



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

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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

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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

draw 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 (Fritsch and Elliott, 2017; Hernandez-Miranda et al., 2017; Millen et al., 2014; Mishima

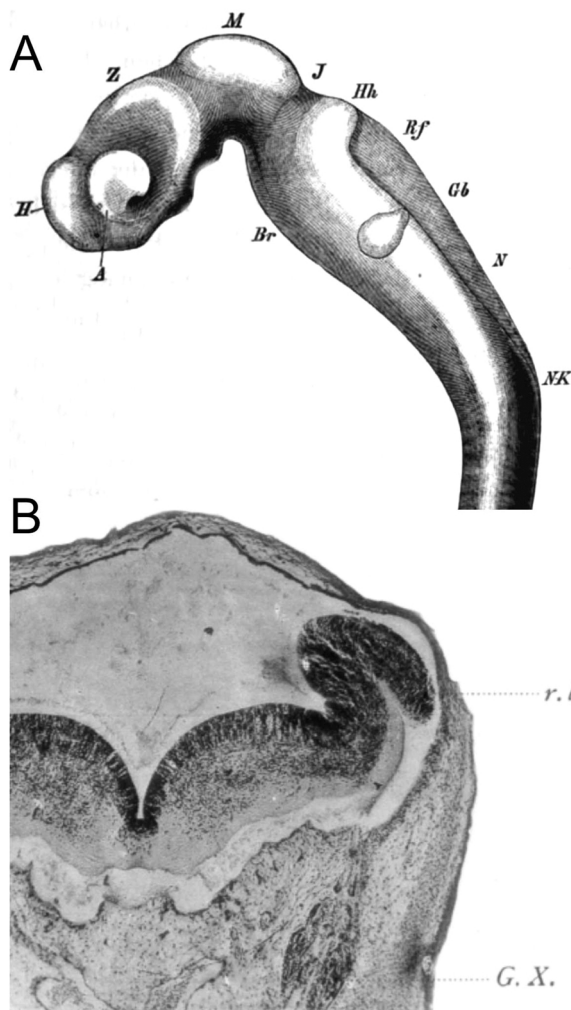


Fig. 1. A whole mount of the brain (A) and coronal section through the rhombencephalon (B) of a human embryo. Abbreviations: A, Augenblase, eye; Br, Brueckenkruemmung, pons; Gb, Gehorblase, ear; H, Hemisphaerenhirn, telencephalon; Hh, Hinterhirn, pons; J, Isthmus; M, Mittelhirn, midbrain; N, Nachhirn, 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).

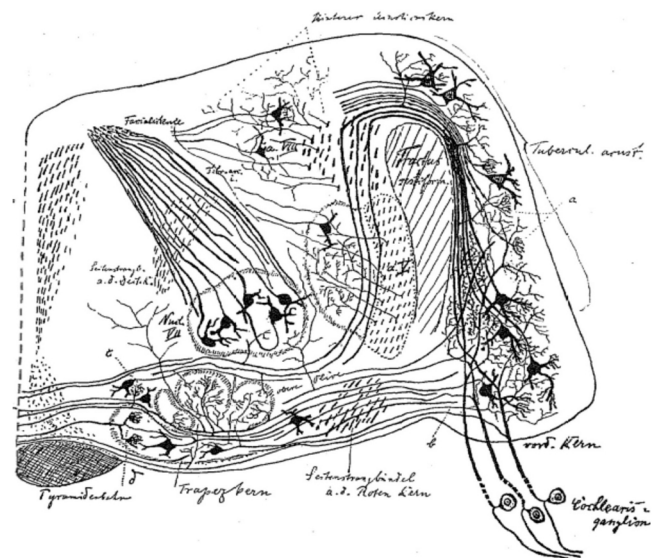


Fig. 2. Silver-stained coronal section of the hindbrain of a cat showing the innervation of the cochlear nuclei (Tuberculum) by afferents of the cochlear ganglion (Cochlearis-ganglion), and second-order projections from the anterior ventral cochlear nucleus (PVCN, root Kern) to the superior olives (Trapezkern).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

