Evaluating efficiency, stability and safety of virus-mediated, neural expression of fast-Chrimson in the mouse cochlea

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Background & Methods

Our previous study (Mager et al., 2018) demonstrated that fast-Chrimson (f-chrimson) channelrhodopsin is a promising candidate for future clinical optogenetic hearing restoration. We were able to optically drive spiral ganglion neurons (SGNs) of mice for at least nine months post transduction without obvious functional or morphological disturbances of the cochlea. In order to investigate possible long-term effects of virus-mediated f-Chrimson expression on SGNs and cochlear microstructure, we now unilaterally injected **rAAV2/6-hSyn-fast-Chrimson-eYFP (titer: 9.9 x 10^{12} vg/ml)** into the **cochlea of p5-7 C57BL6/J mice** and bilaterally sampled the cochleae at **1, 3, 6, 12, and 24 months of age**, covering the average mouse lifespan, for histopathological and immunohistochemical (ihc) analysis.

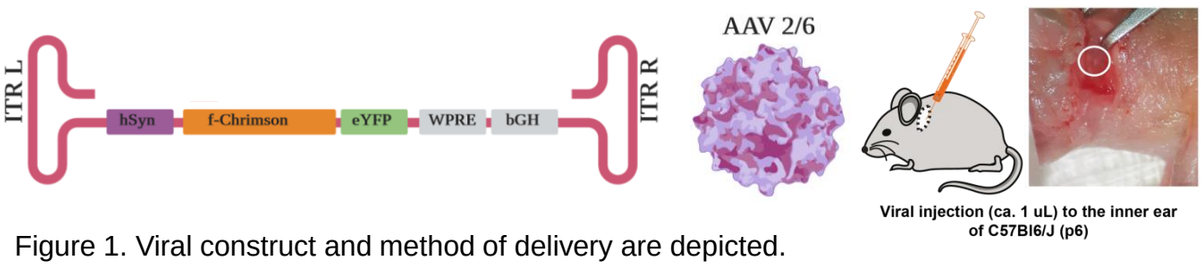

Figure 1. Viral construct and method of delivery are depicted.

All cochleae were processed for serial thin cryosections (16 µm) and stained with **hematoxylin & eosin (HE)** as well as ihc antibodies for the fluorescent reporter protein **GFP**, neuronal marker parvalbumin (**PV**), histiocytic cell marker ionized calcium-binding adapter molecule 1 (**IBA-1**) and general mature T-lymphocyte marker **CD3**.

1. Mouse SGNs show durable expression of f-Chrimson

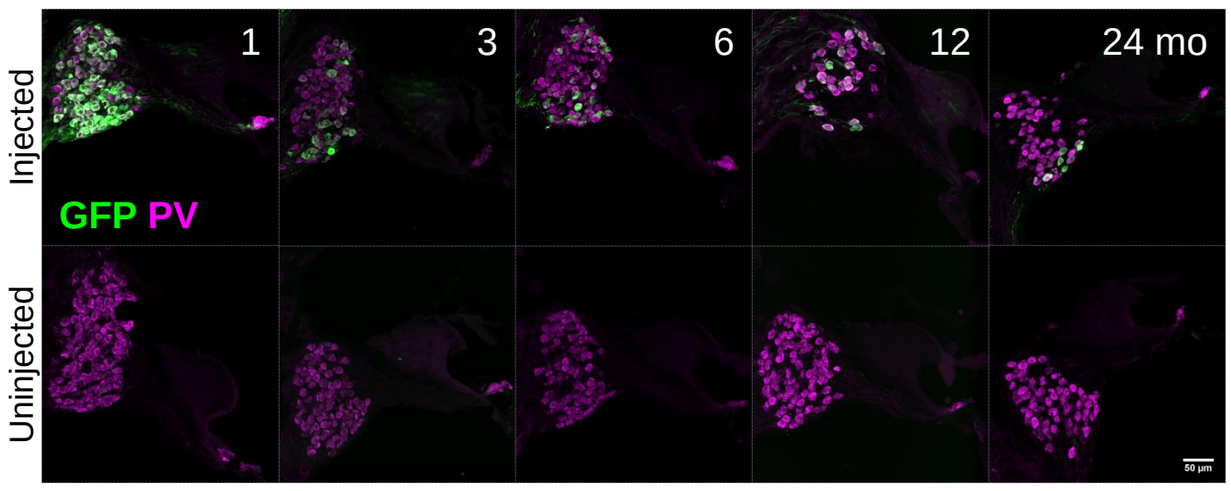

Figure 2. Confocal image stacks from mid-modiolar cross sections of viral construct injected and uninjected contralateral cochleae: GFP expression (green) persists in single SGNs of C57B6 mice up to two years of age. Scale bar: 50 µm.

