High concentrations of divalent cations isolate monosynaptic inputs from local circuits in the auditory midbrain

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INTRODUCTION

Hierarchical processing of sensory information occurs at multiple levels between the peripheral and central pathway. Different extents of convergence and divergence in top down and bottom up projections makes it difficult to separate the various components activated by a sensory input. In particular, hierarchical processing at sub-cortical levels is little understood. Here we have developed a method to isolate extrinsic inputs to the inferior colliculus (IC), a nucleus in the midbrain region of the auditory system, with extensive ascending and descending convergence. By applying a high concentration of divalent cations (HiDi) locally within the IC, we isolate a HiDi-sensitive from a HiDi-insensitive component of responses evoked by afferent input in brain slices and in vivo during a sound stimulus. Our results suggest that the HiDi-sensitive component is a monosynaptic input to the IC, while the HiDi-insensitive component is a local polysynaptic circuit. Monosynaptic inputs have short latencies, rapid rise times, and underlie first spike latencies. Local inputs have variable delays and evoke long-lasting excitation. In vivo, local circuits have variable onset times and temporal profiles. Our results suggest that high concentrations of divalent cations should prove to be a widely useful method of isolating extrinsic monosynaptic inputs from local circuits in vivo.

Keywords: high divalents, inferior colliculus, monosynaptic, local circuits, first spike latency
by the Committee for Animal Care and Use at the Northeast Ohio Medical University and conformed to the guidelines for labora-
tory animal care and use published by the National Institutes for Health.

**BRAIN SLICE RECORDINGS**

Brains from 25- to 35-day-old mice were used for slice record-

ings. The methods are described briefly here since they have been

published in detail (Chandrasekaran et al., 2013). Mice were anes-

thetized with isoflurane and decapitated. The brain was removed and

300 μm thick slices were made through the transverse plane of

the IC. For recordings, slices were transferred to a temperature-

regulated recording chamber and experiments were carried out

at 35°C. The slice was superfused at 2 ml/min with oxygenated

(95% O2/5% CO2) artificial cerebrospinal fluid (ACSF) containing

10 HEPES, 10 phosphocreatine, pH 7.3; free Ca++ was the same as

26 NaH2PO4, 25 glucose, pH 7.35.

Whole-cell patch-clamp recordings were made from the central

ergon of the IC. Recordings were made under visual control

using an upright microscope (Zeiss Axioskop) fitted with a water

immersion objective (×40/NA 0.75) and differential interface

optics. Patch pipettes were made from borosilicate glass

(Kimax, 1.5 mm O.D.), with resistances of 5–7 MΩ when filled

with a recording solution containing, in millimoles: 120 K Glu-

conate, 10 KCl, 0.2 EGTA, 0.1 CaCl2, 4 Mg-ATP, 0.3 Na-GTP,

10 HEPES, 10 phosphocreatine, pH 7.3; free Ca++ = 90 mM.

Series resistances were generally 12–18 MΩ and compensated

by 75–80%. A junction potential correction of ~11 mV was

applied to all voltages; reported resting membrane potentials

include this correction. An EPC-10 amplifier and Patchmas-
ter/Finmaster software (HEKA Elektronik/Instrutech Corpora-
tion) were used respectively for recordings, data collection, and

analyses. Origin software (OriginLab) was used for statistical anal-

ysis and graphing data. Data are reported from 166 cells in 38

slides.

**High-divalent solutions**

For experiments where a HiDi concentration was used, ACSF was

made without NaH2PO4 and MgCl2 was substituted for

MgSO4 (to prevent precipitation of calcium phosphate or sulfate),

and a HiDi concentration reached by increasing the concentra-

tions of CaCl2 and MgCl2, with a compensatory reduction of

the NaCl concentration to balance osmolality. To prevent com-

petitive block of presynaptic Ca channels and allow normal

transmitter release, the ratio of Ca ++ /Mg ++ was the same as

the control (Byrne et al., 1978; e.g., 2.5 HiDi = 5 mM CaCl2,

3.25 mM MgCl2; total increase in divalents = 4.95 mM). We

tested several HiDi concentrations on intrinsic membrane prop-

erties (see Results) to determine an optimal concentration that

would isolate monosynaptic from polysynaptic inputs without

altering intrinsic firing patterns. HiDi was applied either locally

through a second electrode close to the cell (n = 26 slices) or both

applied to control for incomplete effects on dendritic branches

(n = 32 slices). HiDi effects on intrinsic properties and synap-

tic transmission did not differ significantly between these two

application methods (comparison of peak amplitudes of synap-

tic currents evoked by maximum lemniscal stimulation in control

ACSF and HiDi; t(7) = 1.4, p = 0.16), suggesting that HiDi appli-

cation using both methods reached (electrotonically) most of the

synapses on the neuron from which recordings were made. For

complete washout from HiDi, ACSF was perfused for 5–10 min

and intrinsic firing patterns or synaptic responses checked for

recovery.

**Lemminal stimulation**

To restrict the spatial spread of current as much as possible to the

immediate vicinity of the stimulating electrode, synaptic activity

evoked by stimulating the lateral lemniscus (LL) with a con-

centric extracellular bipolar electrode. We used an electrode with

a tip diameter of ~100 μm as a way to recruit the largest possible

number of axons at maximum current strengths. In some experi-

ments, we switched to a bipolar two-pronged stimulating electrode

straddled across the lemniscal tract, to check the response ampli-

tude at maximum stimulus currents. A 100 μm tip diameter would

cover a large percentage of the axons in the lemniscal tract, thus

it would be expected that minimal stimulation, defined by the

recruitment of a single axon, would not be possible. In other

experiments we used concentric electrodes with smaller active

diameters, but these electrodes did not give us the full range of

synaptic activity, suggesting inadequate effective current spread,

which would selectively eliminate axons with smaller diameters

from our data. With the 100 μm tip diameter, responses at minimal

stimulation, which was defined as a 50% failure rate, evoked synap-

tic currents between 30 and 60 pA, which we estimate to be three
to four times the amplitude of a spontaneous miniature synaptic

activity in IC neurons (Sivaramakrishnan and Oliver, 2006). Thus

we think that we were able to isolate a few synapses with minimal

stimulation.

The stimulating electrode was placed ventral to the dorsal

nucleus of the lateral lemniscus and to the IC, allowing stimu-

lation of LL fibers of passage from lower brainstem nuclei and

from the dorsal nucleus of the LL. Stimulus pulses generated by a

stimulator (AMPI, Israel) were passed through a constant current

stimulation unit (A365; WPI, Sarasota, FL, USA) before reaching

the stimulating electrode. Biphasic current pulses were used to

prevent DC build-up on the LL tract during repeated stimula-

tion, preventing repeated activation of the same LL afferent axon

at high stimulus intensities (with a single shock). Minimal cur-

rents (<0.5 mA, 0.1–0.3 ms) were those that evoked posttynaptic

potentials (PSPs) 50% of the time in 40–50 trials. Maximal cur-

rents were those beyond which PSPs did not change in amplitude

or duration and were taken to indicate maximum recruitment of

LL afferent axons (100% LL activation). Current strengths during

stimulus trains were adjusted in each slice to generate the responses

needed. Inter-trial intervals were generally ~500 ms for single

pulse stimulation and at least ~2 s for stimulus trains with <10

pulses.

