Supporting Information

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SI Materials and Methods

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The lateral wall of the LV was dissected then fixed with 2.5% glutaraldehyde and 2% paraformaldehyde. For SEM, samples were dehydrated, critical point dried and infiltrated with gradient hexamethyldisilazane, then mounted on stubs and sputter coated with gold-palladium. Images were collected on a Philips FEI XL30 SEM. For TEM, samples were postfixed in 1% osmium tetroxide, dehydrated, infiltrated, and polymerized. Ultrathin sections were post-stained. Images were taken on a Philips EM400 TEM with a MegaView digital camera (Soft Imaging Systems, Inc.).

Immunohistochemistry. Cultures were fixed with 4% paraformaldehyde in PBS (PBS) for 20 min, then rinsed and immunostained with antibodies directed against mouse and rabbit anti-β tubulin III (Tuj1) (BAbco), mouse anti-neurofilament 200 (Sigma), mouse anti-NeuN (Chemicon), mouse anti-synapsin 1 (SY), rabbit anti-glial fibrillary acidic protein (GFAP) (DAKO), mouse anti-glial fibrillary acidic protein (Chemicon), rabbit anti-myosin VIIA and myosin VI (*Proteus* Biosciences), mouse anti-CtBP2 (BD), chicken anti-GFP (Chemicon) and rat anti-BrdU (Accurate Chemical & Scientific Corporation), following standard protocols. Immunostaining was visualized with secondary antibodies conjugated to Cy3, Cy5, or Alexa Fluor 488, 548, or 647 fluorophores. Regular immunohistochemistry staining followed biotin-streptavidin-diaminobenzidine protocols. Omission of the primary antibodies eliminated staining in all preparations examined. The nucleus was counterstained with DAPI. A Zeiss LSM 510 Meta confocal microscope or an Olympus fluorescent microscope equipped with a digital camera was used to collect images. Imaris Bitplane software was used to generate 3D reconstructions of confocal Z-stack images.

BrdU Administration and Immunofluorescence. To demonstrate the *in vivo* proliferation of ependymal cells, BrdU (100 mg/kg in 0.9% NaCl) was injected i.p. into C57BL/6j mice (8–10 weeks old, Charles River) once a day for 30 days before sacrifice. Animals were then anesthetized and killed by cervical dislocation, whereafter their brains were flash-frozen with dry ice and then cryosectioned. For the *in vitro* proliferation test of ependymal cells, BrdU was added to the culture medium 48 h after initial culture (5 μM) and was maintained for 72 h. BrdU and myosin VIIA double staining followed the protocol as described[5]. Omission of the primary antibodies eliminated staining. Double-labeled ependymal cells were confirmed by serial confocal sectioning.

Assay for Mechanosensory Transduction in Ependymal Cells. Animals were killed by cervical dislocation then the lateral wall of LV was exposed to 5 μM FM1–43FX (Molecular Probes) for 60 s and fixed with 4% formaldehyde. A thin layer of the lateral wall was dissected and mounted to examine the fluorescent intensity to determine dye uptake. To block the entry of FM1–43, the lateral ventricle was preincubated and repeatedly flushed with DHS for 4 min.

Electrophysiology. Hair cells were identified by the presence of green fluorescence under UV light. NSC-derived neurons were identified by their distinct neuronal morphology of a small, round and phase bright cell body with long uneven processes. Spiral ganglion neurons were identified by their much larger round and phase bright cell bodies and unipolar and/or bipolar neurites. The criteria were confirmed by immunostaining in parallel cultures.

Currents were amplified with an Axopatch 200B amplifier (Axon Instruments) and filtered at a frequency of 2–5 kHz through a low-pass Bessel filter. The data were digitized at 5–500 kHz using an analog-to-digital converter (Digidata 1200; Axon Instruments). The sampling frequency was determined by the protocols used. Action potentials were amplified (100×), filtered (bandpass 2–10 KHz), and digitized at 5–500 kHz using the Digidata 1200 as described earlier. The extracellular solution for most experiments contained (in mM) NaCl 145, KCl 6, $MgCl_2$ 1, $CaCl_2$ 0–2, D-glucose 10, and Hepes 10, at pH 7.3. For perforated patch experiments, the tips of the pipettes were filled with an internal solution containing (in mM): KCl 150, Hepes 10, and D-glucose 10, at pH 7.3. The pipettes were front-filled with the internal solution and back-filled with the same solution containing 250 μg/ml amphotericin. Stock solutions were reconstituted and perfused in the recording chamber. CNQX (5 μM) was used to block AMPA receptors.

