992. Development of topographic order in the mammalian retinocollicular projection. J. Neurosci. 12, 1212–1232.
Sobkowicz, H.M., Rose, J.E., Scott, G.E., Slapnick, S.M., 1982. Ribbon synapses in the developing intact and cultured organ of Corti in the mouse. J. Neurosci. 2, 942–957.
Stankovic, K., Rio, C., Xia, A., Sugawara, M., Adams, J.C., Liberman, M.C., Corfas, G., 2004. Survival of adult spiral ganglion neurons requires erbB receptor signaling in the inner ear. J. Neurosci. 24, 8651–8661.
Stevens, C.B., Davies, A.L., Battista, S., Lewis, J.H., Fekete, D.M., 2003. Forced activation of Wnt signaling alters morphogenesis and sensory organ identity in the chicken inner ear. Dev. Biol. 261, 149–164.
Stone, J.S., Shang, J.L., Tomarev, S., 2003. Expression of Prox1 defines regions of the avian otocyst that give rise to sensory or neural cells. J. Comp. Neurol. 460, 487–502.
Taberner, A.M., Liberman, M.C., 2005. Response properties of single auditory nerve fibers in the mouse. J. Neurophysiol. 93, 557–569.
Tritsch, N.X., Bergles, D.E., 2010. Developmental regulation of spontaneous activity in the mammalian cochlea. J. Neurosci. 30, 1539–1550.
Tritsch, N.X., Yi, E., Gale, J.E., Glowatzki, E., Bergles, D.E., 2007. The origin of spontaneous activity in the developing auditory system. Nature 450, 50–55.
Vitelli, F., Viola, A., Morishima, M., Pramparo, T., Baldini, A., Lindsay, E., 2003. TBX1 is required for inner ear morphogenesis. Hum. Mol. Genet. 12, 2041–2048.
Wang, Y., Liu, H., Shen, Y., Wang, Z., Li, H., 2006. Peripherin as a marker for degeneration of spiral ganglion neurons after aminoglycoside ototoxicity. Acta Otolaryngol. 126, 1128–1133.
Wang, S.J., Furusho, M., D'Sa, C., Kuwada, S., Conti, L., Morest, D.K., Bansal, R., 2009. Inactivation of fibroblast growth factor receptor signaling in myelinating glial cells results in significant loss of adult spiral ganglion neurons accompanied by age-related hearing impairment. J. Neurosci. Res. 87, 3428–3437.
Wegner, M., Stolt, C.C., 2005. From stem cells to neurons and glia: a Soxist's view of neural development. Trends Neurosci. 28, 583–588.
Weisz, C., Glowatzki, E., Fuchs, P., 2009. The postsynaptic function of type II cochlear afferents. Nature 461, 1126–1129.
White, P.M., Doetzlhofer, A., Lee, Y.S., Groves, A.K., Segil, N., 2006. Mammalian cochlear supporting cells can divide and trans-differentiate into hair cells. Nature 441, 984–987.
Whitlon, D.S., Zhang, X., Kusakabe, M., 1999. Tenascin-C in the cochlea of the developing mouse. J. Comp. Neurol. 406, 361–374.
Wiechers, B., Gestwa, G., Mack, A., Carroll, P., Zenner, H.P., Knipper, M., 1999. A changing pattern of brain-derived neurotrophic factor expression correlates with the rearrangement of fibers during cochlear development of rats and mice. J. Neurosci. 19, 3033–3042.
Woods, C., Montcouquiol, M., Kelley, M.W., 2004. Math1 regulates development of the sensory epithelium in the mammalian cochlea. Nat. Neurosci. 7, 1310–1318.
Wright, T.J., Mansour, S.L., 2003a. Fgf3 and Fgf10 are required for mouse otic placode induction. Development 130, 3379–3390.
Wright, T.J., Mansour, S.L., 2003b. FGF signaling in ear development and innervation. Curr. Top. Dev. Biol. 57, 225–259.
Wu, D.K., Nunes, F.D., Choo, D., 1998. Axial specification for sensory organs versus non-sensory structures of the chicken inner ear. Development 125, 11–20.
Wu, H., Xiong, W.C., Mei, L., 2010. To build a synapse: signaling pathways in neuromuscular junction assembly. Development 137, 1017–1033.
Xiang, M., Gao, W.Q., Hasson, T., Shin, J.J., 1998. Requirement for Brn-3c in maturation and survival, but not in fate determination of inner ear hair cells. Development 125, 3935–3946.
Xiang, M., Maklad, A., Pirvola, U., Fritzsch, B., 2003. Brn3c null mutant mice show long-term, incomplete retention of some afferent inner ear innervation. BMC Neurosci. 4, 2.
Xu, H., Viola, A., Zhang, Z., Gerken, C.P., Lindsay-Illingworth, E.A., Baldini, A., 2007. Tbx1 regulates population, proliferation and cell fate determination of otic epithelial cells. Dev. Biol. 302, 670–682.
Yang, H., Xie, X., Deng, M., Chen, X., Gan, L., 2010. Generation and characterization of Atoh1-Cre knock-in mouse line. Genesis 48, 407–413.
Youssoufian, M., Couchman, K., Shivdasani, M.N., Paolini, A.G., Walmsley, B., 2008. Maturation of auditory brainstem projections and calyces in the congenitally deaf (dn/dn) mouse. J. Comp. Neurol. 506, 442–451.
Zhao, Z., Wang, Z., Gu, Y., Feil, R., Hofmann, F., Ma, L., 2009. Regulate axon branching by the cyclic GMP pathway via inhibition of glycogen synthase kinase 3 in dorsal root ganglion sensory neurons. J. Neurosci. 29, 1350–1360.
Zheng, J.L., Gao, W.Q., 2000. Overexpression of Math1 induces robust production of extra hair cells in postnatal rat inner ears. Nat. Neurosci. 3, 580–586.
Zheng, J.Q., Poo, M.M., 2007. Calcium signaling in neuronal motility. Annu. Rev. Cell Dev. Biol. 23, 375–404.
Zheng, W., Huang, L., Wei, Z.B., Silvius, D., Tang, B., Xu, P.X., 2003. The role of Six1 in mammalian auditory system development. Development 130, 3989–4000.
Zou, D., Silvius, D., Fritzsch, B., Xu, P.X., 2004. Eya1 and Six1 are essential for early steps of sensory neurogenesis in mammalian cranial placodes. Development 131, 5561–5572.
Zou, D., Silvius, D., Rodrigo-Blomqvist, S., Enerback, S., Xu, P.X., 2006. Eya1 regulates the growth of otic epithelium and interacts with Pax2 during the development of all sensory areas in the inner ear. Dev. Biol. 298, 430–441.
Zou, D., Erickson, C., Kim, E.H., Jin, D., Fritzsch, B., Xu, P.X., 2008. Eya1 gene dosage critically affects the development of sensory epithelia in the mammalian inner ear. Hum. Mol. Genet. 17, 3340–3356.