Recording modes were switched between voltage- and current-

clamp to record postynaptic currents (PSCs) and potentials

(PSPs) respectively. Holding potentials for PSCs were adjusted

for each cell to match the value of the resting membrane poten-

tial measured under current-clamp. PSPs and PSCs were averaged

from 5 to 10 repeated trials, unless otherwise specified. In

some recordings of PSPs under current clamp, we included
A metal pin was cemented onto the skull to secure the head to the stereotaxic apparatus used for recording, and a tungsten electrode was connected to a picospritzer (WPI, Sarasota, FL, USA) through a set of valves that allowed independent control of each barrel. A second port on the picospritzer was connected to a differential amplifier (A-M Systems). One barrel of the multibarreled pipette was filled with ACSF, and the remaining barrels contained synaptic receptor antagonists dissolved in HiDi. Antagonists of inhibitory synaptic receptors, strychnine (8 μM) to block glycine receptors and SR-95531 (Gabazine; 200 nM) to block GABA receptors, were dissolved in HiDi. These concentrations, which were higher than maximally effective concentrations in IC brain slices (Sivaramakrishnan et al., 2004; Chandrasekaran et al., 2013), were used to overcome inconsistent effects on firing rates because of variable diffusive loss caused by different pipette–cell distances in different recording sessions, and so that pressure ejection of the same drug from more than one pipette did not additionally affect firing rate. Gabazine and strychnine concentrations were varied during the day, coinciding with their wake period. Data were reported from 109 cells in 32 animals.

Surgical procedures
Animals were anesthetized with isoflurane inhalation (1.5–2.0% in oxygen; Abbott Laboratories, North Chicago, IL, USA). The head of the animal was aligned in the stereotaxic apparatus at an angle of 20° (with some variations depending on age) to the horizontal. This head angle aligned the region of the midbrain containing the IC to be “flat,” in line with the rest of the brain so that the long axis of the IC was vertical. The hair on the dorsal surface of the animal’s head was removed, a midline incision made in the skin, and the underlying muscles reflected laterally to expose the skull.

The animal was positioned so that the IC was flat with respect to the horizontal and combined with a 90° fixed electrode impalement angle, allowing us to reach almost the whole range (~60 kHz) of characteristic frequencies in the IC (Egorova et al., 2006). Recordings on the same animal were performed for four consecutive days in two 2-h sessions each day, with a rest period of at least an hour between sessions. The animal was offered water from a medicine dropper between electrode penetrations. Signs of discomfort or distress were relieved either by light sedation with acepromazine (0.05 mg/kg) or by terminating the experiment.

Single-unit recordings were combined with pressure injection of HiDi. A glass recording electrode was glued to a five- or seven-barreled multi-pipette system (Haevey and Caspary, 1980), pulled, broken and polished to a total tip diameter of ~20 μm. The recording electrode was filled with 1 M NaCl (15–20 MΩ), or, in later recordings, with ACSF and connected to a differential amplifier (A-M Systems). One barrel of the multi-barreled pipette was filled with 1 M NaCl or ACSF as a control for pressure injection. A second barrel contained HiDi, and the remaining barrels contained synaptic receptor antagonists dissolved in HiDi. Antagonists of inhibitory synaptic receptors, strychnine (8 μM) to block glycine receptors and SR-95531 (Gabazine; 200 nM) to block GABA receptors, were dissolved in HiDi. These concentrations, which were higher than maximally effective concentrations in IC brain slices (Sivaramakrishnan et al., 2004; Chandrasekaran et al., 2013), were used to overcome inconsistent effects on firing rates because of variable diffusive loss caused by different pipette–cell distances in different recording sessions, and so that pressure ejection of the same drug from more than one pipette did not additionally affect firing rate. Gabazine and strychnine concentrations were varied in different experiments to examine their effects on depolarization block (Sivaramakrishnan et al., 2004) with and without HiDi. Recordings were tested for general recovery from drug applications by allowing recovery to occur following diffusive loss of the drug, or by application of normal ACSF or HiDi through another barrel. Complete recovery from strychnine was rapid (5–10 min); recovery from gabazine was slower (>30 min). These recovery times are similar to those in IC brain slices (Sivaramakrishnan and Oliver, 2006). In experiments not reported here, we also tested recovery from bicuculline. Recovery occurred rapidly, within 3–5 min, which is normal in brain slices, thus recovery times from strychnine and gabazine in vivo reflected their binding constants rather than artifacts of pressure injection. Chemicals were obtained from Sigma-Aldrich.

For pressure injection, the back end of a 1 ml plastic syringe was pulled to a fine tip, cut to the right length, inserted into each pipette, and glued at the end with Epoxy. The inlet of the syringe was connected to a pressure control valve, which was then inserted into a syringe tubing (see Figure 5A). The five or seven tubes of the multibarrel electrode were connected to a picospritzer (WPI, Sarasota, FL, USA) through a set of valves that allowed independent control of each barrel. A second port on the picospritzer was connected to a vacuum inlet, which maintained a very low negative pressure (1–2 psi) on all bars. Injection pressures were raised above vacuum pressures, and kept low (4–6 psi, 100–500 ms) to prevent cell damage.
Acoustic stimulation

Sound was delivered through a loudspeaker placed 10 cm in front of the animal at an angle of 15° to the midline, contralateral to the IC from which recordings were made. Acoustic stimuli were digitally synthesized and downloaded onto a digital signal processing card (AP2 Multi-Processor DSP card; Tucker-Davis Technologies, Alachua, FL, USA), converted to analog signals at a sampling rate of 500 kHz (model DA3–2; Tucker-Davis Technologies), filtered (model FT6–2; Tucker-Davis Technologies), attenuated (model PA4; Tucker-Davis Technologies), summed (model SM3; Tucker-Davis Technologies), amplified (model HCA-800I, Parasound, San Francisco, CA, USA), and sent to a loudspeaker (Infinity EMIT-B; Harmon International Industries, Woodbury, NY, USA). The output of the acoustic system was calibrated over a frequency range of 10–120 kHz using a condenser microphone (model 4135; Bruel and Kjaer, Nærum, Denmark) placed in a position normally occupied by the animal’s head. The calibration of speaker output at 0 dB attenuation was as follows: 4 kHz, 109 dB SPL; 40 kHz, 101 dB SPL; 50 kHz, 93 dB SPL; 80 kHz, 69 dB SPL. We needed to use a maximum tone frequency of 64 kHz, the upper limit of characteristic frequency (CFs). Harmonic distortion was not detectable 60 dB below the signal intensity using a fast Fourier analysis of the digitized microphone signal (model AD2; Tucker-Davis Technologies).

Data acquisition and analysis. Custom software (Matlab, Dr. D. Gans, Northeast Ohio Medical University) was used to generate tone bursts, acquire spikes and frequency tuning curves, and display basic spike statistics in real time. Search stimuli consisted of tones, wide-band noise, and narrow-band noise bursts separated by 30–60 ms. Well-isolated single units were characterized by stable amplitude, consistent shape, and a signal-to-noise ratio exceeding 5:1. Once a single unit was isolated, its CF was determined. The CF was defined as the frequency at which the largest sound pressure level consistently elicited stimulus-locked action potentials.

