IBRO Neuroscience Reports 13 (2022) 306–313

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

Molecular mechanisms governing development of the hindbrain choroid plexus and auditory projection: A validation of the seminal observations of Wilhelm His

Joel C. Glover [a,b,c,*], Bernd Fritzsch [d,**]

[a] Department of Molecular Medicine, University of Oslo, Oslo, Norway
[b] Norwegian Center for Stem Cell Research, Oslo University Hospital, Oslo, Norway
[c] Sars International Centre for Marine Molecular Biology, University of Bergen, Bergen, Norway
[d] Department of Biology, University of Iowa, Iowa, IA 52242, USA

ARTICLE INFO

Keywords:
Brainstem
Cochlear nuclei
Ear
Cochlear hair cells

ABSTRACT

Studies by His from 1868 to 1904 delineated the critical role of the dorsal roof plate in the development of the hindbrain choroid plexus, and of the rhombic lips in the development of hindbrain auditory centers. Modern molecular studies have confirmed these observations and placed them in a mechanistic context. Expression of the transcription factor *Lmx1a/b* is crucial to the development of the hindbrain choroid plexus, and also regulates the expression of *Atoh1,* a transcription factor that is essential for the formation of the cochlear hair cells and auditory nuclei. By contrast, development of the vestibular hair cells, vestibular ganglion and vestibular nuclei does not depend on *Lmx1a/b.* These findings demonstrate a common dependence on a specific gene for the hindbrain choroid plexus and the primary auditory projection from hair cells to sensory neurons to hindbrain nuclei. Thus, His' conclusions regarding the origins of specific hindbrain structures are borne out by molecular genetic experiments conducted more than a hundred years later.

Introduction

Wilhelm His Sr (1831–1904) invented the microtome (His, 1870), described the histology of brain and body development (His, 1880), and introduced a new anatomical nomenclature (His, 1895). He described and defined the 'Zwischenstrang' [neural crest (His, 1868)], the rhombencephalon, and some of the principal features of the brain anlage [floor plate, basal plate, alar plate, roof plate, sulcus limitans, isthmus (His, 1890; His, 1904)]. Most notably, His pointed out early on that the geniculate ganglion of cranial nerve VII does not derive from the 'Zwischenstrang', thus indicating a different developmental origin for what he referred to as 'visceral ganglia'. It was only later that the true origins of such ganglia as well as of the ear anlage were identified as placodes (O'Neill et al., 2012; Schlosser, 2017; Von Kupffer, 1891). His defined specific longitudinal domains within the rhombencephalon based on external and internal features, and identified the rhombic lip as the source of migratory cell populations giving rise to major dorsal and ventral nuclei, including the pontine nuclei ('Brueckenkerne'), the superior and inferior olives, and the cochlear nuclei (de No, 1981; Held, 1893; Malmierca, 2015; Muniak et al., 2016).

Our review will concentrate on the insights provided by His into the development of the human hindbrain with an emphasis on its longitudinal regionalization and on the role of the dorsal plate and rhombic lip ('Rautenlippe') in the development of the rhombencephalic choroid plexus and the auditory projection. We extend an excellent previous review (Ray and Dymecki, 2009) by adding more recent molecular insights into the role of specific critical genes (Chizhikov et al., 2021; Elliott et al., 2021; Glover et al., 2018).

His' observations on the hindbrain were based on human embryos

His was a member of the 'Entwicklungsmechanik' school whose aim was to explain how the shape and form of the brain arise during development. His noted that the brain undergoes significant shape changes that open the 4th ventricle. His basic working hypothesis was that the neural tube behaves like a rubber tube cut dorsally. He

* Corresponding author at: Department of Molecular Medicine, University of Oslo, Oslo, Norway.
** Corresponding author.
E-mail addresses: joel.glover@medisin.uio.no (J.C. Glover), bernd-fritzsch@uiowa.edu (B. Fritzsch).

https://doi.org/10.1016/j.ibneur.2022.09.011
Received 29 April 2022; Received in revised form 29 September 2022; Accepted 29 September 2022
Available online 3 October 2022

described how the roof of the 4th ventricle forms the choroid plexus and how its shape depends on the narrowing of the ventricular floor at the calamus scriptorius (Fig. 1). To His, the lateral recesses of the 4th ventricle were formed by the force of the pons pushing against the medulla oblongata: the form of the 'Rautenhirn' then emerging as a tube fully closed at the isthmus and obex but with an increasingly wider gap in between, stretching the thin, overlying roof plate.

His noted that in transverse sections through the rhombencephalon of the human embryo the extremes of the alar plates are demarcated by a longitudinal pial sulcus, forming what he called the 'Rautenlippen', or rhombic lips (Fig. 1B). Much of his subsequent work focused on neuron populations that he believed to originate from the rhombic lips. He realized, based purely on histological evidence, that several hindbrain neuron populations were generated through tangential migrations of neuroblasts, delaminating from the rhombic lips. His was the first to correctly identify these migratory cells as they traverse the rhombencephalon.

Following his early insights into the distinct origins of cranial and spinal sensory ganglia, His provided a detailed reconstruction of a ventral view of the human embryonic brainstem that highlighted all nerve fiber roots and many of their associated central nuclei. He also drew some of the central nuclei he postulated to originate from the rhombic lip, such as the pons and superior olivary nuclei ('Brueckenkern', 'obere Olive'; Fig. 2 (de No, 1981; Held, 1893)). The development of the auditory centers was of particular interest to him, as well as to his son (His Jr, 1889). However, His Sr did not consider the different auditory and vestibular subnuclei and relied on the more sophisticated work of Ramon y Cajal and Gustav Retzius for insights into the peripheral and central projections of auditory afferents as they relate to these subnuclei and to the cerebellum (Retzius, 1884; y Cajal, 1911). His noted in particular that the above-mentioned nuclei as well as the rhombic lip and the choroid plexus of the fourth ventricle are features unique to the hindbrain, as elaborated in detail by de No (de No, 1981).

His explicitly defined distinct longitudinal regions of the brainstem: a) the 'Schaltstueck' (an intermediate segment between the spinal cord proper and the tip of the calamus scriptorius where the rhombic lips start to diverge); b) the calamus scriptorius region containing the gracile and cuneate nuclei; c) the medulla oblongata ('Nachhirn'); d) the pons ('Bruecke' or 'Hinterhirn') and e) the isthmus. Throughout this region, the roof plate ('Deckplatte') in his description is a thin epithelial sheet that elaborates the choroid plexus of the fourth ventricle. His noted that the alar plate ('Fluegelplatte') and basal plate ('Grundplatte') develop into various partially identifiable nuclei (Fig. 1B), with the left and right basal plates connected by the diminutive midline floor plate ('Bodenplatte').

Thus, His described the hindbrain as being composed of longitudinal domains of different character, each containing specific sets of afferent inputs terminating in more or less defined central nuclei. He noted that some nuclei were established at specific sites along the longitudinal axis by migratory neuroblasts derived from the rhombic lip. Later work conducted mainly by the American school of functional neuroanatomists led Herrick to consider these domains as 'neomorphs' of the hindbrain, and he accorded them status as 'special' columns restricted to the rhombencephalon (Glover, et al., 2018; Herrick, 1948).