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 *Ptf1a* 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

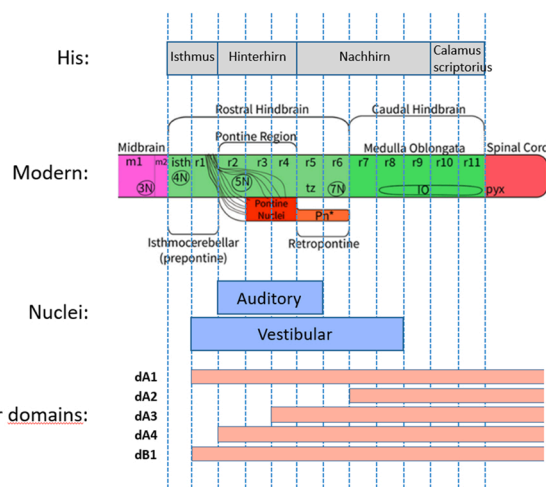


Fig. 3. The relationship between His' hindbrain regions, modern hindbrain neuromeric organization, the auditory and vestibular nuclear complexes, and the 5 most dorsal progenitor domains. Modified after (Diaz and Glover, 2021; Diek, et al., 2022).

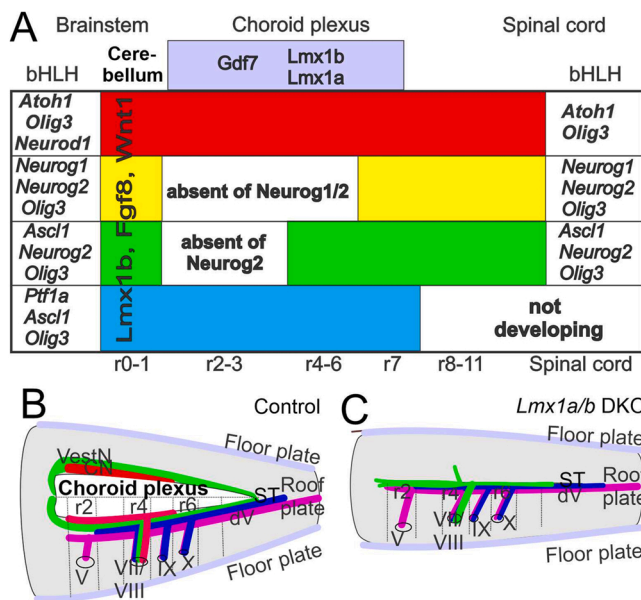


Fig. 4. Dorsal development of the hindbrain depends on *Lmx1a/b*. (A) The choroid plexus originates from r2 to r7 (light purple) and depends on the expression of *Lmx1a/b* and *Gdf7*. The bHLH genes *Atoh1*, *Neurog1*, *Neurog2*, *Neurod1*, *Ascl1*, *Olig3* and *Ptf1a* are expressed combinatorially in 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 *Olig3* (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*, *Ascl1* 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 (Birmingham et al., 2001; Farago et al., 2006; Fritzsich 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 Fritzsich, 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 perturbation 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 Fritzsich, 2013), *Lmx1a/b* (Chizhikov, et al., 2021), *Fgf2* (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 Fritzsich, 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).