Construction and analysis of rate-intensity functions

Rate-intensity functions were constructed by averaging firing rates over the maximum response duration, measured from response onset, which was determined from the asymptote of first spike latency (FSL) plots. For each RIF, we first obtained the average firing rates and SD across all sweeps for each intensity. We then used t-tests to determine whether RIFs were significantly different in HiDi or drugs (p < 0.05). SD error bars are not included in illustrated graphs for clarity. Averages determined over other time windows, such as from the beginning of the sound stimulus or from the value of the median or lowest FSL, did not significantly alter the values of spike frequencies in this study. When comparing RIFs in different conditions, the maximum response duration was obtained from the first response once steady state was reached, defined by little or no change in the RIF, we averaged three to four RIFs to obtain the steady state response.

Statistical tests

First spike latencies were calculated across 12 stimulus presentations, and reported as the minimum value of the median first-spike latency obtained across the sound levels tested in a RIF. We subtrated 0.3 ms to account for travel of sound across the 10 cm distance between the speaker and the animal’s ear and 0.5 ms for the rise time of the tone. Results are expressed as mean ± standard error of the mean. Standard deviation, when used, is indicated in the text. Normality was confirmed (Origin software) and paired t-test or ANOVA with p < 0.05, and Bonferroni correction, were used as a criterion for significance.

RESULTS

Invertebrate systems and in acute mammalian brain slices, HiDi has been used to separate polysynaptic inputs from monosynaptically driven activity (Einum and Buchanan, 2004; Rose and Metherate, 2005). The effects of HiDi in blocking polysynaptic activity are generally attributed to an increase in firing threshold at the successive synapses in a string of synaptic contacts (Berry and Pentreath, 1976). High concentrations of divalent cations in the external bathing fluid can raise spike threshold by shifting sodium channel activation, generally observed at high (>3× normal) divalent concentrations (Campbell and Hille, 1976) or by local screening of ion channels that arises from the increased density of positive charges around their external surfaces (Gilbert and Ehrenstein, 1969; Hille et al., 1975).

To establish the effects of HiDi on intrinsic membrane properties as well as synaptic activity, and to find optimal divalent strengths for the IC, we tested the effects of HiDi in brain slices. We defined an optimal HiDi concentration in three ways. First, it should not result in intrinsic spike failure. This requirement was necessary to establish that HiDi did not prevent the postsynaptic cell from responding to a normally suprathreshold synaptic input. Second, HiDi should produce a clear separation between the synaptic components of an input. That is, to establish that the net synaptic input to a neuron contained a HiDi-sensitive and insensitive component, at least one of the two components had to have features that were invariant in HiDi. Third, under conditions of minimal stimulation of the ascending input fiber
Hidi effects on intrinsic membrane properties

Intrinsic membrane properties were examined by injecting current pulses into the soma and recording voltages with the same electrode (Figure 1A). Firing patterns evoked by depolarizing current steps were used to test the effects of Hidi on spike characteristics. Input resistances and membrane time constants were measured from responses to hyperpolarizing current steps. We report Hidi effects on sustained-regulated cells (n = 14), a common IC cell type, chosen for its low spike thresholds, consistent spike heights during sustained firing, regular inter-spike intervals and negligible active conductances at small hyperpolarizations (Sivaramakrishnan and Oliver, 2001).

Firing patterns remained sustained in 2.5–3 Hidi (Figure 1B, left, middle). At higher divalent strengths (≥3 Hidi), spikes shortened and aborted (Figure 1B, right column). We therefore expected that divalent concentrations between 2.5 and 3× the normal would be optimal.

Figures 1C−E summarizes the effects of different Hidi concentrations on intrinsic membrane properties of sustained-regulated cells. At ≤3 Hidi, spike frequencies, interpulse intervals, and spike heights were normal. At >5 Hidi, spike frequencies dropped, interpulse intervals increased, and spike heights rapidly declined (Figure 1C, ANOVA, p < 0.05). These results suggested that 2.5 Hidi did not cause non-linear effects on voltage-gated conductances that underlie spiking in IC neurons, at least at the macroscopic level tested here. Non-linear effects at divalent strengths >3 Hidi are likely due, among other things, to high levels of charge screening or shifts in activation of voltage-gated sodium currents (Campbell and Hille, 1976).

About 2.5 Hidi did not alter passive membrane properties to significant extents. Input resistances and membrane time constants were measured with responses to small hyperpolarizing currents. Input resistances are illustrated for a single sustained-regulated cell (Figure 1D, top) and averaged over 14 cells (Figure 1D, middle). Input resistances, measured at steady state, were normal up to ≤3 Hidi (ACSF, 2, 2.5, 3 Hidi: F_AAR = 0.99, p = 0.42) but deviated at very large hyperpolarizing currents. Membrane potentials reached at these large hyperpolarizing currents were approximately ~100 mV (30 mV hyperpolarization from a resting potential of ~70 mV, Figure 1D, top and middle panels), which is out of the normal range of inhibitory synaptic potentials in IC neurons. If Hidi raises surface charge (Hille et al., 1975), the membrane would be expected to charge more slowly. In 2.5 Hidi, whole-cell time constants, derived from single exponential fits of voltage responses to small hyperpolarizing currents, increased by ~1–3 ms in different cells (Figure 1D, bottom panel), a very slight increase in the long time constants of IC neurons (11–37 ms in the different cell types; Sivaramakrishnan and Oliver, 2006). When averaged over the sample (n = 56, data pooled from different cell types), changes in time constant were minimized and were not significant in 2.5 Hidi (τ_111 = 1.53, p = 0.13), but showed significant differences at higher Hidi concentrations (e.g., 3.5 Hidi; τ_111 = 3.1, p = 0.002).

To examine the effect of Hidi on threshold for spiking, we measured threshold currents, voltages and the rate of rise of the membrane potential to threshold in response to current pulses injected into the soma. Threshold currents increased by 31 ± 8 pA in 2.5 Hidi (Figure 1E, top, arrowhead) and by 300 ± 69 pA in 4 Hidi (ACSF, 2 Hidi, τ_27 = 1.21, p = 0.23; ACSF, 2.5 Hidi, τ_27 = 3.3, p = 0.003; ACSF, 4 Hidi, τ_27 = 5.41, p < 0.0001). In 2.5 Hidi, threshold voltage increased by 1.1 ± 0.4 mV and the rate of rise of membrane voltage toward threshold slowed by 1.9 ± 0.5 mV/ms in (Figure 1E, middle, bottom). These data suggest that Hidi causes the membrane to charge more slowly, and raises firing threshold.

Identification of Hidi-sensitive and -insensitive components of synaptic responses

We next established the criteria necessary to distinguish a synaptic response as an extrinsic input that ascended through the lemniscal pathway and made monosynaptic contact on an IC neuron. Anatomical evidence suggests different origins for monosynaptic inputs to the IC central nucleus, arising from lemniscal afferents making direct glutamatergic (Loftus et al., 2004) or glycinergic (Moore et al., 1998; Loftus et al., 2004) contact onto neurons, or by corticofugal inputs (Nakamoto et al., 2013). Since corticofugal pathways were not accessible in slices, we restricted the interpretation of our data to lemniscal inputs.

