L-type calcium channels refine the neural population code of sound level

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Grimsley CA, Green DB, Sivaramakrishnan S. L-type calcium channels refine the neural population code of sound level. J Neurophysiol 116: 2550–2563, 2016. First published September 7, 2016; doi:10.1152/jn.00657.2016.—The coding of sound level by ensembles of neurons improves the accuracy with which listeners identify how loud a sound is. In the auditory system, the rate at which neurons fire in response to changes in sound level is shaped by local networks. Voltage-gated conductances alter local output by regulating neuronal firing, but their role in modulating responses to sound level is unclear. We tested the effects of L-type calcium channels (Ca_{L}: Ca_{v}1.1–1.4) on sound-level coding in the central nucleus of the inferior colliculus (ICC) in the auditory midbrain. We characterized the contribution of Ca_{L} to the total calcium current in brain slices and then examined its effects on rate-level functions (RLFs) in vivo using single-unit recordings in awake mice. Ca_{L} is a high-threshold current and comprises ~50% of the total calcium current in ICC neurons. In vivo, Ca_{L} activates at sound levels that evoke high firing rates. In RLFs that increase monotonically with sound level, Ca_{L} boosts spike rates at high sound levels and increases the maximum firing rate achieved. In different populations of RLFs that change nonmonotonically with sound level, Ca_{L} either suppresses or enhances firing at sound levels that evoke maximum firing. Ca_{L} multiplies the gain of monotonic RLFs with dynamic range and divides the gain of nonmonotonic RLFs with the width of the RLF. These results suggest that a single broad class of calcium channels activates enhancing and suppressing local circuits to regulate the sensitivity of neuronal populations to sound level.

rate-level functions; inferior colliculus; auditory midbrain; dynamic range; level tuning; local circuits

NEW & NOTEWORTHY

Responses of auditory neurons to changes in sound level depend on processing by local networks. Voltage-dependent conductances intrinsic to neurons in the network regulate local output, but their effects on sound-level coding are not understood. We show that voltage-gated L-type calcium channels regulate the sensitivity of auditory midbrain neurons to sound level by boosting or suppressing neuronal firing at specific sound levels. These results suggest that calcium channels regulate responses to sound level.

THE CODING OF SOUND PRESSURE LEVEL is a model system to study the processes by which the intensity of a sensory input is incorporated into neural architecture. Sound level is a critical component of object recognition. For example, listeners make decisions about an auditory object by placing greater weight on level than on the reliability of information contained in the sound stimulus (Berg 1990; Lutfi et al. 2008). Neurons in the central auditory system may vary their responses continuously with sound level (level variant), with firing rates that increase monotonically over the physiological range of sound levels, or they may first increase and then decrease their firing rates with sound level, peaking in a small range of levels (level tuned), to produce a nonmonotonic change in firing rate with sound level. Level-variant and level-tuned rate-level functions (RLFs) contribute to object recognition by informing about stimulus context, spectral contrast, and input sensitivity (Barbour and Wang 2003; Bartlett and Wang 2005; Watkins and Barbour 2008). The importance of the central nucleus of the inferior colliculus (ICC) level codes in dynamically shaping object recognition (Chambers et al. 2014; Las et al. 2005; Nelken 2008) is supported by the diversity of ICC RLFs (Ehret and Merzenich 1988; Wallace et al. 2012), their stimulus-dependent plasticity (Dean et al. 2005; Galazyuk et al. 2000; Rees and Palmer 1988), and the level-dependent recruitment of extrinsic and local inputs (Grimsley et al. 2013). Despite their differing representations of sound level, auditory neurons must retain their sensitivity to level. The sensitivity and shapes of sound level codes are governed by the excitatory-inhibitory balance that underlies neuronal discharge rates (Le Beau et al. 1996; Sivaramakrishnan et al. 2004; Wu et al. 2006) and change with sound source or stimulus pattern (Dean et al. 2005; Rees and Palmer 1988; Wallace et al. 2012).