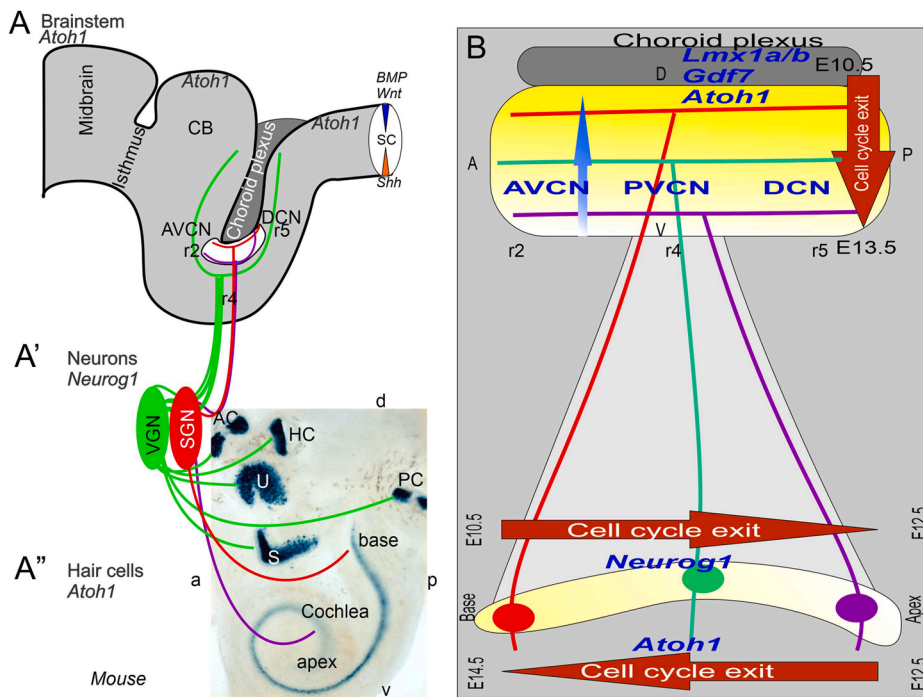


Fig. 5. Cochlear and auditory projection development depends on the expression of specific genes. The development of spiral ganglion neurons (SGN; A') depends on the expression of *Neurog1*. Their projection extends from the cochlea (A'', B) and ends in a topologically organized projection in the auditory 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;

Fritzscht et al., 2019; Macova et al., 2019; Nichols et al., 2008).

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; Fritzscht et al., 2019; Schmidt and Fritzscht, 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 Fritzscht, 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; Milinkevicius and Cramer, 2021; Schmidt and Fritzscht, 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; Fritzscht 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

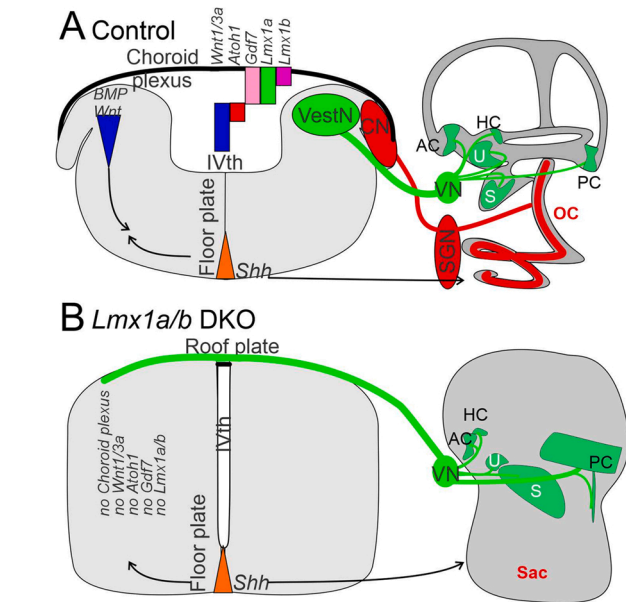


Fig. 6. Central projections of auditory and vestibular sensory afferents depend on proper development of the dorsal hindbrain. In *Lmx1a/b* DKO mice vestibular neurons (VN) project dorsally in the hindbrain as in control mice, whereas auditory projections fail to develop (A, B). However, in *Lmx1a/b* 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 crista; CN, cochlear nuclei; S, saccule; SGN, spiral ganglion neurons; ST, solitary tract; 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).

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 mechano-electrical 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.

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