We first confirmed that the PSC in ACSF contained ascending monosynaptic inputs. The afferent LL tract (Figure 2A) was stimulated with stimulus currents set at 50% above threshold levels to evoke PSCs. Hidi reduced PSCs, but only partially (Figure 2B), which suggested a Hidi-insensitive (monosynaptic, PSC_L) and Hidi-sensitive (local, PSC_C) component. The rise time of the Hidi-insensitive component was rapid (1.5 ± 0.2 ms, Figure 2C). The rise time of the total PSC (4.5 ± 0.23 ms), was less than the...
sum of the rise times of PSCM and PSCL (p = 0.0262) and implies conductance increases when both monosynaptic and local inputs were active. The onset latency of the HiDi-insensitive component, which was short (1.31 ± 0.16 ms, n = 21 cells), remained constant as stimulus currents were increased, with a SD 

\[ t \pm \sigma \]

of 0.997 ± 0.063 ms (p = 0.0262) and implies monosynaptic and polysynaptic sources. The maximum stimulus current strength (100% LL) current is defined as the current beyond which no further increases in PSC amplitude or duration occur. PSC onset latencies had a SD < 1 and remained constant as LL stimulus currents increased (Fig. B, D). The rise time of PSCM was faster than that of PSCL, 1.5 ± 0.2 ms and 5.10 ms, respectively (p = 0.0026). Spike heights: F(2, 21) = 2.87; p = 0.01. (B) Passive membrane properties in HiDi. Top: input resistance (R) plotted for one sustained regular cell. Same cell as in (D). (C) Membrane potentials (Vme) and local component blocked by HiDi. Double arrow, top trace: latency of the PSC peak measured from the stimulus artifact. Dotted vertical line is lined up to the peak of the PSC. (A) Transverse IC slice, SC: superior colliculus, DL: dorsal nucleus of the lateral lemniscus, LL: lemniscal tract. Slices were bathed in different ACSF concentrations (C). Inside, in HiDi-insensitive component to the IC was a HiDi-sensitive component. Since this component is the portion of the PSC that is not a direct ascending monosynaptic input, we refer to it as the local component, PSC_L (Fig. 2B). The rise time of PSC_L was significantly shorter than that of PSCM (1.5 ± 0.2 and 3.6 ± 0.2 ms, t = 3.38; p = 0.0026, Figure 2C). Its onset latencies were much longer and illustrated with stimulus intensity (6.9 ± 0.4 to 3.23 ± 0.5 ms, n = 21 cells; F(2, 21) = 3.7; p = 0.004). The decrease in onset latency with stimulus intensity

\[ F(5, 100) = 3.38; p < 0.001 \]

\[ F(5, 100) = 3.38; p < 0.001 \]

\[ t < 0.004 \]

\[ 0.063 \]
is suggestive of a polysynaptic pathway. Given the placement of our stimulus electrodes on the lemniscal tract, the change in onset latency with stimulus intensity is likely due to a recruitment of multiple polysynaptic pathways onto a given IC neuron (see Discussion).

Of the total population of cells from which recordings were made (n = 165), 127 (76%) had both monosynaptic and local inputs, 11 (6%) had only monosynaptic inputs at all LL stimulus intensities, and in 28 neurons (17%), most or all responsiveness to LL stimulation was abolished by HiDi, suggesting a predominance of local polysynaptic inputs.

Because the HiDi-insensitive (monosynaptic) component did not exhibit changes in onset latency with stimulus intensity, it was unlikely to have included a significant fraction of the polysynaptic component. The largest separation of onset latencies between the HiDi-insensitive and sensitive components was 5.6 ms (1.3 and 6.9 ms) at the lowest lemniscal shock strength. This large separation suggests that a disynaptic input with an intermediate onset latency (Agmon and Connors, 1992) had not been activated. The HiDi-insensitive component, however, did not increase its onset latency with stimulus strength (variations remained < 1 ms), and did not fill in the 1.94 ms gap. The component isolated by its onset latency with stimulus strength (variations remained < 1 ms), and did not fill in the 1.94 ms gap. The component isolated by HiDi was therefore less likely than the HiDi-sensitive component to have included the recruitment of a high-threshold disynaptic input.

Postsynaptic currents peak latencies (7.76 ± 0.009 ms; n = 46 cells) were normally distributed, but latency distributions of both the monosynaptic and local inputs were skewed (Figure 2E). The monosynaptic input preceded the total PSC (by ~3.31 ± 0.036 ms; t/2PSC = 3.72; p = 0.0002), but the local input followed it (by 3.78 ± 0.063 ms; t/2PSC = 2.62; p = 0.009). The most likely interpretation of these early and late latencies is an electronic or spatial segregation of inputs on IC neurons or, given the variable extent of axonal collateralization in the IC (Oliver et al., 1991; Wallace et al., 2012), from sources with different path times.

As a more rigorous criterion for a monosynaptic response, we used minimal stimulation of the LL tract (50% synaptic failure rate) to confirm that the 1 ms onset latency established a boundary within which the HiDi-insensitive synaptic response could be considered to be monosynaptic. Minimal stimulation evoked PSPs that were completely insensitive to HiDi. Figure 3A, n = 42 cells; PSP duration: t/2PSP = 0.71; p = 0.48; PSP amplitude: t/2PSP = 0.48; p = 0.65). We presented single shocks at very low stimulus rates to get a sense of the variability in onset latency between trials (repetition rate of 1/3 s). This variability was within ~1 ms (1.01–1.97 ms; mean 1.48 ms; SD 0.28; n = 60 neurons). Because the SD of this distribution was < 1 (0.28 ms), as was the SD of the HiDi-insensitive current evoked by different stimulus intensities and with trains of different frequencies (as in Figure 2), we concluded that the synaptic response insensitive to HiDi was a monosynaptic response. The tight clustering of onset latencies was surprising, because we expected that stimulation of the lemniscal tract would activate multiple inputs from various brainstem sources and that inputs would be distributed along dendrites, and onset latencies would vary, even within the short electrotonic distances that the somatic recording electrode would sample.

If there was considerable heterogeneity in the monosynaptic population either within release sites in a single monosynaptic input or within a group of monosynaptic inputs, then minimally evoked PSP amplitudes should vary during a stimulus train (Stevens and Wang, 1995). PSPs evoked by minimal-stimulus trains remained insensitive to HiDi throughout the train (Figure 3B; 33 cells; 20–30 Hz trains; PSP amplitudes: E500 = 1.39; p = 0.19). Onset latencies during the successive responses in the train varied by less than one SD (1.21 ± 0.19 ms; 21 cells). PSPs also did not facilitate or depress (t/2PSP = 1.16; p = 0.23). We then raised the current strength of lemniscal stimulation to recruit more afferent axons, and switched to voltage-clamp to avoid postsynaptic non-linearities. The onset latencies of PSCs during stimulus trains varied by < 1 SD (42/51 cells; Figure 3C; 25 cells; Mean and SDs: 10 Hz, 1.23 ± 0.18; 20 Hz, 1.26 ± 0.22; 40 Hz, 1.28 ± 0.38). These data suggested constancy to the monosynaptic input to IC neurons, whether it was a single input or multiple inputs. A clustering of monosynaptic inputs close to the soma is one interpretation of our data, however, we suggest that, alternatively, the lack of variability in monosynaptic onset latency could arise from a dominant monosynaptic input (see Discussion).