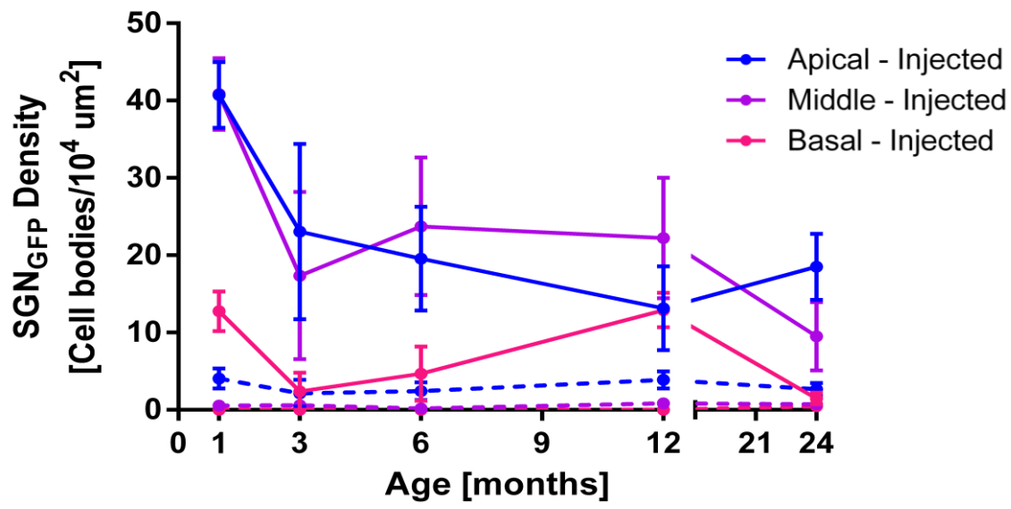

Figure 3. Mean density of GFP⁺ SGN somata in apical, middle and basal parts is plotted against all age groups: successfully transduced, GFP-expressing SGNs show the highest density in the apical and middle turns of the injected ears from 1-month-old mice, which drops to a rather constant level in older groups (3 to 12 mo), while GFP⁺ SGNs in the oldest group (24 mo) predominantly remain apically; there is also certain GFP expression in very few SGNs of the apical modiolar turn at the contralateral cochlea (dashed line).

Conclusion

This is the first study investigating long-term effects of f-chrimson channelrhodopsin expression in cochlear SGNs over the entire mouse lifespan. Some SGNs showed a durable opsin expression up to two years after construct delivery in the cochlea, especially in the apical turns. However, this seems to correlate with a certain degree of accelerated degeneration and neuronal loss in the spiral ganglion.