In the ICC, the auditory midbrain nucleus where most brain stem inputs converge, local synaptic connections predominate compared with brain stem inputs (Saldana and Merchán 2005). In addition, local axons spread extensively within and across frequency laminae (Malmierca et al. 1995; Oliver et al. 1991; Wallace et al. 2012). ICC cytoarchitecture is therefore consistent with substantial regulation of level processing by local networks. The dynamic range of monotonic RLFs and firing rates at the peaks of nonmonotonic RLFs are both increased by the activation of local ICC synapses (Grimsley et al. 2013; Sivaramakrishnan et al. 2013). Local ICC inputs appear to activate at thresholds that are higher than those of inputs from the brain stem (Chandrasekaran et al. 2013), and their effects on firing rates are pronounced at mid-to-high sound levels, with very little effects at low sound levels (Grimsley et al. 2013). These level-dependent effects of local circuit activation suggest that additional nonsynaptic mechanisms, in particular, high-threshold voltage-gated conductances, might boost the responses of ICC neurons to sound level.

We have examined the role of high-threshold L-type calcium channels (Ca_{L}: Ca_{v}1.1–1.4) in sound-level coding in the ICC of awake mice. Ca_{L} has been shown to refine visual and auditory sensory maps by its effects on functional local connectivity and the formation of inhibitory circuits through activity-dependent influx of calcium (Guido 2008; Hirtz et al. 2012). Ca_{L} affects activity in local circuits through its under-
lying effects on intrinsic neuronal firing rates (Putzier et al. 2009; Zheng and Raman 2011). In the ICC, calcium-mediated conductances affect intrinsic firing and synaptically evoked responses (Martinez-Galan et al. 2010; Sivaramakrishnan and Oliver 2001), suggesting that $\text{Ca}_L$ would be important in regulating responses to changes in sound level. We found that $\text{Ca}_L$ increased firing rates at high sound levels in monotonic RLFs and enhanced or suppressed firing at the peaks of nonmonotonic RLFs, implying that $\text{Ca}_L$ differentially activates excitatory and inhibitory local circuits recruited by changes in sound level. The effects of $\text{Ca}_L$ depended on the dynamic range of monotonic RLFs and the width of nonmonotonic RLFs. $\text{Ca}_L$ therefore contributes an activity-dependent, nonsynaptic means of regulating sound level codes.

**METHODS**

*Animals and preparation for in vivo recordings.* Experimental procedures on animals were approved by the Committee for Animal Care and Use at the Northeast Ohio Medical University and conformed to the guidelines published by the National Institutes of Health. CBA/Ca mice of either sex and between 1 and 4 mo old (Jackson Laboratory, Bar Harbor, ME/in-house colonies) were used for recordings from the inferior colliculus (IC) in brain slices (1- to 2-ml-old animals) and in vivo from awake mice (2- to 4-mo-old animals). Animals were housed in a room with a reversed light-dark cycle and were used for recordings during the day.

*Brain slice recordings.* Animals were anesthetized with isoflurane, the brain as removed, and 300-μm-thick coronal slices were made through the IC with a vibratome (Dorsaka, Kyoto, Japan) (Chandrasekaran et al. 2013). Coronal slices contained the central nucleus (ICC) and the dorsal and external cortices of the IC. The ICC was identified as distinct from the surrounding IC nuclei by its relative opacity, which creates visual boundaries from the external and dorsal IC nuclei, by intrinsic responses of cell types and by passive membrane time constants, which are faster than those of the external cortex and slower than those of the dorsal cortex (Sivaramakrishnan S, unpublished observations). Fibers of the lateral lemniscal nerve tract, commissural connections between the two colliculi, and auditory regions ventral to the IC were also present. Slices containing extreme caudal and rostral portions of the IC were not used, and each animal yielded one to two usable slices. Slices were cut and incubated at 35°C in oxygenated (95% O$_2$-5% CO$_2$) artificial cerebrospinal fluid (ACSF), which contained (in mM) 120 NaCl, 3 KCl, 2 CaCl$_2$, 1.3 MgSO$_4$, 1 Na$_2$HPO$_4$, 20 NaHCO$_3$, and 10 glucose, pH 7.3. Recordings were made in a chamber whose temperature was maintained at 35°C, and slices were superfused with ACSF at 2 ml/min.