To get a sense of the “dynamic range” of monosynaptic and local inputs, we changed LL stimulus currents to evoke a minimal to maximal synaptic response (from 50% failures to a response that did not change with further stimulus increases; Figure 4A). Recordings were made with QX-314 in the recording pipette to block sodium- and other voltage-gated currents (Mulle et al., 1985) and switched between voltage- and current-clamp to record PSCs and PSPs.

The PSC in ACaF increased with stimulus intensity and included smaller delayed events. PSCaF did not include the smaller events seen in the control PSC. As expected from temporal delays associated with polysynaptic inputs (Sineau and Buchanan, 2005), the local input included long-latency events (Figure 4A). Peak PSC amplitudes were controlled by monosynaptic inputs (PSC: E500 = 1.72; p = 0.15; PSC, PSCaF: E500 = 4.14; p = 0.003; Figure 4B). PSC durations were more strongly affected by local inputs. At low stimulus currents, the total PSC, the mono- and local polysynaptic inputs had similar durations (e.g., 20% LL current; E500 = 0.74; p = 0.48). With increases in stimulus current, however, the local input increased in duration, and similarly to the PSC, but the duration of the monosynaptic current decreased (e.g., 80% LL current: E500 = 14.6; p < 0.0001; Figure 4C). The different trajectories of PSCaF and PSC with stimulus current verified that the smaller peak amplitudes of the local synaptic current were not due to just activity-dependent depression of the same synaptic pool (Walden et al., 2000) as the monosynaptic input.

Postsynaptic currents area integrals (ΔPSC) showed monosynaptic and local inputs with highest synaptic efficiencies in adjacent stimulus current ranges. ΔPSCaF increased only until ~30% of the maximum LL stimulus current, after which it declined. ΔPSCaF, which had an initially higher threshold than ΔPSCaF (>30% at...
20% LL current), continued to increase with stimulus current. At peak LL currents, Δ\(\Delta P_{\text{SC}}\) was 90% of \(\Delta P_{\text{SC}}\), whereas \(\Delta P_{\text{SC}_{\text{M}}}\) was just 15% of \(\Delta P_{\text{SC}}\), indicating a 75% monosynaptic-local input differential (Figure 4D).

The slow time constants of IC neurons (Sivaramakrishnan and Oliver, 2006) would be expected to favor integration of delayed local inputs. As stimulus current increased, the PSP became multi-peaked and prolonged (Figure 4E). The monosynaptic component, \(\Delta P_{\text{SC}_{\text{M}}}\), was single-peaked, while the local component, \(\Delta P_{\text{SC}_{\text{L}}}\), was multi-peaked, reflecting integration of delayed local inputs. The monosynaptic-local input differential, seen with synaptic currents, was reduced, but not eliminated, by postsynaptic integration. \(\Delta P_{\text{SC}_{\text{M}}}\) amplitudes reached just 63% of the PSP amplitude at 100% LL current, and durations increased only very slightly, saturating by 60% LL current strength (Figure 4F), suggesting active conductances (Sivaramakrishnan and Oliver, 2001). \(\Delta P_{\text{SC}_{\text{L}}}\) amplitudes also did not reach those of PSP, however, their durations, which increased with stimulus current, were closely matched to the PSP duration (e.g., durations at 80% LL stimulus strength: \(\Delta P_{\text{SC}}, \Delta P_{\text{SC}_{\text{L}}}: t_{109} = 1.21, p = 0.11\); Figure 4G). PSP...
HiDi effects in vivo

We used well-isolated (signal to noise >5:1) single unit recordings of neuronal discharge patterns in the IC of head-fixed unanesthetized mice to examine the effects of HiDi in vivo. To separate extrinsic monosynaptic inputs from local activity, HiDi was applied with pressure pulses for several minutes through one barrel of a multi-barrel electrode (Figure 5A). Each pulse was 500–1000 ms long and applied with pressures of 5–12 psi.

HiDi effects on spike characteristics

We performed control experiments to test for possible artifactual effects of HiDi in vivo. In 2.5 HiDi, single unit isolation was not affected and spike heights and widths were normal (p = 0.32; p = 0.226). Parallel to HiDi effects in slices, higher HiDi...
FIGURE 5 | Establishing optimal HiDi concentrations in vivo. (A) Multi-barrel system for pressure injection and attached recording electrode (arrow). Pressure tubes are inserted into each barrel. (B) Spikes during single unit recordings were normal in 2.5 HiDi but shortened and broadened in 4 HiDi. Left: recordings of spiking in control, 2.5 HiDi and 4 HiDi. Top panel: superimposed traces of single tone-evoked spikes. Second through fourth traces: snippets of background activity in the absence of tones. The 50 ms scale bar applies to the second through fourth traces. (C) Quantification of HiDi-induced changes in spike heights and widths. Top: superimposed spikes from one neuron in control (black) and HiDi (red) illustrate that single unit isolation is unaffected by the pressure application of HiDi. Recordings illustrated are at 81 dB SPL, suggesting that changes in spike rates in HiDi at high intensities were not due to false positives or false negatives. Bottom panels: spike heights and widths as a function of two HiDi concentrations. Mean and SD. Comparison between 60 spikes in control and 60 in each concentration of HiDi. Eight neurons. Spike heights: control; 2.5 HiDi: $t_{119} = 1.01; p = 0.32$; control, 4 HiDi: $t_{119} = 2.56; p = 0.01$. Spike widths: control, 2.5 HiDi: $t_{119} = 1.2; p = 0.23$; control, 4 HiDi: $t_{119} = 3.01; p = 0.003$. (D) Strong firing in HiDi does not produce spike loss due to additional depolarization block. Left and right columns show spikes at two intensities. Left: depolarization block at 46 dB SPL is not evident in either Gabazine alone or in HiDi/gabazine (left). The first spike latency in Gabazine alone occurs earlier than in HiDi, suggesting that some of the FSL is due to excitatory local circuits released from inhibition. Firing in HiDi is reduced, leaving the monosynaptic excitatory input. Right: depolarization block at 76 dB SPL is also observed in HiDi/Gabazine. HiDi does not produce additional depolarization block. 100 ms tone.

HiDi did not increase the likelihood of postsynaptic depolarization block that occurs in the IC at high sound intensities when inhibitory GABAergic input is blocked (Sivaramakrishnan et al., 2004). At sound intensities that did not elicit depolarization block in the GABA antagonist, gabazine, no block occurred in HiDi. At intensities that produced depolarization block in gabazine, HiDi evoked the same amount of block ($n = 24$; Figure 5D). This result suggests that 2.5 HiDi did not cause non-linear changes in the neuronal spike generator in vivo.
The criterion for a complete separation of HiDi-sensitive and insensitive components of firing rate was a steady-state response in HiDi. To establish parameters for HiDi’s pressure injection that would produce steady-state responses, we constructed RIFs by varying tones between 0 and 90 dB SPL. This allowed us to test the effects of HiDi under conditions where the number of inputs and the range of frequencies recruited change with acoustic stimulation (Xiang et al., 1965). Since 2.5 HiDi did not produce spike shortening or depolarization block by itself, we were confident that changes in spike rates in HiDi could be attributed to changes in the relative complement of monosynaptic and local inputs, which would include the effects of postsynaptic integrating mechanisms. Steady-state firing rates were reached gradually through each subsequent HiDi pulse that produced intermittent changes (Figure 6). HiDi effects were reversible, which allowed us to record from several depths in the IC during one experiment. The reversibility of HiDi also suggested no long-lasting effects on membrane integrity.

