Supplemental Text for "Control of interneuron firing by subthreshold synaptic potentials in principal cells of the dorsal cochlear nucleus."

Methods

Electrophysiology

Experiments were performed with prior approval from Oregon Health & Science University's IACUC. 250-300 µm thick coronal sections of the DCN were prepared from p15-p25 mice of either sex from the following strains: C57/Bl6, Thy1-ChR2-YFP, VGluT2-ChR2-YFP, and connexin36-/- mice (or their wild-type littermates). The brain was submerged and sliced in an ice-cold cutting solution containing (in mM): 87 NaCl, 25 $NaHCO_3$, 25 glucose, 75 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 7 $MgCl_2$, 0.05 R-CPP, and bubbled with 5% CO_2/95% O_2. Slices were subsequently transferred to a holding chamber and recovered for 30-45 min at 34^o C in an artificial cerebrospinal fluid (ACSF) solution containing (in mM): 130 NaCl, 2.1 KCl, 1.7 $CaCl_2$, 1 $MgSO_4$, 1.2 KH_2PO_4, 20 $NaHCO_3$, 3 Na-HEPES, 10-12 glucose, 0.05 R-CPP, bubbled with 5% CO_2/95% O_2 (300-310 mOsm). After recovery, slices were stored at 22-24^o C until recording and experiments were performed within 5 hours of slice preparation.

Slices were placed in a recording chamber and perfused with the ACSF solution described above, heated to 31-33^o C using an inline heater (3-5 ml/min). All experiments except for those in Figure 3C were performed in the presence of inhibitory synaptic blockers (10 µM SR95531 and 1-2 µM strychnine). Additionally, NMDA receptors were blocked in most experiments with 5 µM R-CPP. Stellate and fusiform cells were visually identified using previously established criteria (Apostolides and Trussell, 2013). The pipette internal solution for voltage-clamped stellate cells contained (in mM) 64.5 $CsMeSO_3$, 30 CsF, 5 TEA-Cl, 5 QX314-Cl, 5 Cs_4BAPTA, 4.8 $MgCl_2$, 4 ATP, 0.5 GTP, 10 Tris-phosphocreatine, 10 HEPES, pH 7.2-7.3 with CsOH. In some experiments, CsF was replaced with 15 $CsMeSO_3$ and 15.5 CsCl. The internal solution for current clamp experiments contained 113 K-gluconate (Or 113 $KMeSO_3$), 4.8 $MgCl_2$, 4 ATP, 0.5 Tris-GTP, 14 Tris-phosphocreatine, 0.1 EGTA, 10 HEPES, pH 7.2-7.3 with KOH,

~290 mOsm. In some experiments, 15.5 KCl was substituted for equimolar K-Gluconate or $KMeSO_3$. For paired recordings, Alexa488 (30 µM) was added to the internal solution to visualize the dendrites of fusiform cells. Optogenetic stimulation was performed as in Apostolides and Trussell (2013, 2014). Series resistance (<30 MOhm) was compensated in the majority of voltage-clamp experiments (60-80% correction, 90% prediction. Bandwith = 3 kHz). Data were discarded if the series resistance varied >20-25% over the course of the recording. In current clamp experiments, the pipette capacitance was canceled and bridge balance was maintained.

Parallel Fiber stimulation

A bipolar metal or theta glass stimulating electrode was placed in the DCN molecular layer >100 µm from the recorded cell. The distance between the two tips of the stimulating electrodes was ~20-50 µm (glass electrode) and 100 µm (metal electrode). Size of the stimulating electrode and distance from the recorded cell were critical for whether we recruited gap junction mediated components of parallel fiber transmission. Indeed, our previous study describing chemical synapses onto DCN stellate cells did not report slow inward or outward components of parallel fiber transmission (Apostolides & Trussell, 2014). In that paper, small theta glass stimulating electrodes (<10 µm distance between tips) were placed in close proximity (20-40 µm) to the stellate cell, preferentially activating synapses onto the recorded stellate cells, and minimally activating synapses on prejunctional fusiform cells. This was confirmed by varying the distance of the stimulating electrode from the recorded stellate cell: EPSCs evoked 20-40 µm from the cell had significantly smaller half-widths than EPSCs evoked >100 µm away (n=5 cells. EPSC half-width at 20-40 µm: 2.2±0.7 ms. 100-200 µm: 43.1±9.0 ms, p=0.01, paired t-test).

Data Acquisition and analysis

Data were acquired in PClamp9 with a Multiclamp 700B amplifier and Digidata 1322A A/D converter. Traces were sampled at 50 kHz, online filtered at 10-20 kHz, and subsequently filtered at 1-10 kHz during offline analysis. All traces are averages of multiple trials unless stated otherwise. EPSC waveforms

in Figures 2-4 were generated using Axograph X (rise: 0.2-0.7 ms, monoexponential decay: 3-7 ms). Statistical significance was set at $p<0.05$ and determined using methods listed in the main text and legends.

Reagents

R-CPP, SR95531, NBQX, and TTX were purchased from Abcam. Strychnine was from Sigma-Aldrich.

References

Apostolides, P.F., and Trussell, L.O. (2013). Regulation of interneuron excitability by gap junction coupling with principal cells. Nat Neurosci *16*, 1764-1772.

Apostolides, P.F., and Trussell, L.O. (2014). Chemical synaptic transmission onto superficial stellate cells of the mouse dorsal cochlear nucleus. J Neurophysiol. *111*, 1812-1822