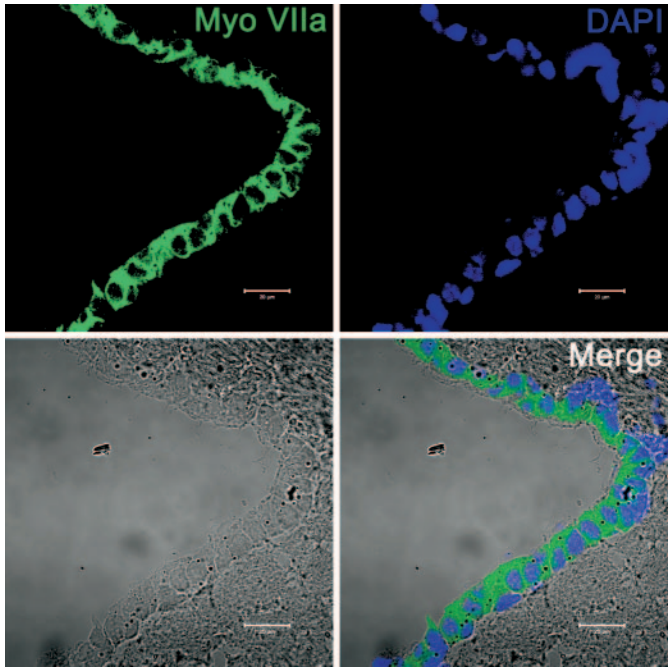


Fig. S1. Adult brain ependymal cells take the shape of polarized columnar epithelial cells and express myosin VIIA. (Scale bar, 20 μm.)

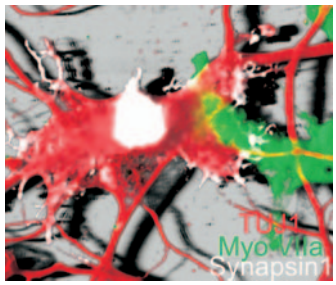


Fig. S2. Accumulation of synapsin 1 at the nerve fibers focused around the ependymal cells revealed that contacts among SGNs and ependymal cells have the potential to develop into synapses. Shown is an enlarged region of Fig. 4*A*2, depicting the accumulation of synapsin 1 at the adult SGN nerve endings. (Scale bar, 20 μm.)

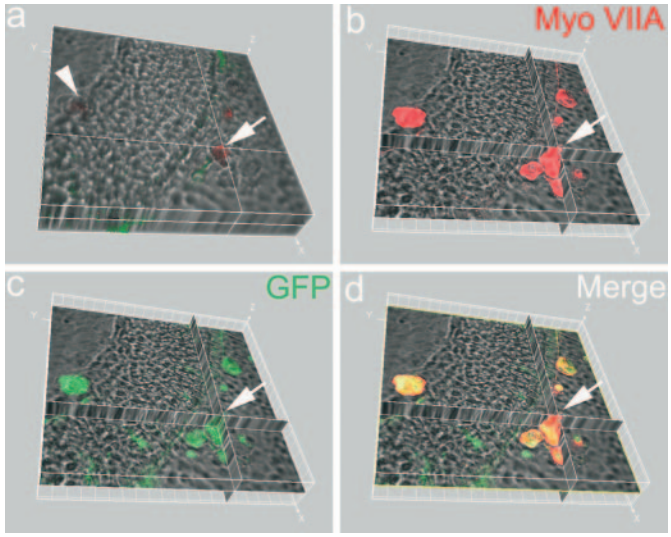


Fig. S3. Incorporation of ependymal cells into cochlear sensory epithelia. Cochlear sensory epithelia were dissected from wild type mouse (C57 BL/6), and residual HCs were eliminated using streptomycin treatment. Ependymal cells were isolated from myosin VIIA-GFP transgenic mouse line. Cochlear sensory epithelia and ependymal cells were co-cultured for 5 days. (*A*) 3D reconstruction of co-cultured inner ear sensory epithelia and ependymal cells. Small patch of ependymal cells incorporated into the inner (arrowhead) and outer (arrow) marginal portion of the sensory epithelia. To demonstrate this incorporation clearly, we made an *x* axis (indicated by red line) and a *y* axis (indicated by green line) section across the incorporated ependymal cells. We also made a section along the mid point of the *z* axis (indicated by blue line). The detailed demonstration was illustrated in (*B*–*D*). (*B*) incorporated ependymal cells were stained with hair cell marker myosin VIIA, to show that the myosin VIIA-positive cells were incorporated ependymal cells, not the remaining cochlear hair cells which are also myosin 7a positive. We also stained the co-cultures with GFP, as shown in (*C* and *D*), the myosin VIIA-positive cells were also labeled with GFP, demonstrating that they are ependymal cells derived from myosin VIIA-GFP transgenic mouse.