**Temporal activation of local circuits**

To examine temporal variations in the HiDi-sensitive local component of the response, we measured the effects of HiDi on firing rates in successive time windows during a tone. Figure 7 uses two cells to illustrate temporal variations in HiDi effects at different sound intensities. The cell in Figure 7A responded throughout the 100 ms tone. HiDi had no effect on firing rates at low sound intensities (e.g., 30 dB SPL), but decreased firing at high intensities (e.g., 70 dB SPL). At 70 dB SPL, the reduction in firing rate was most noticeable >50 ms after tone onset. The monosynaptic component overlapped the control RIF at very early times after tone onset (Figure 7A, right column, top panel), but contributed less at later times (Figure 7A, right column, middle, bottom panels). The firing rate due to the HiDi-sensitive local component was obtained by subtracting the monosynaptic RIF from the RIF in control conditions. This method of deriving local circuit effects on RIFs is simplistic, and assumes that the firing rate in HiDi includes interaction of local synaptic inputs with postsynaptic properties. It is used here merely to illustrate changes in firing rate in HiDi. The HiDi-sensitive component was not activated at tone onset, but increased at later times. In this cell, therefore, the local component turned on at 52 ms, and continued to increase with sound intensity. The cell in Figure 7B showed more temporally uniform HiDi effects. HiDi did not affect firing rates at low (15 dB SPL) and high (40 dB SPL) intensities. Its effect at 25 dB SPL, which corresponded to the peak firing rate of the control RIF, was distributed throughout the tone, with no particular distinction between tone onset and later times.

**HiDi isolates monosynaptic inputs in vivo**

As a first requirement that extrinsic inputs to the IC would persist in HiDi, we tested its effects on first spike latencies (FSLs). Most FSLs in the IC arise from ascending input (Heil and Neubauer, 2001). FSLs involve direct monosynaptic pathways to the IC from the cochlear nucleus as well as ascending multi-synapse pathways (Mauger et al., 2010). We measured an average control FSL of 15.34 ± 6.15 ms (n = 88). The large SD of our population data reflects the wide distribution of FSLs (~5–30 ms), which has been previously reported in the unanesthetized IC (Sivaramakrishnan et al., 2004; Sanchez et al., 2007; Sayegh et al., 2012). If this wide range of FSLs reflects a range of monosynaptic, disynaptic, or polysynaptic inputs then, by parallels with brain slice recordings, a shortening of FSLs with increases in sound intensity should imply that a polysynaptic pathway within the IC was included in determining the FSL for a given neuron. Two aspects of the data in Figure 8 suggest that most FSLs arose from monosynaptic inputs. First, in the two cells shown (Figure 8, left column), FSLs in HiDi did not shorten at high intensities (ANOVA, p < 0.05). This finding was consistent across the sample of IC neurons (60 neurons analyzed; ANOVA, p < 0.05) and suggested that the shortest latency input to an IC neuron was monosynaptic. Second, as an average in the population and in within-neuron comparisons, FSLs in HiDi were not altered (14.88 ± 6.03; 84/88 neurons; p = 0.118, Figure 8, middle, right panels). In four neurons with FSLs > 25 ms, HiDi decreased FSLs by 2–6 ms. Local inhibition would be one source of long FSLs and could occur through recurrent inputs between IC neurons, and indicates that in a small population of IC neurons, FSLs involve local dis- or polysynaptic connections. These data suggested that most FSLs in the IC arise from monosynaptic connections and further, that HiDi application did not compromise spike invasion into monosynaptic nerve terminals in vivo.
FIGURE 7 | Local circuits in vivo show temporal variations. (A,B)

Examples of HiDi effects on two neurons. (A) At 30 dB SPL, which was 15 dB above threshold for this neuron, HiDi did not affect spiking. At 70 dB SPL, HiDi reduced spiking, but mainly at later times during the tone. Rate-level functions plotted in different time windows during the tone for this cell (right column) show little effect of HiDi immediately following tone onset, but a larger effect ~20 ms after tone onset and 20 dB above threshold. (B) In a second neuron, HiDi does not affect firing at 15 dB SPL, but reduces firing at 25 dB SPL, which corresponds to the peak of the control RIF (right column). HiDi has almost no effect at intensities corresponding to the downward limb of the RIF. HiDi does not affect responses soon after onset (top graph, right column), but its effect in successive time windows is similar.

Isolating monosynaptic inputs to the IC in vivo has not been simple because both inhibition and excitation ascend through the lemniscal pathway. Using high local levels of GABAergic agonists raises the threshold for local excitation (Zhang and Kelly, 2003) however it is not clear what the suppressive effect would be on ascending excitation. To test whether we could use HiDi to isolate monosynaptic input to the IC, we tested its effects on glycinergic inputs, which are well-established as being monosynaptic (Moore et al., 1998; Loftus et al., 2004). We measured firing rates in HiDi, and again in a HiDi/strychnine combination. Firing rates in HiDi/strychnine increased beyond control rates. This increase was observed in a number of neurons with different patterns of glycinergic input ($n = 17$; Figure 9) and provided one piece of evidence that we could use HiDi to isolate monosynaptic inputs in vivo. We chose the two cells in Figure 9 to illustrate that the preservation of monosynaptic inputs by HiDi is not necessarily a reflection of their strengths. The neuron in Figure 9A retained sustained monosynaptic excitation during the tone (Figure 9A, middle panel), thus glycinergic inhibition was not strong enough to completely block monosynaptic excitation. Glycinergic input did decrease monosynaptic excitation, however, since the firing rate in HiDi/strychnine was greater than in HiDi alone. In the neuron in Figure 9B, glycinergic inhibition was strong enough to completely block the monosynaptic excitatory input. Had the balance been toward monosynaptic excitation, part of the firing observed in HiDi/strychnine should have occurred during the tone in HiDi alone.
recruitment of lemniscal inputs on proximal dendrites. The gesting large glutamatergic terminals or dense terminal arbors total synaptic current, but it also reached its peak earlier, sug-

naptic current in brain slices, determined the amplitude of the rapidly rising, short latency, monosynaptic response. Its onset latency shows little jitter during repeated acti-

tic input appears to have the characteristics of a monosynaptic input. Its onset latency shows little jitter during repeated acti-

