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 \textit{Lmx1a/b} is crucial to the development of the hindbrain choroid plexus, and also regulates the expression of \textit{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 \textit{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...
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...
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 (Gru 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 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 (Merzendorf 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 sharpen 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 P浩x2b 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; Lunde 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 (Lovenstein et al., 2021; Pan et al., 2009), defines the cerebellum (Fig. 4). A delayed expression of NeuroD 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
Atoh1 and Olig3 are expressed in the spinal cord, the hindbrain and the cerebellum (Bermingham et al., 2001; Farago et al., 2006; Fritzsche 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 Olig3-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 Fritzsche, 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 Neurog2**

Spiral ganglion neurons (SGNs) have been classified into type Ia, Ib, Ic and type II (Elliott et al., 2021; Petitprêtre 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-neurosensorial 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 Neurog2 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 Fritzsche, 2013), Lmx1a/b (Chizhikov et al., 2021), Fgfr2 (Pirvola et al., 2000), Shh (Riccomagno et al., 2002) and Dicer (Kersgo et al., 2011). Partial loss of VGNs occurs in the absence of Fgfg10 (Pauley et al., 2003) and Foxq1 (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; Petitprêtre et al., 2022; Petitprêtre 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 Nhh1/2 (Krüger et al., 2004), Isl1 (Filova et al., 2022), and Ebf2 (Petitprêtre et al., 2022) to initiate the formation of type I and II SGNs (Petitprêtre 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 Fritzsche, 2013; Karis et al., 2001; Luo et al., 2013; Petitprêtre et al., 2022), all of which depend additionally on the expression of Cox2 and Pou4f2. Id2 regulates expression of Calretinin and Phox2b 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; Petitprêtre et al., 2022; Shrestha et al., 2018; Sun et al., 2018).
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). SGNs 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).

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 thevestibular 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). Choroidplexus 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).

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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 Wnt (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), Pouf3 (Li et al., 2020; Xiang et al., 2003), Gfi1 (Hertzano et al., 2004), Srm/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; Ozkiblo 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, Celer1 and Gal2, regulated through the planar cell polarity (PCP) pathway (Tarchini et al., 2013). Ema2 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 profoundly new insight into the importance of the adjacent rhombic lip in the formation of central neuron populations novel to the hindbrain. He thus found insight into the importance of the adjacent rhombic lip in understanding the neural crest as the origin of much of the peripheral nervous system. 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, Olig2, 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.
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