Below we will expand on Wilhelm His Sr.'s observations by describing how his hindbrain regions correspond to molecular discontinuities in longitudinal progenitor domains in the dorsal hindbrain, and relating these to the neuromeric origins of the choroid plexus and the primary auditory nuclei (see Section 2 and 3; and (Fritzsch and Elliott, 2017; Hernandez-Miranda et al., 2017; Millen et al., 2014; Mishima

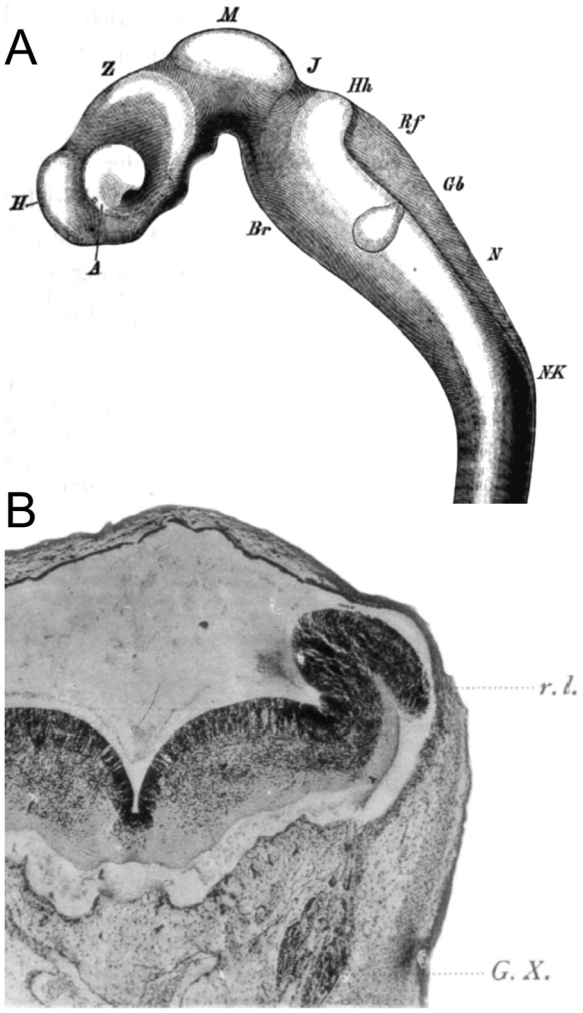

Fig. 1. A whole mount of the brain (A) and coronal section through the rhombencephalon (B) of a human embryo. Abbreviations: A, Augenblase, eye; Br, Brueckenkruemmung, pons; Gb, Gehorblase, ear; H, Hemisphaerenhirn, telencephalon; Hh, Hinterhirn, pons; J, Isthmus; M, Mittelhirn, midbrain; N, Nachhirn, medulla oblongata; NK, Nachhirnkruemmung, bridge of spinal cord; Rf, Rautenfeld, rhombomeric choroid plexus; Z, Zwischenhirn, diencephalon; rl = rhombic lip; G.X. = ganglion of the vagus nerve. Taken from His (1897).

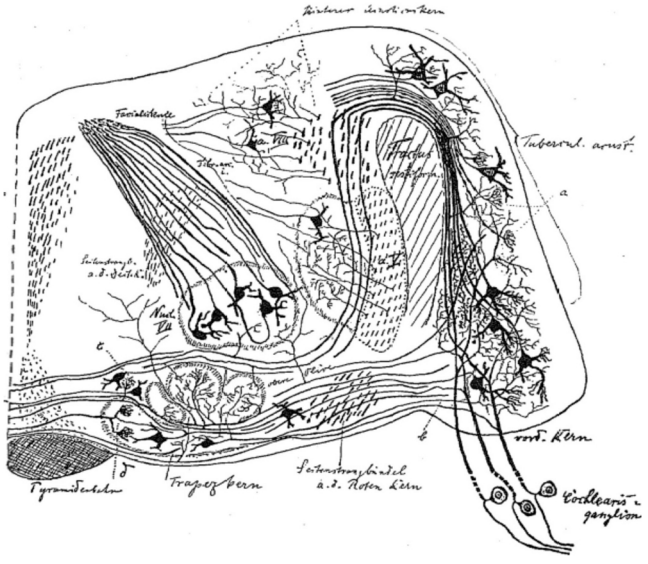

Fig. 2. Silver-stained coronal section of the hindbrain of a cat showing the innervation of the cochlear nuclei (Tuberculum) by afferents of the cochlear ganglion (Cochlearis ganglion), and second-order projections from the anterior ventral cochlear nucleus (PVCN, root Kern) to the superior olives (Trapezkern). Taken from Held (1893).

et al., 2009; Ray and Dymecki, 2009). We pinpoint the transcription factor Lmx1a/b as a key regulator of the development of these hindbrain structures, and further describe the role of additional transcription factors that specify the cochlear hair cells and sensory neurons and the hindbrain auditory nuclei. Most of the information presented has been obtained from studies on mice, both wild type and various genetic mutant lines.

The longitudinal regionalization of the hindbrain

The rostrocaudal extent of the hindbrain (Fig. 1A) is defined by visible external morphological features in craniate vertebrates. The sulcus isthmus encompasses the midbrain/hindbrain boundary (MHB) and adjacent isthmus, and the caudal limit of the calamus scriptorius sets the hindbrain/spinal cord boundary (Fritzsch and Glover, 2006; Watson et al., 2017). Of these two boundaries, the MHB is best understood in a molecular context. The MHB forms through a sequential and partially overlapping expression of specific genes. For example, *Lmx1b* (Millen et al., 2014; Mishima et al., 2009) is needed to stabilize the expression of *Wnt1* (McMahon and Bradley, 1990) and to upregulate and maintain the expression of *Fgf8* (Guo et al., 2007; Lee et al., 1997; Watson et al., 2017), which initiates the proliferation of neuronal progenitors specific to the isthmus.

Between these two extremes lie His' calamus scriptorius region (containing the gracile and cuneate nuclei), 'Nachhirn' (medulla oblongata), and 'Hinterhirn' (pons), which he evidently distinguished in large part based on the shape and appearance of the 4th ventricle (Fig. 1A). The differential specification of their constituent neuron populations is driven by a complex interaction of signaling systems and transcription factors in which retinoic acid, fibroblast growth factors (*Fgf8*) and *Hox* genes play pivotal roles (Glover et al., 2006; Parker and Krumlauf, 2017). A similar mechanism, albeit without the elements that instate physical segmentation, is likely to underlie the specification of the cryptic pseudorhombomeres which continue in sequence from the last true rhombomere (rhombomere 6, r6) to pseudorhombomere 11 (Tomás-Roca et al., 2016). Based on a comparison of His' drawings and contemporary studies of overt and cryptic hindbrain segmentation, the first rhombomere lies within what His described as the isthmus, r2-r4 correspond to the Hinterhirn (pons), r5 and r6 to the upper part of the Nachhirn (medulla oblongata), and r7-r11 to the lower part of the Nachhirn and the calamus scriptorius region (Fig. 3). A recent study indicates that, in its modern definition, the isthmus is a domain separate and immediately rostral to r1 (thus receiving the monicker "r0"), and

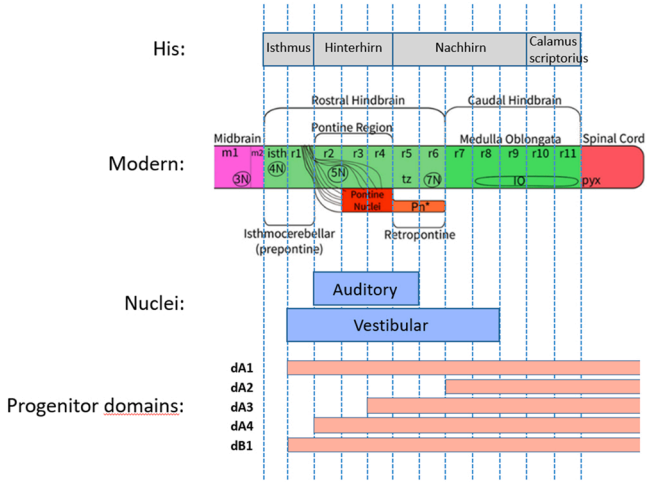

Fig. 3. The relationship between His' hindbrain regions, modern hindbrain neuromeric organization, the auditory and vestibular nuclear complexes, and the 5 most dorsal progenitor domains. Modified after (Diaz and Glover, 2021; Diek, et al., 2022).

specified by the expression of *Fgf8* [(Watson, et al., 2017)].

The Hinterhirn, or pons, is of particular interest, since recent work has shown that its traditional definition, based on the outward ventral bulge associated with the basilar pontine nuclei, does not represent a core division of the neural tube, but is rather a ventral appendix to the rhombencephalon (Nieuwenhuys and Puelles, 2015; Puelles and Ferran, 2012). Recent studies have shown that the pontine nuclei originate from the rhombic lips through migration, with the neurons eventually settling on the ventral surface of the hindbrain several rhombomeres more rostral. Moreover, their final location along the series of rhombomeres varies among species, and supernumerary pontine nuclei, generated through genetic manipulation of migratory mechanisms, can settle at ectopic ventral locations (Di Bonito et al., 2017; Di Meglio et al., 2013).

