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Review

Development and regeneration of vestibular hair cells in mammals

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ABSTRACT

Vestibular sensation is essential for gaze stabilization, balance, and perception of gravity. The vestibular receptors in mammals, Type I and Type II hair cells, are located in five small organs in the inner ear. Damage to hair cells and their innervating neurons can cause crippling symptoms such as vertigo, visual field oscillation, and imbalance. In adult rodents, some Type II hair cells are regenerated and become re-innervated after damage, presenting opportunities for restoring vestibular function after hair cell damage. This article reviews features of vestibular sensory cells in mammals, including their basic properties, how they develop, and how they are replaced after damage. We discuss molecules that control vestibular hair cell regeneration and highlight areas in which our understanding of development and regeneration needs to be deepened.

Contents

1. Introduction

Vestibular hair cells are sensory receptors in the inner ear that detect head motion and thereby enable animals to orient their bodies and coordinate movements. In mammals, vestibular hair cells and their innervating neurons degenerate with age [1–3], and they can be destroyed by therapeutic drugs such as aminoglycoside antibiotics [4,5]. Extensive loss of vestibular sensory cells is highly debilitating and can elicit nauseating bouts of dizziness, imbalance, and incapacitation. Vestibular deficits are prevalent in the human population. They are estimated to affect 35% of the U.S. population >40 years old, and they increase significantly with age [6]. Although mammals compensate after vestibular hair cell loss by invoking visual and proprioceptive senses, functional deficits can persist and affect balance throughout life.

The pathology of vestibular aging and toxicity is complex, affecting various cell types and structures in the sensory organs, neurons of the vestibular ganglion, and the central pathways to which the neurons project [1–4,7]. Indeed, the degree to which losses of vestibular hair cells and neurons contribute to vestibular dysfunction in humans is not well understood. Regeneration of vestibular hair cells is one treatment strategy being explored for some forms of vestibular dysfunction. The majority of hair cells in mammalian vestibular organs are formed during embryogenesis. However, adult mammals can regenerate a subpopulation of these cells after damage, increasing the likelihood that cellular repair or replace-

Abbreviations: K, potassium; CDKIs, cyclin dependent kinase inhibitors; PCP, planar cell polarity.

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ment could potentially benefit millions of people suffering from vestibular deficits. This review summarizes the current state of knowledge on development, damage, and regeneration of sensory cell types in the mammalian vestibular system and highlights critical information gaps that must be addressed before new therapies for vestibular dysfunction can be defined.

2. The mammalian vestibular organs contain a diverse array of cell types

The sense of balance is achieved by integrating vestibular, visual, and somatosensory inputs. Five vestibular organs located in the inner ear sense head position and movements in different directions (Fig. 1). Mechanosensitive hair cells are receptor cells located in the sensory epithelium of each vestibular organ. Hair cells and their innervating neurons detect head velocity and acceleration when a specialized bundle of stiff finger-like projections (stereocilia) located at their apical surface is deflected in response to head movement. There are two types of vestibular sensory epithelia. *Maculae* are found in the utricle and the saccule. The stereocilia of macular hair cells are weighted by small stones (otoconia), enabling the cells to sense linear head acceleration and gravity. *Cristae* (lateral, anterior, and posterior) lie at the end of the three semicircular canals and sense head rotations.

Each vestibular sensory epithelium is composed of hair cells and supporting cells (Fig. 1, bottom right), which share similarities with epithelial and glial cells. Each macula has two anatomical zones: a central *striola* in which specialized afferent terminals are located and a surrounding *extrastriola*. In or around the striola, hair cells are divided along a line of polarity reversal in which the vector of maximum sensitivity of their stereocilia reverses direction. Polarity reversal allows for detection of linear acceleration in opposing directions. Each crista is also divided into central and peripheral zones, but in contrast to the maculae, all hair bundles in the crista share a common orientation.

Vestibular hair cells are divided into two subtypes, Type I and Type II [8–14]. Type I and Type II hair cells are found in both central and peripheral zones of all five vestibular organs, usually in almost equal ratios, of all mammals that have been examined, including humans. This has sometimes been confused with findings from birds and reptiles, in which Type I hair cells are only found in central zones [15,16]. The functional differences between hair cell subtypes and regions are still being elucidated, although accumulating evidence indicates that a subset of Type I hair cells located centrally within the sensory epithelium may be better suited for detecting acceleration during high frequency head movements [17,18].