The amplitude of the monosynaptic and local inputs

SOURCES OF MONOSYNAPTIC AND LOCAL INPUTS

The amplitude of the monosynaptic and local inputs

DISCUSSION

The ability to isolate inherited inputs from local circuitry allows a dissection of hierarchical processing in systems. Our results suggest that the use of HiDi in vivo succeeds in isolating the monosynaptic component of the response to an acoustic stimulus. Because we applied HiDi locally, the component that is sensitive to HiDi is most likely a local component. Although parallels between brain slices and in vivo recordings are subject to interpretation, our measurement of HiDi effects in vitro and in vivo provide strong evidence that HiDi distinguishes at least two synaptic pools, one a monosynaptic pool ascending through the lemniscal pathway into the auditory midbrain, and a second pool that consists of local di-

and polysynaptic pathways. The component of HiDi-insensitive responses in the IC evoked by electrical stimulation of the lateral lemniscus or by acous-
tic input appears to have the characteristics of a monosynaptic input. Its onset latency shows little jitter during repeated acti-

vation of inputs, and it gives rise to the FSL in vivo. Increasing recruitment of lemniscal inputs in vitro or increasing the tone duration recruits additional inputs that are HiDi-sensitive and change their onset latencies with intensity, suggesting a polysynap-
tic effect of local IC connections. While the limited jitter in onset latency FSLs, which were shortened in HiDi, could imply local inhibitory influence. The decrease in onset latency of HiDi-sensitive local inputs suggests that increased stimulus intensity at the lemniscus recruits multiple local pathways whose final synapses impinge on the den-

drites of an IC neuron at different distances from the soma, or that summation of different local inputs produces a response that reaches the soma faster. The distal dendritic location of local input suggested by the delayed response in brain slices could arise from axonal collateralizations of IC neurons within frequency laminae or more widespread (Oliver et al., 1991; Wallace et al., 2012) local inter-neuronal connections. Polysynaptic activity generates plateau potentials in the IC and elsewhere (Rose and Metherate, 2005) and local circuit regulation of dendritic excitability involving glutamate receptors or voltage-gated channels (Yu and Salter, 1999; Ohtsuki et al., 2012; Lee et al., 2013) would be expected to provide an increased gain associated with large and prolonged local potentials. The longer, slower polysynaptic responses most likely reflect the fact that local inputs are less synchronously activated. The long pathways involved in com-

m issial influences on local processing (Orton et al., 2012) or the more spatially restricted dendritic arbors within anatomical laminae (Morest and Oliver, 1984; Oliver and Morest, 1984; Servien et al., 1984; Brown et al., 1997) could underlie the variability in the temporal activation of local inputs.

In vivo, most FSLs did not change in HiDi, also suggesting a dominant monosynaptic lemniscal input to each neuron. Unlike the slice recordings, however, FSLs were widely distributed in IC neurons 5–30 ms, strongly suggesting that inputs to different IC neurons arose from axons with different diameters (Malmierca et al., 2005), conduction velocities or path distances. The few long latency FSLs, which were shortened in HiDi, could imply local inhibitory influence. The decrease in onset latency of HiDi-sensitive local inputs suggests that increased stimulus intensity at the lemniscus recruits multiple local pathways whose final synapses impinge on the den-

drites of an IC neuron at different distances from the soma, or that summation of different local inputs produces a response that reaches the soma faster. The distal dendritic location of local input suggested by the delayed response in brain slices could arise from axonal collateralizations of IC neurons within frequency laminae or more widespread (Oliver et al., 1991; Wallace et al., 2012) local inter-neuronal connections. Polysynaptic activity generates plateau potentials in the IC and elsewhere (Rose and Metherate, 2005) and local circuit regulation of dendritic excitability involving glutamate receptors or voltage-gated channels (Yu and Salter, 1999; Ohtsuki et al., 2012; Lee et al., 2013) would be expected to provide an increased gain associated with large and prolonged local potentials. The longer, slower polysynaptic responses most likely reflect the fact that local inputs are less synchronously activated. The long pathways involved in com-

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In summary, high concentrations of divalent cations, which have been used to separate monosynaptic inputs from local circuits in invertebrates and mammalian brain slices, appears to be a viable tool to isolate monosynaptic extrinsic inputs from local circuits in vivo.

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AUTHORS CONTRIBUTION
Shobhana Sivaramakrishnan performed slice experiments. Calum Alex Grimsley, Jason Tait Sanchez, and Shobhana Sivaramakrishnan collected in vivo data. Calum Alex Grimsley and Shobhana Sivaramakrishnan analyzed in vivo data. Shobhana Sivaramakrishnan designed the study and wrote the paper.

REFERENCES
Agmon, A., and Connors, B. W. (1992). Correlation between intrinsic firing patterns and thalamocortical synaptic responses of neurons in mouse barrel cortex. J. Neurosci. 12, 319–329.

Babadi, B., and Abbott, L. F. (2010). Intrinsic stability of temporally shifted spike timing dependent plasticity. PLoS Comput. Biol. 6:e100961. doi: 10.1371/journal.pcbi.1000961

Berry, M. S., and Pentreath, V. W. (1976). Criteria for distinguishing between monosynaptic and polysynaptic transmission. Brain Res. 105, 1–20. doi: 10.1016/0006-8993(76)90919-7

Brown, M., Webster, W. R., and Martin, B. L. (1979). Criteria for distinguishing between monosynaptic and polysynaptic transmission. Brain Res. 105, 1–20. doi: 10.1016/0006-8993(76)90919-7

Bucy, R., and Albrecht, K. (1995). Intrinsic firing patterns and thalamocortical synaptic responses of neurons in mouse barrel cortex. J. Neurosci. 12, 319–329.