Hindbrain dorsoventral patterning is an elaboration of spinal cord dorsoventral patterning

The hindbrain rhombomeres and pseudorhombomeres exhibit the same basic pattern of dorsoventral gene expression as the spinal cord (Diek et al., 2022; Hernandez-Miranda et al., 2017; Lai et al., 2016), but with several features that are unique to r0-r7. One example is *Wnt* gene expression, which shows greater variation in the rhombencephalon than in the spinal cord (Merzdorf and Forecki, 2018), thereby influencing the shape changes associated with IVth ventricle formation. *Wnt* genes play a major role in dorsoventral patterning by establishing, with *BMPs*, a dorsal signaling gradient that interacts with an opposing ventral signaling gradient established by *Shh* and *Gli*. Wnt genes and their downstream targets are therefore instrumental in specifying dorsal hindbrain structures such as the choroid plexus and neuron populations such as the oculomotor and trochlear motoneurons (Jahan et al., 2021).

Complex interactions among a set of basic Helix-Loop-Helix (bHLH) genes establish a series of dorsal progenitor domains (dA1-dA4 and dB1-dB4, see Fig. 3). These domains arise through cross-repressive reciprocal interactions among genes that are selectively expressed at different dorsal levels, which sharpens their mutual expression boundaries (Lai, et al., 2016). Such interactions define the boundaries of the most dorsal progenitor domains dA1-dA4, characterized by the expression of (in sequence starting from the roof plate) *Atoh1, Neurog1/2, Ascl1* and *Ptf1a,* each in combination with *Olig3*. Additional transcription factors such as Phox2b contribute to this hindbrain-specific dorsal patterning. The end result is that the hindbrain shares with the spinal cord six dorsal progenitor domains, but has two additional dorsal progenitor domains not found in the spinal cord (Hernandez-Miranda et al., 2017; Lunde et al., 2019).

In addition, roof plate development is regulated by *Lmx1a/b* and *Gdf7,* whose expression is triggered by high *BMP* and *Wnt* levels. Recent work has shown that the unique dorsal morphology of the hindbrain and the formation of the rhombencephalic choroid plexus depend on these two genes (Glover, et al., 2018;Lee et al., 2000;Mishima, et al., 2009). For example, in *Lmx1a/b* double null mice, the choroid plexus never forms, and the dorsal part of the hindbrain is transformed into a rostral elongation of the spinal cord (Chizhikov et al., 2021; Elliott et al., 2021).

Several features of gene expression lead to rhombomere-specific modifications of the dorsal progenitor domains. *Ptf1a* is expressed in apparent hindbrain-specific duplicate variants (Hernandez-Miranda et al., 2017), a situation that results in an alteration of dorsal cell fates in r0-r7 in *Ptfi1a* null mice (Diek et al., 2022; Iskusnykh et al., 2016; Lowenstein et al., 2021). Specifically, there is a loss of *Neurog1/2* in r1-r6 and a partial loss of *Ascl1* in r1-r3 (Hernandez-Miranda et al., 2017). A unique combinatorial interaction of *Atoh1, Neurog1/2, Olig3* and *Ptf1a,* among other genes (Lowenstein et al., 2021; Pan et al., 2009), defines the cerebellum (Fig. 4). A delayed expression of *Neurod1* adds to the interaction by providing a cerebellum-specific negative feedback of *Atoh1* (Kersigo et al., 2021). This helps to establish the rostral limit of the auditory nuclei, whose development depends on a higher level of *Atoh1* expression (Pan et al., 2009). *Lmx1a/b, Fgf8* and *Wnt1* are also

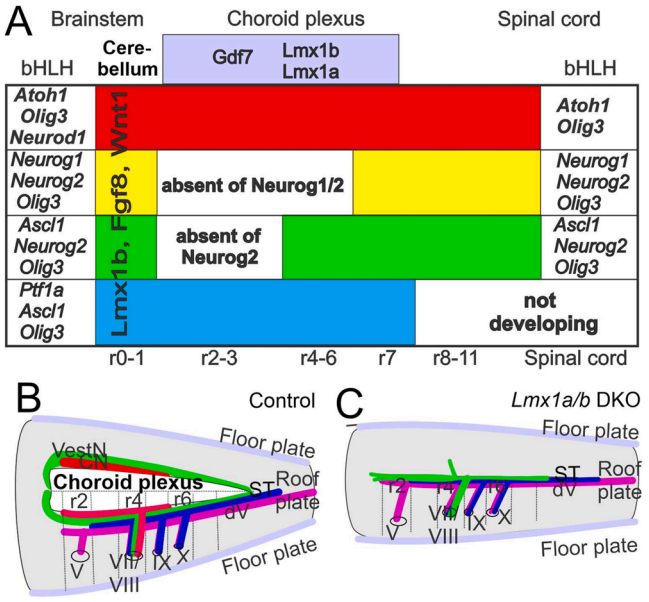

Fig. 4. Dorsal development of the hindbrain depends on *Lmx1a/b*. (A) The choroid plexus originates from r2 to r7 (light purple) and depends on the expression of *Lmx1a/b* and *Gdf7*. The bHLH genes *Atoh1*, *Neurog1*, *Neurog2, Neurod1, Ascl1, Olig3* and *Ptf1a* are expressed combinatorially in different dorsoventral domains with different longitudinal extents. The domain expressing the combination of Atoh1, Olig3 and Neurod1 (dA1, red) is continuous from the spinal cord up to the cerebellum. The domain expressing the combination of Neurog1, Neurog2 and Olig 3 (dA2, yellow) is interrupted in r2–6 where expression of Neurog1 and 2 is absent. The domain expressing the combination of Ascl1, Neurog2 and Olig3 (dA3, green) is interrupted at r2-3 where expression of Neurog2 is absent. The domain expressing the combination of Ptf1a, Ascl2 and Olig3 (dA4, blue) is continuous from r7 up to the cerebellum (Ptf1a expression is absent more caudally). All of these genes as well as *Lmx1b, Fgf8* and *Wnt1* are expressed in the cerebellum at r0-r1. (B, C) Depictions of structures and axon projections in hindbrains that have been slit along the ventral midline and folded out. (B) Normal hindbrain. (C) Hindbrain lacking *Lmx1a/b* expression (Lmx1a/b DKO). In this case, the choroid plexus and auditory nuclei do not form, the roof plate remains narrow, and vestibular sensory neurons project axons across it. Abbreviations: dV, trigeminal axons; ST, solitary tract axons; V, VII, VIII, IX, X, afferent fibers. Reprinted with permission from (Chizhikov et al., 2021; Elliott et al., 2021; Hernandez-Miranda et al., 2017).

important in regulating cerebellar development (Glover et al., 2018; Jahan et al., 2021; Watson et al., 2017).

Atoh1 and *Olig3* are expressed in the spinal cord, the hindbrain and the cerebellum (Bermingham et al., 2001; Farago et al., 2006; Fritzsch et al., 2006; Hernandez-Miranda et al., 2017; Pan et al., 2009). Complete knockout of *Atoh1* expression using *Wnt1-cre* upstream of *Atoh1* (Wang et al., 2005) leads to the loss of all hindbrain neurons that depend on *Atoh1/Olig3,* leaving only the choroid plexus (Elliott et al., 2017). In contrast, some *Atoh1*-positive neurons develop in *Olig3*-null mice, indicating that *Olig3* is less critical than *Atoh1* in mediating dorsal hindbrain neurogenesis (Lowenstein et al., 2021). Lack of *Gdf7* (Lee et al., 2000) or *Lmx1a/b* expression (Mishima et al., 2009) abolishes *Atoh1* expression and leads to the same phenotype (Figs. 4, 5).

In the absence of *Lmx1a/b,* projections to the cerebellum are perturbed, including unusual central vestibular and solitary tract projections, which are free to cross the dorsal midline due to the aberrantly closed roof plate [Figs. 4, 6; (Elliott et al., 2021)]. Neither electroreception nor auditory projections reach the cerebellum (Fig. 4), although in other specific gene mutations auditory fibers are perturbed but can be transiently traced to the cerebellum (Schmidt and Fritzsch, 2019).

In summary, the development of dorsal hindbrain structures including the auditory nuclei depends on complex interactions among specific bHLH genes. Lack of expression of the key gene *Lmx1a/b* leads to a loss of the choroid plexus and of *Atoh1* expression. The latter effect impacts on the expression of other dorsal patterning genes (*Neurog1, Neurog2, Neurod1, Olig3, Ascl1 and Ptf1a*) resulting in additional perturbance of the development of dorsal hindbrain nuclei.