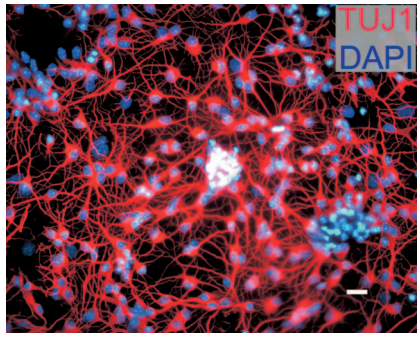


Fig. S4. Neuronal differentiation of adult NSCs. (Scale bar, 20 μm.)

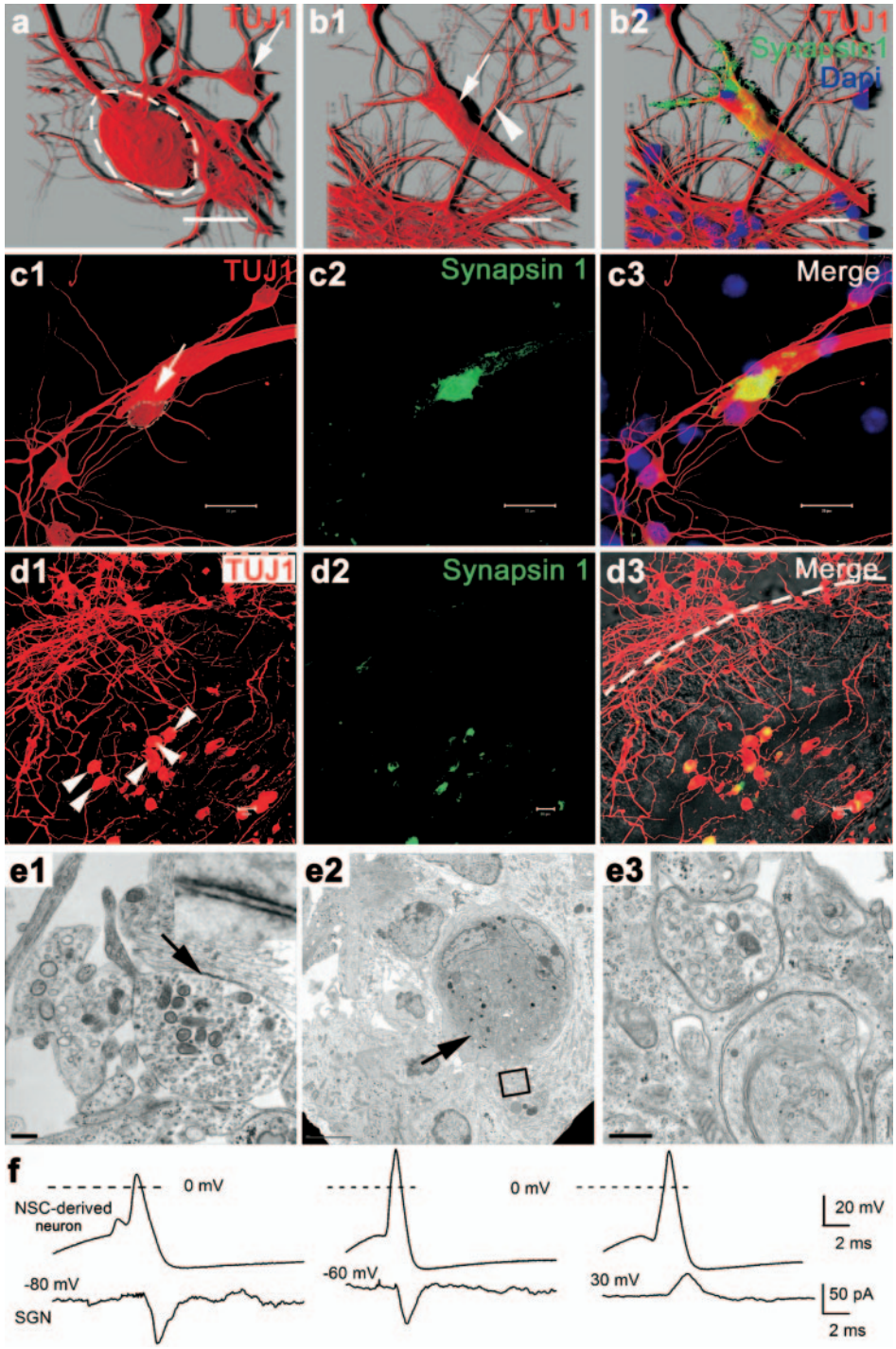


Fig. S5. NSC derived neurons functionally rewire deafferented SGNs. (*A*) SGN and NSC derived neurons were labeled with TUJ1. White dashed lines indicate a co-cultured adult SGN, which is about 4 times larger than a NSC-derived neuron (arrow). Various contacts were found between SGNs and NSC derived neurons. (*B*1) The nerve fibers of adult SGNs (arrow) were much thicker than that of NSC-derived neurons (arrowhead). (*B*2) Presynaptic protein synapsin 1 accumulated at the nerve endings of SGNs. (*C*1–3) A dendro-somatic-like contact between a large SGN nerve ending (arrow) and the cell body of a NSC-derived neuron (marked with white dashed line). Accumulations of synapsin 1 at the nerve ending of a SGN are shown. DAPI was used to show the nuclei. (*D*1–3) SGNs and NSC-derived neurons could also establish synapse-like contacts at the organ level. Adult NSCs were co-cultured with cochlear explants. Nerve fibers of NSC-derived neurons passed through the cochlea, integrating into the neuronal circuit of SGNs and establishing synapse-like contacts. The arrowhead indicates co-cultured SGNs (*D*1). The white dashed line marks the margin of the co-cultured cochlea. (*E*1) Ultra structure of a bouton from an adult NSC-derived neuron was filled with synaptic vesicles and the neuron was