Type I hair cells are unique to amniotes. They are classically defined by the presence of cup-shaped, calyceal afferent innervation, whereas Type II hair cells synapse upon discrete bouton afferent terminals [10]. Distinct morphological differences such as cell shape and stereocilia width and length have also been linked to each subtype [11,19–21]. Relatively little is known about what separates vestibular hair cell subtypes at the molecular level, but differences in Sox2 transcription factor expression [22] and calcium binding protein expression [9,23] can be reliable indicators. Rapid progress on this front will likely soon be made as high throughput profiling methods like single-cell RNA-sequencing should allow for characterization of differences at the whole transcriptome level [24]. A growing list of electrophysiological differences also distinguish Type I and Type II hair cells [25–34], and regional differences in physiological recordings suggest that Type I and Type II hair cells may be further subdivided based on central versus peripheral location [25,26,35–41].

Diversity amongst the afferent vestibular ganglion neurons that innervate hair cells is somewhat more complex. Morphologically, terminals of the neurons can be broken into two types: calyces and boutons. Each neuron usually branches multiple times and innervates several hair cells [42,43]. Neurons whose arbors exclusively form bouton endings are least common and only found in peripheral zones, whereas neurons whose arbors exclusively form calyceal endings are only found in central zones. The vast majority of afferent neurons are dimorphic; they branch to form both calyceal and bouton endings. Dimorphs are found in both central and peripheral zones, but individual neurons do not cross zones. Finally, some central calyces are complex, meaning that one calyx from the same branch can extend to innervate multiple neighboring hair cells, whereas the peripheral calyces that originate from dimorphs only innervate a single hair cell.

In mature mice, peripheral afferents that innervate the maculae project centrally to distinct regions of the brain, depending on the zone from which they arise. Afferent nerves from the medial half of the utricle project almost exclusively to vestibular nuclei, while afferents in the lateral half travel primarily to the cerebellum [44]. The converse is true in the saccule. These zones have been termed “cerebellar macula” and “vestibular macula”. The functional significance of this segregation of projections is not known. However, since a given stimulus activates one macular zone and not the other, it has been proposed that this would result in facilitation of one nucleus and defacilitation of the other. Given the central circuitry, this could result in enhanced vestibular tuning [44].

Another major feature distinguishing individual vestibular ganglion neurons is their background firing pattern [45–47]. The calyx-only and dimorphic neurons in the central zones exhibit irregular discharge of action potentials, whereas the bouton-only afferents and dimorphs in peripheral zones are more regular. Irregular afferents show larger gain at higher frequency stimuli than do regular afferents, suggesting central afferents are better at detecting the onset of rapid movements [48,49]. A clear connection between hair cell subtype, afferent terminal type, and discharge regularity is obscured, however, by the fact that Type I/Type II hair cells and calyces/boutons are found in both central and peripheral zones. Therefore, mechanisms by which each cell type in each region encodes head movements must be elucidated before we learn how to reconstruct injured vestibular organs such that function is restored. Experiments showing that low and mid frequency components of the vestibulo-ocular reflex remain intact after silencing irregular afferents suggest that these inputs to the central nervous system may be dispensable for some forms of vestibular function [50]. However, the full impact of removing and replacing distinct neural and hair cell subtypes on vestibular function is still actively under investigation.

While sensory hair cells and neurons might be the most essential cell types for vestibular function, there are other cell types present within vestibular organs that have key roles. Supporting cells anchor hair cells into the sensory epithelium, generate material for the overlying structures that are essential for stereocilia displacement, clear dead hair cells and debris, and help maintain ion homeostasis (supporting cell features and functions are reviewed in [51,52]). Supporting cells also play the key role of serving as precursors to new hair cells in adulthood (discussed in detail below). Clear evidence exists that, at least during development, there are two subtypes of vestibular supporting-cell-like progenitors separated by the central and peripheral regions, particularly within the maculae [24,53–55]. Whether these differences persist in mature supporting cells at adulthood remains to be determined.

Surrounding the sensory epithelium is a transitional region, followed by a thinner epithelium that arches up to form a roof and enclose the lumen. Dark cells in this thinner epithelium, and to a lesser extent cells from the transitional region, are essential for secreting potassium (K) and maintaining the high K concentration of the endolymph in utricles and cristae. In addition, macrophages

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