Generation of cochlear sensory neurons depends on Eya1, Sox2, Neurog1 and Neurod1

Spiral ganglion neurons (SGNs) have been classified into type Ia, Ib, Ic and type II (Elliott et al., 2021; Petitpré et al., 2022). The development of SGNs depends on a set of genes that collectively regulate both proliferation and specification. In contrast, expression of a different set of genes defines vestibular ganglion neurons (Sun et al., 2022) that also depend on *BDNF* and *TrkB* for normal development and viability (Elliott et al., 2021).

In the earliest stage of neurogenesis, *Eya1* interacts with *Brg1* to initiate pro-neurosensory development (Xu et al., 2021). In the absence of *Eya1* there is no neuronal development whatsoever, leading to formation of an inner ear in which neither neurons nor hair cells differentiate (Xu, et al., 2021). A crucial next step is the initiation of *Sox2* expression, which is needed to upregulate *Neurog1* (Kageyama et al., 2019; Riddiford and Schlosser, 2016). *Neurog1* (Ma et al., 2000), *Pax2* and *Lmx1a/b* (Bouchard et al., 2010; Chizhikov, et al., 2021) are all essential for SGN development (Fig. 3), but the effect of knocking out or knocking down these and other genes is weaker in the vestibular ganglion than in the cochlear ganglia. Early-differentiating vestibular hair cells and sensory neurons are generated in the absence of *Sox2* and *Neurog1*, whereas their later-differentiating auditory counterparts are not (Dvorakova et al., 2020). A complete loss of SGNs, but only a partial loss of VGNs, occurs in the absence of *Pax2* (Bouchard, et al., 2010), *Gata3* (Duncan and Fritzsch, 2013), *Lmx1a/b* (Chizhikov, et al., 2021), *Fgfr2* (Pirvola et al., 2000), *Shh* (Riccomagno et al., 2002) and *Dicer* (Kersigo et al., 2011). Partial loss of VGNs occurs in the absence of *Fgf10* (Pauley et al., 2003) and *Foxg1* (Hwang et al., 2009;Pauley et al., 2006). In *Sox10* null mice, VGNs develop virtually normally whereas SGNs become disorganized (Mao et al., 2014). In the absence of *Erb2* expression nearly all SGNs are lost but VGNs are only reduced (Morris et al., 2006).

Following the initial formation of neurons triggered by *Eya1, Sox2, Pax2* and *Neurog1/2,* further neuronal differentiation is regulated by another set of genes, starting with *Neurod1* (Alsina, 2020; Macova et al., 2019) and followed by *Isl1, Foxg1, Pou4f1* and *Phox2b* (Alsina, 2020; Filova et al., 2022;Moody and LaMantia, 2015;Sun, et al., 2022) and their interactions with *Shh, BMPs* and *Wnts* (Muthu et al., 2019). Regional regulation of distinct VGN and SGN populations is further defined by downstream genes involved in distinct patterns of innervation (Sun, et al., 2022). For example, the expression of *Calbindin, Calretinin, Pou4f1* and *Peripherin* distinguish connections from the inner and outer hair cells (Elliott et al., 2021; Petitpré et al., 2022; Petitpré et al., 2018; Shrestha et al., 2018; Sun et al., 2018).

Additional interactions regulate differentiation of the different SGN classes. *Neurod1* (Jahan et al., 2010) interacts with *Nhlh1/2* (Krüger et al., 2004), *Isl1* (Filova, et al., 2022), and *Ebf2* (Petitpré, et al., 2022) to initiate the formation of type I and II SGNs (Petitpré et al., 2022; Shrestha et al., 2018; Sun et al., 2018). Several other genes, including *Gata3, Zfhx2* and *Dpf1,* interact to regulate the differentiation of the other three SGN classes (type Ia, type Ib, type II) (Appler et al., 2013; Duncan and Fritzsch, 2013; Karis et al., 2001; Luo et al., 2013; Petitpré et al., 2022), all of which depend additionally on the expression of *Cux2* and *Pou4f2*. *Id2* regulates expression of *Calretinin* and *Pcdh20* in the differentiation of type Ia SGNs. *Runx1* regulates expression of *Lypd1, Calbindin, Calretinin*, and *Pou4f1* in the differentiation of type Ib SGNs (Huang et al., 2001; Petitpré et al., 2022; Shrestha et al., 2018; Sun et al., 2018).

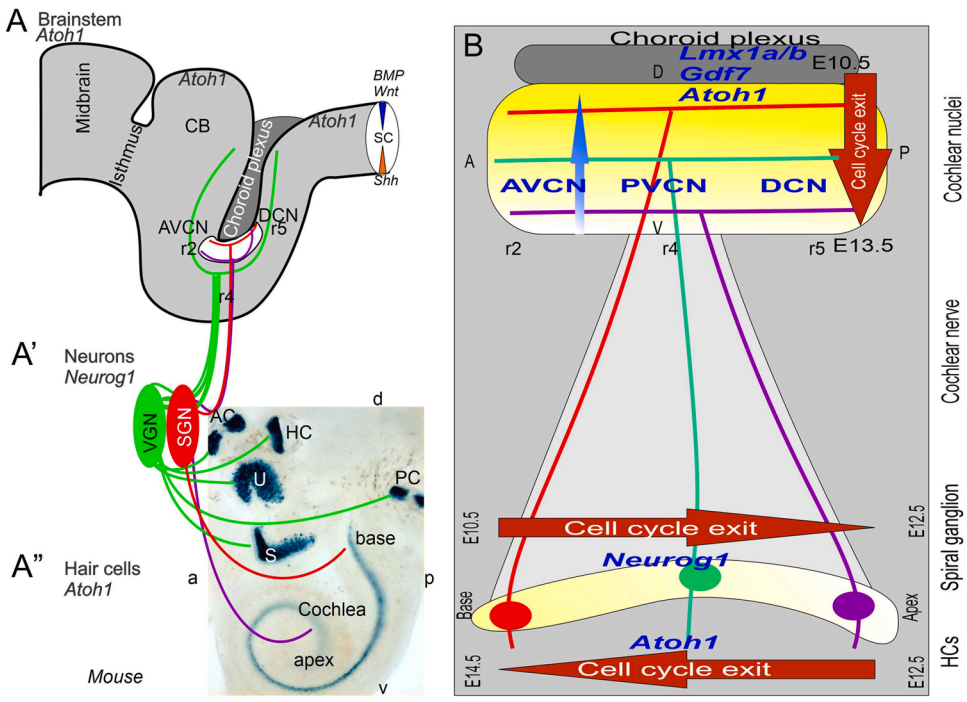

Fig. 5. Cochlear and auditory projection development depends on the expression of specific genes. The development of spiral ganglion neurons (SGN; A') depends on the expression of *Neurog1*. Their projection extends from the cochlea (A", B) and ends in a topologically organized projection in the auditory nuclei (AVCN, PVCN, DCN) whose development depends on the expression of *Atoh1* (A, B). Also shown are the vestibular ganglion neurons (VGN, A'), which project from the 5 vestibular sensory endorgans (anterior, posterior and horizontal semicircular canals, AC, PC, HC, and utricle and saccule, U, S; A") to the central vestibular nuclei and cerebellum (CB; A). SGNs proliferate in a spatiotemporal gradient from the base to the apex of the cochlea during the embryonic period E10.5-E12.5 (B). Their central projections develop topologically from dorsal to ventral positions within the central auditory nuclei over approximately the same period (E10.5.E13.5; B). Later, hair cells proliferate in a gradient from the apex to the base of the cochlea during the period E12.5-E14.5 (B), and contact the peripheral terminals of the SGNs. AC, anterior crista; AVCN, anteroventral cochlear neurons; CB, cerebellum; aLL, pLL, anterior/posterior lateral line neurons; DCN, dorsal cochlear neurons; HC, horizontal crista; PC, posterior crista; r2/4/6, rhombomeres; S, saccule; SC, spinal cord; U, utricle. Modified from (Filova et al., 2022; Fritzsch et al., 2019; Macova et al., 2019; Nichols et al., 2008).