able to establish synaptic contacts with another adult NSC-derived neuron. The postsynaptic thickening (arrow) is enlarged at the upper right corner of this panel. (*E*2 and 3) The nerve ending of a NSC-derived neuron developed into a bouton with synaptic vesicles as it established a synapse with a SGN (arrow). The boxed area in panel (*E*2) is enlarged in panel (*E*3) to show the synaptic contact. (*F*) Functional analyses of synapses between SGNs and NSC derived neurons. Shown are examples of action potentials evoked from a NSC-derived neuron using negative current (≈300 pA) injection. Below are simultaneous voltage-clamp recordings of synaptic currents from a SGN making synaptic contact with the NSC-derived cell. The magnitude of the synaptic current reduced as the SGN was held from −80 mV and −60 mV. In addition, the current reversed from negative to positive holding potentials. The mean synaptic delay was 0.6 ± 0.3 ms; $n = 9$. (Scale bars: *A*–*D*3, 20 μm; *E*1 and 3, 0.5 μm; *E*2, 5 μm.)

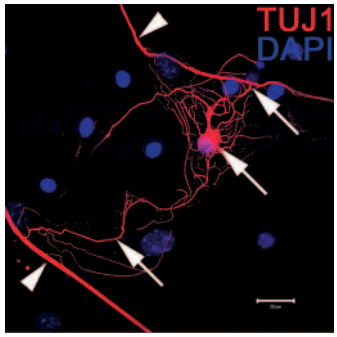
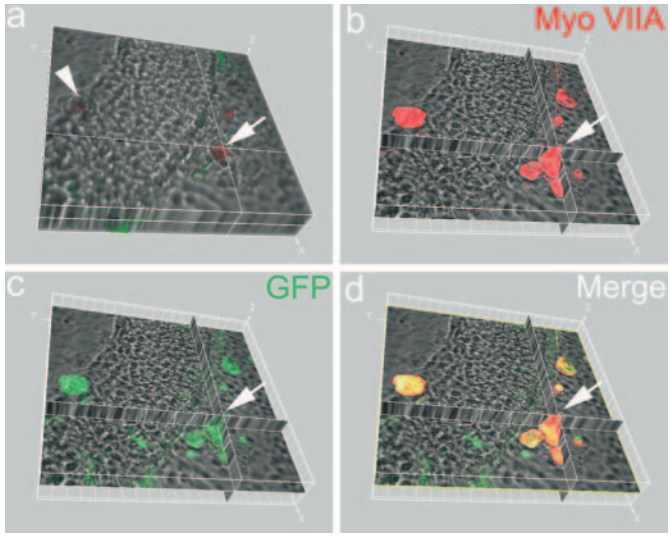


Fig. S6. Example of NSC-derived neuron serving as an interneuron between SGNs. The neurite of a NSC derived neuron (arrows) projected to and linked the neurites of two deafferentated SGNs (arrowhead). (Scale bar, 20 μm.)



Movie S1. A movie file that provides more information on how ependymal cells incorporate into cochlear sensory epithelia.

Movie S1 (AVI)