Whole cell patch recordings were made from ICC neurons under visual control through a water-immersion objective (×40/NA 0.75) attached to an upright microscope (Zeiss Axioskop) fitted with differential interference optics. Patch pipettes made of borosilicate glass had resistances of 3–5 MΩ when filled with recording solutions. Series resistances of 6–10 MΩ were compensated by 75–80%, and junction potentials were corrected by −11 mV. K$^+$ currents were measured in ACSF containing very low calcium (0.1 mM), and NaCl was replaced by N-methyl-d-glucamine (NMDG$^+$). Ca$^{2+}$ currents were recorded in ACSF with the NaCl and KCl replaced by TEA-Cl (140 mM) and CsCl (10 mM), respectively, and with addition of 2 mM 3,4-diaminopyridine (3,4-DAP) to block K$^+$ channels. K$^+$ currents were recorded with pipette solutions containing (in mM) 120 K-gluconate, 10 KCl, 0.2 EGTA, 0.1 CaCl$_2$, 4 Mg-ATP, 0.3 Na-GTP, 10 HEPES, and 10 phosphocreatine, pH 7.3. Ca$^{2+}$ currents were recorded with pipette solutions containing (in mM) 70 CsMeSO$_4$, 10 CsCl, 0.2 EGTA, 0.1 CaCl$_2$, 4 Mg-ATP, 0.3 Na-GTP, 10 HEPES, and 10 phosphocreatine, pH 7.3.

Recordings of K$^+$ and Ca$^{2+}$ currents were made under voltage clamp, and spontaneous synaptic potentials were measured under current clamp. Recordings were made with an EPC-10 amplifier and Patchmaster software, and recordings were analyzed using Fitmaster software (HEKA Elektronik/Instrucheck). For K$^+$ and Ca$^{2+}$ currents, cells were held at −60 and −90 mV, respectively. Spontaneous synaptic potentials were recorded at normal resting potentials (−66.7 ± 2.62 mV).

nimodipine, a blocker of L-type Ca$^{2+}$ channels (Ca$_V$ 1.1–1.4; Bar et al. 1990), was stocked in DMSO, diluted to final concentrations (1.5% DMSO in external solution), and applied to the slice through bath perfusion at the same rate as the external solution (2 ml/min). DMSO did not affect responses (4 cells; normality was confirmed with the Shapiro-Wilk test; resting potentials: $t_s = 0.002, P = 0.99$; average intrinsic firing frequency evoked by 300-ms, 0.15-nA current step: $t_s = 0.03, P = 0.97$; peak amplitudes of lemiscally evoked synaptic potentials: $t_s = 0.1, P = 0.92$). ω-Conotoxin GVIA (ω-Ctx-GVIA), a blocker of N-type Ca$^{2+}$ channels (Lewis 2004), was stocked in distilled water and diluted to its final concentration in ACSF before it was added to the slice. Bovine serum albumin (1 mg/ml) was mixed with the toxin to prevent its adhesion to the glass surface of the recording chamber (Sivaramakrishnan and Laurent 1995), and the mixture was added directly to the well containing the slice. The final concentration of ω-Ctx-GVIA was determined from the volumes of the recording well and the aliquot of the toxin added to the well. NNC 55-0396 hydrochloride (NNC-HCl), an antagonist of T-type Ca$^{2+}$ channels (Alvina et al. 2009), was diluted into ACSF from a stock solution in water and bath applied. Except for ω-Ctx-GVIA (Alomone Labs, Jerusalem, Israel), chemicals were obtained from Sigma Aldrich.

Nimodipine, particularly at high concentrations, can affect GABA$_A$ receptors (Das et al. 2004). We therefore tested the effects of 50 μM nimodipine on spontaneous miniature inhibitory postsynaptic potentials (mIPSPs) recorded in the presence of 1 μM tetrodotoxin to block Na$^+$ currents and spike-evoked transmitter release. One hundred micromolar D-α-aminophosphononic acid (APV) and 10 μM 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline (NBQX) were included to block glutamatergic NMDA and AMPA receptors, respectively. Recording pipettes contained normal intracellular solution (as used for K$^+$ current recordings) without modification of the chloride concentration. mIPSPs did not appear to be affected by 50 μM nimodipine (n = 14; 500 mIPSPs analyzed; mIPSP amplitude $t_{998} = 0.29, P = 0.77$; mIPSP duration: $t_{998} = 0.61, P = 0.54$), suggesting that nimodipine did not affect GABA$_A$ receptors in the ICC.