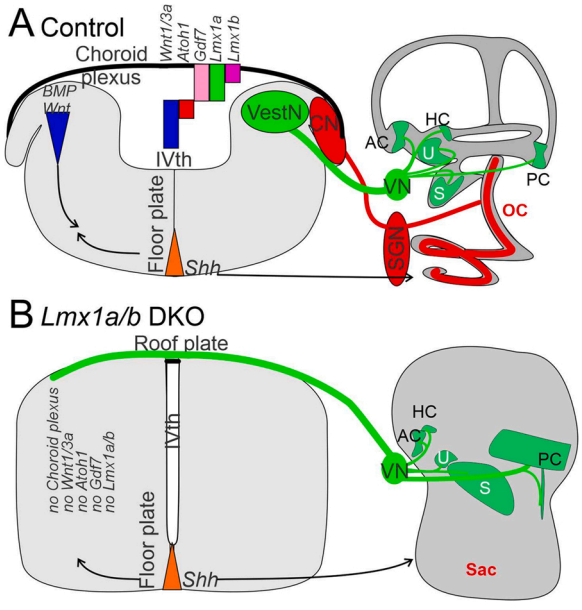

Fig. 6. Central projections of auditory and vestibular sensory afferents depend on proper development of the dorsal hindbrain. In *Lmx1a/b* DKO mice vestibular neurons (VN) project dorsally in the hindbrain as in control mice, whereas auditory projections fail to develop (A, B). However, in *Lmx1a/b* DKO mice, vestibular projections cross the midline through the roof plate, which is continuous across the midline due to the absence of the choroid plexus (B). Choroid plexus development depends on the expression of *Atoh1*, *Gdf7* and *Wnt1/3a* which is lost in the absence of *Lmx1a/b* expression (A, B). AC, HC, PC, anterior, horizontal, posterior cristae; CN, cochlear nuclei; S, saccule; SGN, spiral ganglion neurons; ST, solitary tact; U, utricle; VestN, vestibular nuclei; VN, vestibular ganglion neurons. Reprinted with permission from (Chizhikov et al., 2021; Elliott et al., 2021; Glover et al., 2018; Lee et al., 2000).

A spatiotemporal pattern of SGN development has been demonstrated in mammals (de No, 1981), with differentiation first of basal turn neurons that innervate the anteroventral, posteroventral, and dorsal cochlear nuclei (AVCN, PVCN, DCN) followed, after a delay, by differentiation of apical turn neurons (Filova, et al., 2022; Fritzsch et al., 2019; Schmidt and Fritzsch, 2019) (Fig. 5). SGN neurons develop prior to the cochlear hair cells and central auditory nuclei. SGNs can establish central projections even in the absence of target hair cells (Elliott et al., 2017; Kersigo and Fritzsch, 2015), but expression of *Neurod1, Wnts, Fzd, Npr2* and *Ephrins* is required for proper targeting of the central projections (Duncan et al., 2019; Macova et al., 2019; Milinkeviciute and Cramer, 2021; Schmidt and Fritzsch, 2019). Additional work is needed to characterize selectively the development of the central terminations of the different SGN classes (Filova et al., 2022; Filova, et al., 2022; Muniak, et al., 2016; Muniak and Ryugo, 2014).

In summary, the SGNs are generated and diversified through the action of a set of genes downstream of *Neurog1*. Shortly after their generation, SGNs innervate their target hair cells, but they can differentiate and establish their central projections even in the absence of these peripheral targets. Segregation of the central projections follows a spatiotemporal, topological sequence that is also dependent on the expression of specific genes by the neurons of the central auditory nuclei (Fig. 5).

Hair cell differentiation depends on several genes including Lmx1a/b

Mechanosensory hair cells are utilized in vestibular, cochlear, lateral line, electroreceptor and Merkel cell-mediated somatosensory signaling (Chagnaud et al., 2017; Elliott et al., 2021). Evolutionary evidence suggests that hair cells derived from the unicellular choanoflagellates (Arendt et al., 2016; Fritzsch and Straka, 2014), through a transformation of the single flagellum surrounded by villi of choanoflagellates into the kinocilium/stereocilia bundle that distinguishes hair cells. The specification of inner ear hair cells begins with the actions of

Eya1/Six1 (Ahmed et al., 2012), *Pax2/8* (Bouchard, et al., 2010), *Shh* (Muthu, et al., 2019), *BMPs* (Ohyama et al., 2010) and *Wnts* (Wright et al., 2015) during formation of the otocyst. Upregulation of *Sox2* (Dvorakova, et al., 2020) sets up hair cell differentiation within the otic sensory epithelium. This depends on interactions between *Atoh1* and *Neurod1* (Filova et al., 2020), *Pou4f3* (Li et al., 2020; Xiang et al., 2003), *Gfi1* (Hertzano et al., 2004), *Srrm/Rest* (Nakano et al., 2020) and *Barhl1* (Chellappa et al., 2008).

Lmx1a null mutant mice (Huang et al., 2018; Koo et al., 2009; Nichols et al., 2020; Nichols et al., 2008; Steffes et al., 2012) and various human *LMX1A* mutations (Lee et al., 2020; Oziębło et al., 2022; Schrauwen et al., 2018; Wesdorp et al., 2018) exhibit auditory and vestibular defects that can be linked to impaired hair cell differentiation. *Lmx1a* null mouse mutants appear to be completely deaf (Steffes et al., 2012), and humans with *LMX1A* mutations exhibit partial hearing loss (Lee et al., 2022; Schrauwen et al., 2018; Wesdorp et al., 2018). *Lmx1a/b* double KO mice completely lack the cochlea (Chizhikov et al., 2021; Elliott et al., 2021) (Fig. 5).

The differentiation of functionally mature hair cells depends on several genes that encode key functional proteins. The stereocilia are interconnected by tip link proteins such as PCDH15 and CDH23, which regulate hair cell morphogenesis during differentiation and function as the mechanical transducers for opening the mechanoelectrical transduction channels (METs) in mature hair cells. MET opening permits endolymphatic potassium to enter and depolarize the hair cells (Elliott et al., 2018). METs are composed in part by the transmembrane proteins *Tmc1* and, transiently, *Tmc2* (Marcovich and Holt, 2020; Shibata et al., 2016). Auditory hair cells in mammals depend further on the expression of *Vangl2*, *Dvl1*, *Celsr1* and *Gal2,* regulated through the planar cell polarity (PCP) pathway (Tarchini et al., 2013). *Emx2* and *Jag1* are both required for the normal development of outer hair cells (Holley et al., 2010; Jiang et al., 2017).

Summary and Conclusion

In summary, Wilhelm His Sr. not only laid the foundation for understanding the neural crest as the origin of much of the peripheral sensory nervous system (Glover et al., 2018), but also contributed profound insight into the importance of the adjacent rhombic lip in the formation of central neuron populations novel to the hindbrain. He thus made seminal contributions to our understanding of the evolutionary elaboration of a basic spinal cord organization into the more complex and functionally diverse hindbrain region. Contemporary molecular studies have defined the critical dependence of central hindbrain nuclei and specific cell types on the expression of particular genes (including *Atoh1, Neurog1/2, Olig3, Ascl1* and *Ptf1a* for central nuclei, *Neurog1* for neurons, and *Atoh1* for hair cells) (Hernandez-Miranda et al., 2017; Lunde, et al., 2019). Development of dorsal structures such as the roof plate and choroid plexus depends on the expression of *Lmx1a/b, BMPs* and *Wnts* (Chizhikov et al., 2021; Elliott et al., 2021; Glover et al., 2018). In the absence of *Lmx1a/b* the choroid plexus and auditory nuclei do not form (Fig. 6). The elegant and detailed anatomical descriptions that His made of hindbrain-specific specializations are thus being validated at the molecular level over a century later.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

"Compliance with Ethical Statements"

N/A.

Conflict of interest statement

The corresponding author states that there are no conflicts of interest.

Acknowledgements

This work was supported by the NIH (R01 AG060504).