Byrne, J. H., Castellucci, V. F., and Kan del, E. R. (1978). Contribution of individual mechanoreceptor sensory neurons to defensive gill-withdrawal
rollen in Aplysia. J. Neurophysiol. 41, 418–437.
Campbell, D. T ., and Henkel, C. K. (1987). Kinetic and pharmacological properties of the somatodendritic channel of frog skeletal muscle. J. Gen. Physiol. 67, 509–525. doi: 10.1085/jgp.67.5.509
Cant, N. B., and Benson, C. G. (2003). Parallel auditory pathway: projection patterns of the different neuronal populations in the dorsal and ventral cochlear nuclei. Brain Res. Bull. 60, 407–476. doi: 10.1016/S0361-9230(03)00093-0
Chandiramani, L., Xiao, Y., and Sivaramakrishnan, S. (2013). Functional architecture of the inferior colliculus revealed with voltage-sensitive dyes. Front. Neural Circuits 7:41. doi: 10.3389/fncir.2013.00041
Doyle, M. W ., and Andresen, M. C. (2005). Reliability of monosynaptic sensory transmission in brain stem neurons in vivo. J. Neurophysiol. 93, 2213–2223.
Eisworth, M., Vartanian, L., and Ehrat, G. (2006). Frequency response areas of mouse inferior colliculus neurons. Cereb. Cortex 17, 1783–1786. doi: 10.1093/cercor/bhj308
Einum, J. F ., and Buchanan, J. T . (2005). Retinocollicular neurons receive direct spinocerebellar inputs during locomotor activity in rat. J. Neurosci. 25, 1386–1390. doi: 10.1523/JNEUROSCI.5484-04.2005
Einhorn, J. F ., and Buchanan, J. T . (2005). Membrane potentials oscillations in retinocollicular and spinocerebellar neurons during locomotor activity. J. Neurophysiol. 94, 2772–2784. doi: 10.1152/jn.00895.2004
Frankenhaeuser, B., and Hodges, A. L. (1957). The action of calcium on the electrical properties of squid axons. J. Physiol. 137, 154–177.
Gilbert, D. L., and Henriquez, G. (1998). Effect of depolarizing current on potassium conductance of squid axon: determination of surface charge. Biophys. J. 79, 447–461. doi: 10.1016/S0006-3495(98)77396-4
Harris, D. J ., and Cowley, M. A. (1993). A simple technique for constructing ‘piggy-back’ multibarrel microelectrodes. Electroencephalogr. Clin. Neurophysiol. 86, 249–251.
Holl, F ., and Neubauer, H. (2001). Temporal integration of sound pressure determines thresholds of auditory-nerve fibers. J. Neurosci. 21, 7404–7415.
Hille, B., Woodhull, A. M., and Shapiro, D. L. (1969). The action of calcium on the thalamus in the rat. J. Neurosci. 10, 1598–1606. doi: 10.1016/0016-6473(80)90313-2
Kiang, N. Y . S., Watanabe, T., Thomas, C., and Clark, L. B. (1965). Discharge Patterns of Single Fibers in the Cat’s Auditory Nerve. Cambridge, MA: MIT Press.
Lee, K. I., Quaasen, R. N., Roes- boom, A. M., Bellmore, R., Lim, S. T., Vicini, S., et al. (2013). Mossy fiber-CAT synapses mediate homeo- static plasticity in mature hippocampal neurons. Neuron 79, 99–114. doi: 10.1016/j.neuron.2012.10.035
Liu, R. B., Wu, G. K., Arbuszka, R., Tan, H. W., and Zhang, L. I. (2007). Defining cortical frequency tuning with recurrent excitatory circuitry. Nat. Neurosci. 10, 1599–1606. doi: 10.1038/nn2012
Lorita, W ., Bishop, D. C., Saint Marie, R., and Oliver, D. L. (2004). Organization of binned excitatory and inhibitory inputs to the inferior colliculus from the superior olives. J. Comp. Neurol. 472, 220–236. doi: 10.1002/cne.20305
Malmierca, M. S., Haberkorn, J., Henkel, C. K., and Oliver, D. L. (2013). SSAK channel modulation contributes to compartment-specific dendritic plasticity in cortical pyramidal cells. Front. Neurosci. 7, 1783–1786. doi: 10.3389/fnins.2013.0164
Malmierca, M. S., Saint Marie, R., and Oliver, D. L. (2009). Monosynaptic, chemical and electrical connections between sensory and motor cells in the central nervous system of the leech. J. Physiol. 580, 647–667.
Mizuno, S., Oishi, T., and Otsuka, S. (2004). Dendritic and axonal morphologies of HRP-injected neurons in the inferior colliculus of the cat. J. Comp. Neurol. 472, 226–239. doi: 10.1002/cne.20305
Malmierca, M. S., Meredith, A. M., Henkel, C. K., and Oliver, D. L. (2002). Direct projections from cochlear nucleus complex to auditory thalamus in the rat. J. Neurosci. 22, 10891–10897.
Malmierca, M. S., Saint Marie, R. L., Merchán, M. A., and Oliver, D. L. (2005). Laminar inputs from dorsal cochlear nucleus and ventral cochlear nucleus to the central nucleus of the inferior colliculus form two patterns of convergence. Neuroscience 136, 883–894. doi: 10.1016/j.neuroscience.2005.04.040
Markham, H., Liebs, J., Friedman, M., Roth, A., and Umemoto, B. (1997). Physiological and anatomy of synap- tic connections between thick tuber pyramidal neurons in the developing rat neocortex. J. Physiol. 500(Pt 1), 485–510.
Mauge, S. J., Shvetsova, M. N., Roth- broome, G. D., Argent, R. E., and Paolini, A. G. (2010). An in vivo investigation of first spike lincencies in the inferior colliculus in response to multichannel penetrating auditory brainstem implant stimulation. J. Neurol. Eng. 7, 046004. doi: 10.1049/jnet:20100064
Moor, D. R., Kotak, V. C., and Sanes, D. H. (1998). Commensural and lom- ninal synaptic input to the gerbil inferior colliculus. J. Neurophysiol. 80, 2229–2318.
Merret, D. K., and Oliver, D. L. (1994). The neuronal architecture of the inferior colliculus in the cat: defining the functional anatomy of the auditory midbrain. J. Comp. Neurol. 322, 204–236. doi: 10.1002/cne.903220206
Mulli, C., Sterbing-M., and Donde- smes, M. (1985). The effects of QX314 on tlimatic neurons. Brain Res. 355, 350–354. doi: 10.1016/0006-899X(85)91791-4
Nakamoto, K. T ., Malott, J. G., Kikusa, J., Stoney-White, M. E., Sorwich, C. S., and Scharf, D. B. (2013). Analysis of excitatory synapses in the guinea pig inferior colliculus: a study using electron microscopy and GABA immunocytochemistry. Neuroscience 217, 179–189. doi: 10.1016/j.neuroscience.2013.01.041
Nichells, J. G., and Purves, D. (1970). Monosynaptic, chemical and electrical connections between sensory and motor cells in the central nervous system of the leech. J. Physiol. 209, 467–477.
Ohsako, G., Peschon, C., Adelman, J. P., and Hensel, C. (2012). SK2 channel modulation contributes to compartmentspecific dendritic plasticity in cortical pyramidal cells. Front. Neurosci. 6, 1783–1786. doi: 10.3389/fnins.2012.00178
Palacios, A., Reiter, S., and Sakmann, B. (2004). GABA(A) receptor shape and sound intensity control the inferior colliculus. J. Neurosci. 24, 5031–5044. doi: 10.1523/JNEUROSCI.0228-04.2004
Stevens, C. F ., and Wang, Y. (1995). Facilitation and depression at single central synapses. Neuron 14, 795–802. doi: 10.1016/0896-6273(95)90225-3
Talavera, H., and Magee, J. C. (2009). Pathway interactions and synaptic plasticity in the dynamic thalamic tuft regions of CA1 pyramidal neu- rons. Neuron 62, 102–111. doi: 10.1016/j.neuron.2009.05.007
Weliky, P. F ., Peraza, A., and Faber, D. S. (2000). Properties and plasticity of paired-pulse depression at a central synapse. J. Neurosci. 20, 5322–5330.
Sivaramakrishnan, S., Sterbing-M., et al. (2013). Mossy fibers and parvalbumin neurons. Front. Neural Circuits 7:41. doi: 10.3389/fncir.2013.00175
**fncir-07-00175** — 2013/10/28 — 14:13 — page 16 — #16
of laminar cells in the central nucleus of the inferior colliculus. Front. Neural Circuits 6:55. doi: 10.3389/fncir.2012.00055

Winer, J. A. (2005). Decoding the auditory corticofugal systems. Hear. Res. 207, 3–9. doi: 10.1016/j.heares.2005.06.007

Yu, S. H., Ma, C. L., and Kelly, J. B. (2004). Contribution of AMPA, NMDA, and GABA(A) receptors to temporal pattern of postsynaptic responses in the inferior colliculus of the rat. J. Neurosci. 24, 4625–4634. doi: 10.1523/JNEUROSCI.0318-04.2004

Yu, X. M., and Salter, M. W. (1999). Src, a molecular switch governing gain control of synaptic transmission mediated by N-methyl-D-aspartate receptors. Proc. Natl. Acad. Sci. U.S.A. 96, 7967–7972. doi: 10.1073/pnas.96.14.7967

Zhang, H., and Kelly, J. B. (2005). Glutamatergic and GABAAergic regulation of neural responses in inferior colliculus to amplitude-modulated sounds. J. Neurophysiol. 90, 477–490. doi: 10.1152/jn.01084.2002

Yu, X. M., and Salter, M. W. (1999). Src, a molecular switch governing gain control of synaptic transmission mediated by N-methyl-D-aspartate receptors. Proc. Natl. Acad. Sci. U.S.A. 96, 7967–7972. doi: 10.1073/pnas.96.14.7967

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