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Targeted deletion and in vivo rescue uncover critical roles of the neuronal t-SNARE SNAP-25 in hearing function

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Abstract:
The auditory sensory inner hair cells (IHCs) encode sound into nerve impulses with high temporal precision and sensitivity over a wide range of stimulus intensities[1] . A striking structural and functional feature of IHC synapses is the presence of an electron-dense presynaptic organelle of submicron diameter called a ribbon, marking the centre of the synaptic active zone. Despite their structural differences, IHC ribbon synapses and conventional central nervous system (CNS) synapses share several key mechanisms of regulated synaptic vesicle exocytosis. IHC and CNS synapses are both equipped with the presynaptic scaffold proteins, bassoon and RIM. The presence of the neuronal SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) complex in auditory hair cell is well documented, including syntaxin 1, SNAP25 and synaptobrevin1/2 [3–6].

These observations led to the notion that vesicle fusion at both synapses involves similar SNARE proteins [1,7,8]. However, the study of null mutant mouse models failed to reveal functional role for the neuronal SNARE proteins in IHC synaptic transmission. This finding led to the proposal that IHC synapse operate without neuronal SNARE [3]. It worthy to note that the null mutant mouse models for many of the neuronal SNARE proteins, die at birth or shortly after. Therefore, the IHC function in these mutants could only be analysed in organotypic culture, wherein the findings may not reproduce the functioning of IHC synapse in a healthy and mature hearing organ. To untangle this issue, we focused on SNAP-25, a key component of the canonical synaptic SNARE complex. We generated a hair cell-specific *Snap-25* knockout (*Snap-25* cKO) mouse model allowing to study the effect of acute inactivation of *Snap-25* in IHCs at both neonatal and mature stages. We found that the mice subjected to *Snap-25* inactivation at mature

stage, i.e., after the hearing onset, developed a severe to profound deafness which was associated with a defective exocytosis at IHC synapses followed by ribbon degeneration and a progressive loss of hair cells. Viral mediated transfer of SNAP25 cDNA rescued all these phenotype aspects. These results demonstrated that SNAP-25 is essential for normal hearing function by ensuring the rapid fusion of vesicles at the IHC ribbon synapse and strongly suggest that this fusion is governed by the canonical neuronal SNARE complex

Bio:
Auditory physiopathology has been Dr. Saaid Safieddine's area of research for almost 30 years now. In the 1990's, Saaid Safieddine spent seven years as staff scientist in Robert Wenthold's laboratory at the National Institutes of Health in Bethesda, Maryland, USA. During that time, he made a determinant contribution toward the elucidation of the molecular architecture of the auditory hair cell synapse. In the late 1990's he joined the Pasteur Institute in Paris, wherein he has been working during the last 19 years. His achievements during that time have had still greater impact on our understanding of the molecular anatomy and function of hearing organ. In addition to pursing pioneering basic research aimed at understanding the molecular genetics of hearing and deafness, Dr. Safieddine's recently started a translational research project focusing on the development of innovative therapies for deafness, especially gene therapy. His team conducted several proof-of-concept studies demonstrating for the first time that not only gene therapy can prevent deafness, but that it can also treat it once installed. These studies are raising unexpected new hopes for gene replacement therapy in deaf patients and open the way to future clinical trials. He is now the leader of the team Technologies and gene therapy for deafness at the Institut de l'Audition, Paris. Safieddine's team is one of the three investigators of the University Hospital Research Project (RHU) *AUDINNOVE*. The goal is to develop gene therapy for one of the most frequent form of congenital deafness. This project has been selected for funding under the French State's Major Investment Programs involves tight collaboration between Dr. Safieddine's team and ENT clinicians at the Necker Hospital of Paris (Necker-Sick Children Hospital, Public Assistance - Hospitals of Paris).

Education:
Since 2011: Accreditation to supervise research, Neuroscience Sorbonne université (Paris, France)
1990-1993: Ph.D in "Biology & Health", (Université de Montpellier II, France)
1988-1990: Master's degree in cell biology (Université de Montpellier II, France)

Research & Work History:
2019-present: Visiting Researcher, University of Sheffield, UK
2019-present: group leader "Technologies and gene therapy for deafness"
2012-present: Scientific Director CNRS, Institut Pasteur
2017-present: President of the "Intranational Society for Inner Ear Therapeutics"
2000-2012: Researcher at CNRS, Institut Pasteur (Paris, France)
1997-1999: Researcher associate, NIH (Bethesda, USA)
1994-1997: Visiting Fellow, NIH (Bethesda, USA)

Relevant Bibliography:

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