References

Ahmed, M., Xu, J., Xu, P.-X., 2012. EYA1 and SIX1 drive the neuronal developmental program in cooperation with the SWI/SNF chromatin-remodeling complex and SOX2 in the mammalian inner ear. Development 139, 1965–1977.
Alsina, B., 2020. Mechanisms of cell specification and differentiation in vertebrate cranial sensory systems. Curr. Opin. Cell Biol. 67, 79–85.
Appler, J.M., Lu, C.C., Druckenbrod, N.R., Yu, W.M., Koundakjian, E.J., Goodrich, L.V., 2013. Gata3 is a critical regulator of cochlear wiring. J. Neurosci. 33, 3679–3691.
Arendt, D., Musser, J.M., Baker, C.V., Bergman, A., Cepko, C., Erwin, D.H., Pavlicev, M., Schlosser, G., et al., 2016. The origin and evolution of cell types. Nat. Rev. Genet. 17, 744–757.
Bermingham, N.A., Hassan, B.A., Wang, V.Y., Fernandez, M., Banfi, S., Bellen, H.J., Fritzsch, B., Zoghbi, H.Y., 2001. Proprioceptor pathway development is dependent on Math1. Neuron 30, 411–422.
Bouchard, M., de Caprona, D., Busslinger, M., Xu, P., Fritzsch, B., 2010. Pax2 and Pax8 cooperate in mouse inner ear morphogenesis and innervation. BMC Dev. Biol. 10:89.
Chagnaud, B.P., Engelmann, J., Fritzsch, B., Glover, J.C., Straka, H., 2017. Sensing external and self-motion with hair cells: a comparison of the lateral line and vestibular systems from a developmental and evolutionary perspective. Brain, Behav. Evol. 90, 98–116.
Chellappa, R., Li, S., Pauley, S., Jahan, I., Jin, K., Xiang, M., 2008. Barhl1 regulatory sequences required for cell-specific gene expression and autoregulation in the inner ear and central nervous system. Mol. Cell. Biol. 28, 1905–1914.
Chizhikov, V.V., Iskusnykh, I.Y., Fattakhov, N., Fritzsch, B., 2021. Lmx1a and Lmx1b are Redundantly Required for the Development of Multiple Components of the Mammalian Auditory System. Neuroscience 452, 247–264.
Di Bonito, M., Studer, M., Puelles, L., 2017. Nuclear derivatives and axonal projections originating from rhombomere 4 in the mouse hindbrain. Brain Struct. Funct. 222, 3509–3542.
Di Meglio, T., Kratochwil, C.F., Vilain, N., Loche, A., Vitobello, A., Yonehara, K., Hrycaj, S.M., Roska, B., et al., 2013. Ezh2 orchestrates topographic migration and connectivity of mouse precerebellar neurons. Science 339, 204–207.
Diaz, C., Glover, J.C., 2021. The vestibular column in the mouse: a rhombomeric perspective. Front. Neuroanat. 15.
Diek, D., Smidt, M.P., Mesman, S., 2022. Molecular organization and patterning of the medulla oblongata in health and disease. Int. J. Mol. Sci. 23, 9260.
Duncan, J.S., Fritzsch, B., 2013. Continued expression of GATA3 is necessary for cochlear neurosensory development. PloS One 8, e62046.
Duncan, J.S., Fritzsch, B., Houston, D.W., Ketchum, E.M., Kersigo, J., Deans, M.R., Elliott, K.L., 2019. Topologically correct central projections of tetrapod inner ear afferents require Fzd3. Sci. Rep. 9, 10298.
Dvorakova, M., Macova, I., Bohuslavova, R., Anderova, M., Fritzsch, B., Pavlinkova, G., 2020. Early ear neuronal development, but not olfactory or lens development, can proceed without SOX2. Dev. Biol. 457, 43–56.
Elliott, K.L., Fritzsch, B., Duncan, J.S., 2018. Evolutionary and Developmental Biology Provide Insights Into the Regeneration of Organ of Corti Hair Cells. Front. Cell. Neurosci. 12, 252.
Elliott, K.L., Kersigo, J., Pan, N., Jahan, I., Fritzsch, B., 2017. Spiral ganglion neuron projection development to the hindbrain in mice lacking peripheral and/or central target differentiation. Front. Neural Circuits 11, 25.
Elliott, K.L., Kersigo, J., Lee, J.H., Yamoah, E.N., Fritzsch, B., 2021. Sustained Loss of Bdnf affects peripheral but not central vestibular targets. Front. Neurol. 12, 768456.
Elliott, K.L., Pavlínková, G., Chizhikov, V.V., Yamoah, E.N., Fritzsch, B., 2021. Development in the Mammalian auditory system depends on transcription factors. Int. J. Mol. Sci. 22, 4189.
Elliott, K.L., Kersigo, J., Lee, J.H., Jahan, I., Pavlinkova, G., Fritzsch, B., Yamoah, E.N., 2021. Developmental changes in peripherin-eGFP expression in spiral ganglion neurons. Front. Cell. Neurosci. 15, 181.
Farago, A.F., Awatramani, R.B., Dymecki, S.M., 2006. Assembly of the brainstem cochlear nuclear complex is revealed by intersectional and subtractive genetic fate maps. Neuron 50, 205–218.
Filova, I., Bohuslavova, R., Tavakoli, M., Yamoah, E.N., Fritzsch, B., Pavlinkova, G., 2022. Early deletion of neurod1 alters neuronal lineage potential and diminishes neurogenesis in the inner ear. Front Cell Dev. Biol. 10, 845461.
Filova, I., Dvorakova, M., Bohuslavova, R., Pavlinek, A., Elliott, K.L., Vochyanova, S., Fritzsch, B., Pavlinkova, G., 2020. Combined Atoh1 and Neurod1 deletion reveals autonomous growth of auditory nerve fibers. Mol. Neurobiol. 57, 5307–5323.
Filova, I., Pysanenko, K., Tavakoli, M., Vochyanova, S., Dvorakova, M., Bohuslavova, R., Smolik, O., Fabriciova, V., et al., 2022. ISL1 is necessary for auditory neuron development and contributes toward tonotopic organization. Proc. Natl. Acad. Sci. USA 119, e2207433119.