2. Injection- and age-related loss of SGNs

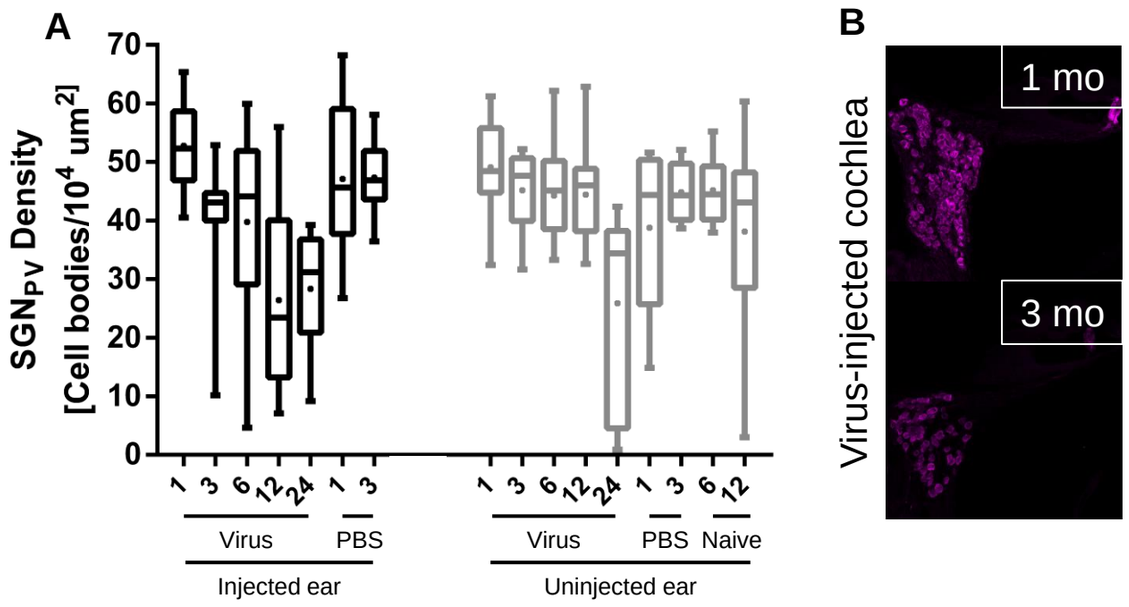

Figure 4. Quantitative analysis of SGN density related to the area of rosenthals canal in injected and uninjected cochleae of all age and control groups: (A) besides a clear age-related decline of SGN density in both, injected and uninjected cochleae at 24 months, there is also an injection-related effect with the neuronal loss starting earlier, in some mice already at 3 months after viral delivery of f-Chrimson (B).

