Temporal Changes in Neuronal Innervation during Human Inner Ear Organoid Development

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Background and Hypothesis:
Ribbon synapses are specialized synapses in sensory systems, crucial for tonic neurotransmitter release. Neuronal refinement, involving synaptic elimination and strengthening, is essential for nervous system development. While vestibular neuronal refinement is not well understood, cochlear maturation in mice provides reference for investigation. This study aims to test whether neuronal refinement/pruning, similar to those seen in the mouse inner ear, takes place in human pluripotent stem cell-derived inner ear organoids during development.

Experimental Design or Project Methods:
A 3D differentiation strategy developed in the laboratory was used to generate inner ear organoids, followed by tissue clearing and immunofluorescence labeling. Confocal microscopy was used to capture 3D volumetric images of vestibular hair cells and neurons at days 80, 120, and 200. Hair cell innervation was quantified by manually counting direct contact points between neurons and hair cells in 3D. NIS Elements annotation software was employed for accurate quantification.

Results:
The mean number of neuron contacts per hair cell significantly decreases from day 80 to day 200 ($\bar{x}$ = 2.56, 1.89, and 1.60) respectively. The variability in these contacts reduces, as indicated by the standard deviations (σ = 1.131, 0.8570, and 0.7107). Despite this, there is persistent non-normal distribution of neuronal contact.

Conclusion and Potential Impact:
This study identifies temporal changes in neuronal contacts with hair cells in the human in vitro model of inner ear development, which is similar to neuronal refinement/pruning in the mouse inner ear during development. Observations include decreased neuronal contacts after differentiation day 80, branching refinement at day 120, and stabilization until day 200. The findings suggest varied maturation rates but increasing system uniformity over time. These results suggest that human inner ear organoids can be used

to recapitulate normal and pathological development of ribbon synapses and neuronal innervation, essential components for sensory transduction in the human inner ear.