Fritzsch B., Glover J. (2006), Evolution of the Deuterostome Central Nervous System: An Intercalation of Developmental Patterning Processes with Cellular Specification Processes-2.01.
Fritzsch, B., Straka, H., 2014. Evolution of vertebrate mechanosensory hair cells and inner ears: toward identifying stimuli that select mutation driven altered morphologies. J. Comp. Physiol. A 200, 5–18.
Fritzsch, B., Elliott, K.L., 2017. Gene, cell, and organ multiplication drives inner ear evolution. Dev. Biol. 431, 3–15.
Fritzsch, B., Elliott, K.L., Pavlinkova, G., 2019. Primary sensory map formations reflect unique needs and molecular cues specific to each sensory system. F1000Research 8.
Fritzsch, B., Pauley, S., Feng, F., Matei, V., Nichols, D., 2006. The molecular and developmental basis of the evolution of the vertebrate auditory system. Int. J. Comp. Psychol. 19, 1–25.
Glover, J.C., Renaud, J.S., Rijli, F.M., 2006. Retinoic acid and hindbrain patterning. Dev. Neurobiol. 66, 705–725.
Glover, J.C., Elliott, K.L., Erives, A., Chizhikov, V.V., Fritzsch, B., 2018. Wilhelm His' lasting insights into hindbrain and cranial ganglia development and evolution. Dev. Biol. 444, S14–S24.
Guo, C., Qiu, H.-Y., Huang, Y., Chen, H., Yang, R.-Q., Chen, S.-D., Johnson, R.L., Chen, Z.-F., et al., 2007. Lmx1b is essential for Fgf8 and Wnt1 expression in the isthmic organizer during tectum and cerebellum development in mice. Development 134, 317–325.
Held, H., 1893. Die centrale gehörleitung. Arch. Anat. Physiol. Anat. Abt. 17, 201–248.
Hernandez-Miranda, L.R., Müller, T., Birchmeier, C., 2017. The dorsal spinal cord and hindbrain: From developmental mechanisms to functional circuits. Dev. Biol. 432, 34–42.
Herrick, C.J., 1948. The brain of the tiger salamander. Ambystoma tigrinum.
Hertzano, R., Montcouquiol, M., Rashi-Elkeles, S., Elkon, R., Yucel, R., Frankel, W.N., Rechavi, G., Moroy, T., et al., 2004. Transcription profiling of inner ears from Pou4f3 (ddl/ddl) identifies Gfi1 as a target of the Pou4f3 deafness gene. Hum. Mol. Genet 13, 2143–2153.
His, W., 1868. Untersuchungen über die erste Anlage des Wirbelthierleibes: die erste Entwickelung des Hühnchens im Ei. FCW Vogel.
His, W., 1870. Beschreibung eines Mikrotoms. Arch. für Mikrosk. Anat. 6, 229–232.
His, W., 1880. Anatomie menschlicher embryonen. FCW Vogel.
His Jr, W., 1889. Zur Entwicklungsgeschichte des Acoustico-Facialis Gebietes beim Menschen. Arch. F. Anat. U. Physiol. supp:1.
His W. (1890) Histogenese und Zusammenhang der Nervenelemente.
His, W., 1895. Die anatomische nomenclatur: nomina anatomica. Veit.
His, W., 1897. Address upon the development of the brain. Trans. R. Acad. Med. Irel. 15, 1.
His, W., 1904. Die Entwickelung des menschlichen Gehirns während der ersten Monate. S. Hirzel.
Holley, M., Rhodes, C., Kneebone, A., Herde, M.K., Fleming, M., Steel, K.P., 2010. Emx2 and early hair cell development in the mouse inner ear. Dev. Biol. 340, 547–556.
Huang, E.J., Liu, W., Fritzsch, B., Bianchi, L.M., Reichardt, L.F., Xiang, M., 2001. Brn3a is a transcriptional regulator of soma size, target field innervation and axon pathfinding of inner ear sensory neurons. Development 128, 2421–2432.
Huang, Y., Hill, J., Yatteau, A., Wong, L., Jiang, T., Petrovic, J., Gan, L., Dong, L., et al., 2018. Reciprocal Negative Regulation Between Lmx1a and Lmo4 Is Required for Inner Ear Formation. J. Neurosci. 38, 5429–5440.
Hwang, C.H., Simeone, A., Lai, E., Wu, D.K., 2009. Foxg1 is required for proper separation and formation of sensory cristae during inner ear development. Dev. Dyn. 238, 2725–2734.
Iskusnykh, I.Y., Steshina, E.Y., Chizhikov, V.V., 2016. Loss of Ptf1a leads to a widespread cell-fate misspecification in the brainstem, affecting the development of somatosensory and viscerosensory nuclei. J. Neurosci. 36, 2691–2710.
Jahan, I., Kersigo, J., Pan, N., Fritzsch, B., 2010. Neurod1 regulates survival and formation of connections in mouse ear and brain. Cell Tissue Res. 341, 95–110.
Jahan, I., Kersigo, J., Elliott, K.L., Fritzsch, B., 2021. Smoothened overexpression causes trochlear motoneurons to reroute and innervate ipsilateral eyes. Cell Tissue Res. 384, 59–72.
Jiang, T., Kindt, K., Wu, D.K., 2017. Transcription factor Emx2 controls stereociliary bundle orientation of sensory hair cells. Elife 6, e23661.
Kageyama, R., Shimojo, H., Ohtsuka, T., 2019. Dynamic control of neural stem cells by bHLH factors. Neurosci. Res. 138, 12–18.
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.
Kersigo, J., Fritzsch, B., 2015. Inner ear hair cells deteriorate in mice engineered to have no or diminished innervation. Front. Aging Neurosci. 7, 33.
Kersigo, J., D'Angelo, A., Gray, B.D., Soukup, G.A., Fritzsch, B., 2011. The role of sensory organs and the forebrain for the development of the craniofacial shape as revealed by Foxg1–cre-mediated microRNA loss. Genesis 49, 326–341.
Kersigo, J., Gu, L., Xu, L., Pan, N., Vijayakuma, S., Jones, T., Shibata, S.B., Fritzsch, B., et al., 2021. Effects of Neurod1 Expression on Mouse and Human Schwannoma Cells. Laryngoscope 131, E259–e270.
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.
Krüger, M., Ruschke, K., Braun, T., 2004. NSCL-1 and NSCL-2 synergistically determine the fate of GnRH-1 neurons and control necdin gene expression. Embo J. 23, 4353–4364.
Lai, H.C., Seal, R.P., Johnson, J.E., 2016. Making sense out of spinal cord somatosensory development. Development 143, 3434–3448.
Lee, K.J., Dietrich, P., Jessell, T.M., 2000. Genetic ablation reveals that the roof plate is essential for dorsal interneuron specification. Nature 403, 734–740.
Lee, S., Danielian, P.S., Fritzsch, B., McMahon, A.P., 1997. Evidence that FGF8 signalling from the midbrain-hindbrain junction regulates growth and polarity in the developing midbrain. Development 124, 959–969.
Lee, S.Y., Han, J.H., Carandang, M., Kim, M.Y., Kim, B., Yi, N., Kim, J., Kim, B.J., et al., 2020. Novel genotype-phenotype correlation of functionally characterized LMX1A variants linked to sensorineural hearing loss. Hum. Mutat. 41, 1877–1883.
Lee, S.Y., Soon Yoo, H., Hee Han, J., Hee Lee, D., Soo Park, S., Hwan Suh, M., Ho Lee, J., Oh, S.H., et al., 2022. Novel Molecular Genetic Etiology of Asymmetric Hearing Loss: Autosomal-Dominant LMX1A Variants. Ear Hear.
Li, J., Zhang, T., Ramakrishnan, A., Fritzsch, B., Xu, J., Wong, E.Y.M., Loh, Y.E., Ding, J., et al., 2020. Dynamic changes in cis-regulatory occupancy by Six1 and its cooperative interactions with distinct cofactors drive lineage-specific gene expression programs during progressive differentiation of the auditory sensory epithelium. Nucleic Acids Res 48, 2880–2896.
Lowenstein, E.D., Rusanova, A., Stelzer, J., Hernaiz-Llorens, M., Schroer, A.E., Epifanova, E., Bladt, F., Isik, E.G., et al., 2021. Olig3 regulates early cerebellar development. Elife 10, e64684.
Lunde, A., Okaty, B.W., Dymecki, S.M., Glover, J.C., 2019. Molecular profiling defines evolutionarily conserved transcription factor signatures of major vestibulospinal neuron groups. Eneuro 6.
Luo, X.J., Deng, M., Xie, X., Huang, L., Wang, H., Jiang, L., Liang, G., Hu, F., et al., 2013. GATA3 controls the specification of prosensory domain and neuronal survival in the mouse cochlea. Hum. Mol. Genet 22, 3609–3623.
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.
Macova, I., Pysanenko, K., Chumak, T., Dvorakova, M., Bohuslavova, R., Syka, J., Fritzsch, B., Pavlinkova, G., 2019. Neurod1 is essential for the primary tonotopic organization and related auditory information processing in the midbrain. J. Neurosci. 39, 984–1004.
Malmierca, M.S., 2015. Auditory system. The Rat Nervous System. Elsevier,, pp. 865–946.
Mao, Y., Reiprich, S., Wegner, M., Fritzsch, B., 2014. Targeted deletion of Sox10 by Wnt1-cre defects neuronal migration and projection in the mouse inner ear. PloS One 9, e94580.
Marcovich, I., Holt, J.R., 2020. Evolution and function of Tmc genes in mammalian hearing. Curr. Opin. Physiol. 18, 11–19.
McMahon, A.P., Bradley, A., 1990. The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. Cell 62, 1073–1085.
Merzdorf, C., Forecki, J., 2018. Amphibian Zic Genes. Zic family. Springer,, pp. 107–140 (vol.).
Milinkeviciute, G., Cramer, K.S., 2021. Development of the ascending auditory pathway. In: Fritzsch, B. (Ed.), The Senses, vol. 2. Elsevier, pp. 337–353.
Millen, K.J., Steshina, E.Y., Iskusnykh, I.Y., Chizhikov, V.V., 2014. Transformation of the cerebellum into more ventral brainstem fates causes cerebellar agenesis in the absence of Ptf1a function. Proc. Natl. Acad. Sci. 111, E1777–E1786.
Mishima, Y., Lindgren, A.G., Chizhikov, V.V., Johnson, R.L., Millen, K.J., 2009. Overlapping function of Lmx1a and Lmx1b in anterior hindbrain roof plate formation and cerebellar growth. J. Neurosci. 29, 11377–11384.
Moody, S.A., LaMantia, A.-S., 2015. Transcriptional regulation of cranial sensory placode development. In: Current Topics in Developmental Biology, vol. 111. Elsevier,, pp. 301–350.
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.
Muniak, M.A., Ryugo, D.K., 2014. Tonotopic organization of vertical cells in the dorsal cochlear nucleus of the CBA/J mouse. J. Comp. Neurol. 522, 937–949.
Muniak, M.A., Connelly, C.J., Suthakar, K., Milinkeviciute, G., Ayeni, F.E., Ryugo, D.K., 2016. Central projections of spiral ganglion neurons. The primary auditory neurons of the mammalian cochlea, vol. Springer,, pp. 157–190.
Muthu, V., Rohacek, A.M., Yao, Y., Rakowiecki, S.M., Brown, A.S., Zhao, Y.-T., Meyers, J., Won, K.-J., et al., 2019. Genomic architecture of Shh-dependent cochlear morphogenesis. Dev. (Camb., Engl.) 146, dev181339.
Nakano, Y., Wiechert, S., Fritzsch, B., Bánfi, B., 2020. Inhibition of a transcriptional repressor rescues hearing in a splicing factor–deficient mouse. Life Sci. Alliance 3, e202000841.
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.
Nichols, D.H., Bouma, J.E., Kopecky, B.J., Jahan, I., Beisel, K.W., He, D.Z.Z., Liu, H., Fritzsch, B., 2020. Interaction with ectopic cochlear crista sensory epithelium disrupts basal cochlear sensory epithelium development in Lmx1a mutant mice. Cell Tissue Res 380, 435–448.
Nieuwenhuys, R., Puelles, L., 2015. Towards a New Neuromorphology. Springer.
de No, R.L., 1981. The Primary Acoustic Nuclei. Raven Press.
O'Neill, P., Mak, S.-S., Fritzsch, B., Ladher, R.K., Baker, C.V., 2012. The amniote paratympanic organ develops from a previously undiscovered sensory placode. Nat. Commun. 3, 1041.
Ohyama, T., Basch, M.L., Mishina, Y., Lyons, K.M., Segil, N., Groves, A.K., 2010. BMP signaling is necessary for patterning the sensory and nonsensory regions of the developing mammalian cochlea. J. Neurosci. 30, 15044–15051.
Ozięblo, D., Lee, S.Y., Leja, M.L., Sarosiak, A., Bałdyga, N., Skarżyński, H., Kim, Y., Han, J.H., et al., 2022. Update on CD164 and LMX1A genes to strengthen their causative role in autosomal dominant hearing loss. Hum. Genet 141, 445–453.

