Supplementary Methods

Slice procedure. Coronal slices (300–400 µm) were cut at 8-15 °C on a vibrating microtome (DTK-1500E, Ted Pella, Redding, California). Slices containing both the LSO and the MNTB were selected and allowed to recover in an interface-type chamber under 95%O₂/5%CO₂ atmosphere for 1~2 hours at RT (23°C) before recording. For slice preparation and incubation, 1 mM Kynurenic acid was present in the ACSF (composition in mM: NaCl 124, NaHCO₃ 26, Glucose 10, KCl 5, KH₂PO₄ 1.25, MgSO₄ 1.3, CaCl₂ 2, pH = 7.4 when bubbled with 95% O₂/5% CO₂). For recordings, slices were transferred to a submerged-type chamber and superfused with oxygenated ACSF at RT at a rate of ~3-4 ml/min.

Input resistance of LSO neurons. Minimum stimulation data indicated that single input-evoked synaptic responses had amplitudes > 10 pA. Given the input resistance of LSO neurons of >> 100 MΩ (P1 - P4: 751.7 ± 112.0 Mohm, n = 11; P11 - P14: 214.8 ± 33.8 Mohm, n = 14) single fibers should elicit PSPs of > 1 mV, which is well above our detection threshold.

Stimulus–response experiments. The maximal stimulus intensities tested were 506.7 µA ± 35.8 at P1-P5 (n = 16) and 304.2 µA ± 48.4 at P9-P14 (n = 18). The lower values in older animals most likely reflect the lower thresholds of MNTB fibers at this age (intensities necessary for eliciting responses with >50% failure rates were 42.1 ± 5.5 at P9–P14, n = 19 vs. 60.4 ± 11.7 at P1–P5, n = 23). In additional experiments, we tested whether the lower stimulus intensities in P9 - P14 animals indeed were sufficient to stimulate all intact connections. In 13 LSO neurons from P9-P14 animals, stimulation intensities were increased up to 1,000 µA. The maximum currents elicited with these high stimulus intensities was 5.0 nA ± 0.56 nA (n = 13), which is very similar to the average current elicited during the stimulus response experiments (4.5 nA, P = 0.51, Student’s t-test). In 4 cells, we further increased stimulus intensity to 2,000 µA and in no case did this elicit an additional increase in response amplitudes.
**Photolysis of caged glutamate.** The optical fiber approached the slice at an angle of ~40°, and the end of the fiber was bent an additional ~35° so that the light-emitting end of the fiber approached the slice at ~80°. This resulted in well-circumscribed, almost circular UV spots (diameter ~ 25 µm) on the surface of the slice. To minimize variability in light spot size and uncaging resolution, the fiber was carefully lowered at each location until it slightly touched the surface of the slice. For exact positioning of the light spot, a green band-pass filter was inserted in the light path before the optical fiber.