3. Histological changes of the spiral ganglion

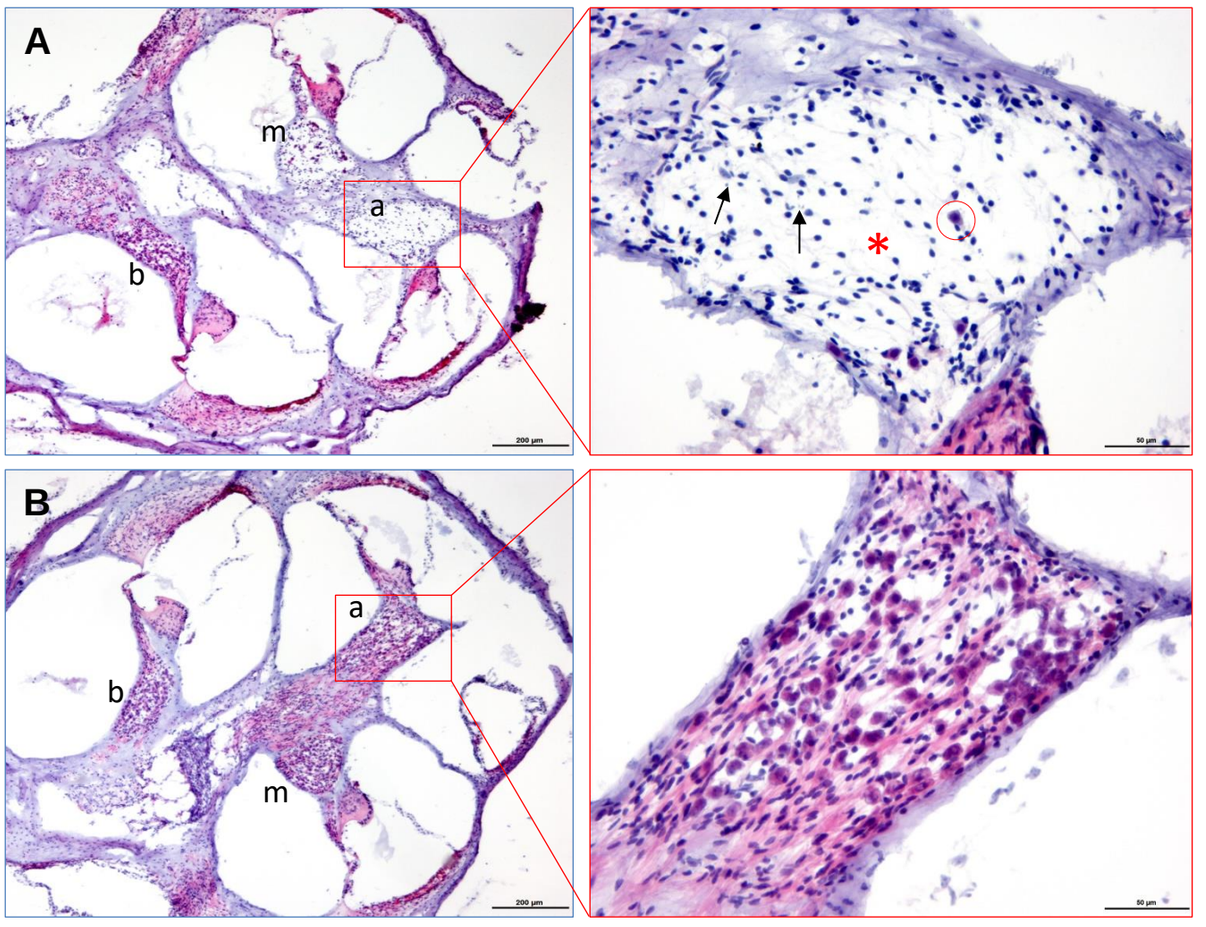

Figure 5. Representative light micrographs of the virus-injected (A) and uninjected (B) cochlea of a 12-month-old mouse showing considerable loss of SGNs (red circle) together with interstitial vacuolation (asterisk) and some cellular debris (arrows), especially in the apical part (a), compared to middle (m) and basal (b) modiolar turns of the spiral ganglion as well as to the contralateral, uninjected side; scale bars left: 200 µm; right: 50 µm; HE stain.

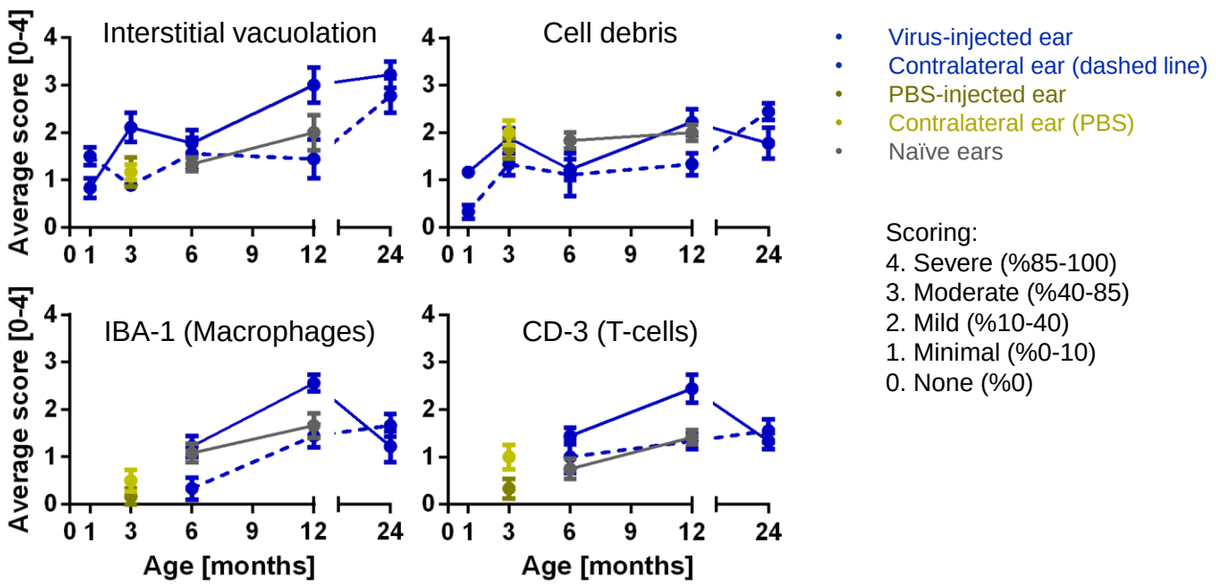

Figure 6. Semiquantitative scoring of histological changes in the spiral ganglia of injected and uninjected cochleae of all age and control groups: the age related increase in interstitial vacuolation corresponds to the observed loss of SGNs (see fig. 4), especially in apical turns of the injected cochlea (data not shown), and there is also a correlating mild increase in inflammatory cell markers (IBA-1 and CD3), compared to the uninjected side and controls.

This project OptoHear has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No.670759), the German Research Foundation through the Collaborative Research Center 889, the Gottfried-Wilhelm-Leibniz Program and BMBF.

Reference: Mager T, Lopez de la Morena D. et al. High frequency neural spiking and auditory signaling by ultrafast red-shifted optogenetics. NatCommun. 2018 May1;9(1):1750.