Pan, N., Jahan, I., Lee, J.E., Fritzsch, B., 2009. Defects in the cerebella of conditional Neurod1 null mice correlate with effective Tg (Atoh1-cre) recombination and granule cell requirements for Neurod1 for differentiation. Cell Tissue Res. 337, 407–428.

Parker, H.J., Krumlauf, R., 2017. Segmental arithmetic: summing up the Hox gene regulatory network for hindbrain development in chordates. Wiley Interdiscip. Rev.: Dev. Biol. 6.

Pauley, S., Lai, E., Fritzsch, B., 2006. Foxg1 is required for morphogenesis and histogenesis of the mammalian inner ear. Dev. Dyn.: Off. Publ. Am. Assoc. Anat. 235, 2470–2482.

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.: Off. Publ. Am. Assoc. Anat. 227, 203–215.

Petitpré, C., Wu, H., Sharma, A., Tokarska, A., Fontanet, P., Wang, Y., Helmbacher, F., Yackle, K., et al., 2018. Neuronal heterogeneity and stereotyped connectivity in the auditory afferent system. Nat. Commun. 9, 3691.

Petitpré, C., Faure, L., Uhl, P., Fontanet, P., Filova, I., Pavlinkova, G., Adameyko, I., Hadjab, S., et al., 2022. Single-cell RNA-sequencing analysis of the developing mouse inner ear identifies molecular logic of auditory neuron diversification. Nature. Communications 13, 1–15.

Pirvola, U., Spencer-Dene, B., Xing-Qun, L., Kettunen, P., Thesleff, I., Fritzsch, B., Dickson, C., Ylikoski, J., 2000. FGF/FGFR-2 (IIIb) signaling is essential for inner ear morphogenesis. J. Neurosci. 20, 6125–6134.

Puelles, L., Ferran, J.L., 2012. Concept of neural genoarchitecture and its genomic fundament. Front. Neuroanat. 6, 47.

Ray, R.S., Dymecki, S.M., 2009. Rautenlippe Redux—toward a unified view of the precerebellar rhombic lip. Curr. Opin. Cell Biol. 21, 741–747.

Retzius, G., 1884. Das Gehörorgan der Reptilien, der Vögel und der Säugethiere. Samson &, Wallin, pp. 1–368.

Riccomagno, M.M., Martinu, L., Mulheisen, M., Wu, D.K., Epstein, D.J., 2002. Specification of the mammalian cochlea is dependent on Sonic hedgehog. Genes Dev. 16, 2365–2378.

Riddiford, N., Schlosser, G., 2016. Dissecting the pre-placodal transcriptome to reveal presumptive direct targets of Six1 and Eya1 in cranial placodes. Elife 5, e17666.

Schlosser, G., 2017. From so simple a beginning–what amphioxus can teach us about placode evolution. Int. J. Dev. Biol. 61, 633–648.

Schmidt, H., Fritzsch, B., 2019. Npr2 null mutants show initial overshooting followed by reduction of spiral ganglion axon projections combined with near-normal cochleotopic projection. Cell Tissue Res. 1–18.

Schrauwen, I., Chakchouk, I., Liaqat, K., Jan, A., Nasir, A., Hussain, S., Nickerson, D.A., Bamshad, M.J., et al., 2018. A variant in LMX1A causes autosomal recessive severe-to-profound hearing impairment. Hum. Genet 137, 471–478.

Shibata, S.B., Ranum, P.T., Moteki, H., Pan, B., Goodwin, A.T., Goodman, S.S., Abbas, P. J., Holt, J.R., et al., 2016. RNA interference prevents autosomal-dominant hearing loss. Am. J. Hum. Genet. 98, 1101–1113.

Shrestha, B.R., Chia, C., Wu, L., Kujawa, S.G., Liberman, M.C., Goodrich, L.V., 2018. Sensory neuron diversity in the inner ear is shaped by activity. Cell 174, 1229–1246 e1217.

Steffes, G., Lorente-Cánovas, B., Pearson, S., Brooker, R.H., Spiden, S., Kiernan, A.E., Guénet, J.L., Steel, K.P., 2012. Mutanlallemand (mtl) and Belly Spot and Deafness (bsd) are two new mutations of Lmx1a causing severe cochlear and vestibular defects. PLoS One 7, e51065.

Sun, S., Babola, T., Pregernig, G., So, K.S., Nguyen, M., Su, S.-S.M., Palermo, A.T., Bergles, D.E., et al., 2018. Hair cell mechanotransduction regulates spontaneous activity and spiral ganglion subtype specification in the auditory system. Cell 174, 1247–1263 e1215.

Sun, Y., Wang, L., Zhu, T., Wu, B., Wang, G., Luo, Z., Li, C., Wei, W., et al., 2022. Single-cell transcriptomic landscapes of the otic neuronal lineage at multiple early embryonic ages. Cell Rep. 38, 110542.

Tarchini, B., Jolicoeur, C., Cayouette, M., 2013. A molecular blueprint at the apical surface establishes planar asymmetry in cochlear hair cells. Dev. Cell 27, 88–102.

Tomás-Roca, L., Corral-San-Miguel, R., Aroca, P., Puelles, L., Marín, F., 2016. Crypto-rhombomeres of the mouse medulla oblongata, defined by molecular and morphological features. Brain Struct. Funct. 221, 815–838.

Von Kupffer C, 1891. The development of the cranial nerves of vertebrates. J. Comp. Neurol. 1, 246–264.

Wang, V.Y., Rose, M.F., Zoghbi, H.Y., 2005. Math1 expression redefines the rhombic lip derivatives and reveals novel lineages within the brainstem and cerebellum. Neuron 48, 31–43.

Watson, C., Shimogori, T., Puelles, L., 2017. Mouse Fgf8–Cre-LacZ lineage analysis defines the territory of the postnatal mammalian isthmus. J. Comp. Neurol. 525, 2782–2799.

Wesdorp, M., de Koning Gans, P.A.M., Schraders, M., Oostrik, J., Huynen, M.A., Venselaar, H., Beynon, A.J., van Gaalen, J., et al., 2018. Heterozygous missense variants of LMX1A lead to nonsyndromic hearing impairment and vestibular dysfunction. Hum. Genet 137, 389–400.

Wright, K.D., Rogers, A.A.M., Zhang, J., Shim, K., 2015. Cooperative and independent functions of FGF and Wnt signaling during early inner ear development. BMC Dev. Biol. 15, 1–15.

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, J., Li, J., Zhang, T., Jiang, H., Ramakrishnan, A., Fritzsch, B., Shen, L., Xu, P.X., 2021. Chromatin remodelers and lineage-specific factors interact to target enhancers to establish proneurosensory fate within otic ectoderm. PNAS 118, 1–12.

y Cajal, S.R., 1911. Histologie du système nerveux de l'homme & des vertébrés: Cervelet, cerveau moyen, rétine, couche optique, corps strié, écorce cérébrale générale & régionale, grand sympathique. A. Maloine.