In vivo recordings. In vivo recordings from the ICC were made in head-fixed awake mice (Grimsmo et al. 2013; Sivaramakrishnan et al. 2013). Surgical procedures to attach a stainless steel head post and perform the craniotomy were conducted under isoflurane anesthesia (1.5–2.0% in oxygen; Abbott Laboratories, North Chicago, IL). Animals recovered for at least 24 h before being used for experiments. Animals were habituated to the recording tube by the presence of tubes placed in their cages in the animal housing unit for several weeks before recordings. Craniotomies were <0.5 mm in diameter and were performed over one IC. The opening was covered with sterile bone wax between recording sessions to prevent the brain from drying out. Recording sessions lasted for 2–3 h. Animals were monitored periodically during the recording session to ensure that they remained awake and were hydrated as necessary. Discomfort was assessed by particular physical signs. An uncomfortable animal would vocalize excessively and exhibit body movements suggestive of struggling and/or shivering. These signs of discomfort were visualized on the video monitor outside the sound chamber or by entering the sound chamber at regular intervals. Excessive physical movements also caused large changes in the baseline during physiological recordings. Discomfort was assessed by raising the temperature to prevent shivering, sedating the mouse with a subdermal injection of acepromazine (2 mg/kg), or terminating the experiment for the day.

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Recordings on each animal were performed once per day on alternate days. Each animal was used for three to four recording sessions.

Single-unit recordings were made with a glass recording pipette attached to a seven-barrel injection system in a piggy-back configuration (Havey and Caspary 1980) modified for pressure injection of drugs (Grimsley et al. 2013; Sivaramakrishnan et al. 2013). Recording electrodes were inserted along the dorsoventral axis of the IC, and acoustically driven responses were first recorded ~200 μm below the surface of the IC to bypass the IC dorsal cortex. The locations of neurons in the ICC were confirmed with the use of criteria that included a position of the recording electrode >250 μm below the brain surface to bypass the dorsal cortex of the IC, the neuron’s characteristic frequency (CF; 4–64 kHz; Egorova et al. 2006; Grimsley et al. 2013), and locked first-spike latencies in response to tones delivered to the contralateral ear. The lateral edge of the ICC was avoided during recordings.

Extracellular recording pipettes were filled with ACSF (same composition as for slice recordings) and had resistances of 15–18 MΩ. At least two barrels of the multibarrel were filled with nimodipine to make sure that a lack of effect was not attributable to blocked barrels. Nimodipine was dissolved from its stock solution made in DMSO into ACSF, for a final concentration of 0.15% DMSO applied to the neuron. ACSF was pressure injected from additional barrels to control for injection artifacts and to aid in drug washout. Each barrel was independently controlled through valves attached to a picospritzer (WPI, Sarasota, FL). Vacuum (1–2 psi) was placed on all barrels to prevent drug leakage, and drugs were injected with positive pressures (4–6 psi, 100–500 ms) that overrode the vacuum. Recovery from drug application occurred through diffusive loss or ACSF application.

Acoustic stimuli were digitally synthesized and downloaded onto a digital signal processing card (AP2 Multi-Processor DSP card; Tucker-Davies Technologies, Alachua, FL), converted to analog signals (500-KHz sampling rate; DA3-2; Tucker-Davies Technologies), filtered (FT6-2; Tucker-Davies Technologies), attenuated (PA4; Tucker-Davies Technologies), summed (SM3; Tucker-Davies Technologies), amplified (HCA-800II; Parasound, San Francisco, CA), and sent to a loudspeaker (Infinity EMIT-B; Harmon International Industries, Woodbury, NY). The output of the acoustic system was calibrated over 10–120 kHz with a condenser microphone (Briel and Kjaer, Nærum, Denmark) placed in a position typically occupied by the opening on the animal’s ear. Sound pressure levels were corrected for speaker drop-off at different frequencies. We used a maximum tone frequency of 64 kHz, but most CFs were <50 kHz. Harmonic distortion was not detectable 60 dB below the signal intensity with a fast Fourier analysis of the digitized microphone signal. Although we cannot rule out the possibility that the animals detected harmonic distortions at high sound levels, there were no sudden changes in firing rates, suggesting that harmonic distortion was not significant. However, an increasing influence of binaural interactions at high sound levels remained a possibility.

The speaker used to deliver sound was placed 10 cm in front of the animal at an angle of 15° to the midline and contralateral to the IC from which recordings were made. In-house software (Batlab) was used to synthesize acoustic stimuli and acquire data. Tones and wide- and narrow-band noise bursts were used as search stimuli to isolate single-unit recordings. The CF of each neuron, defined as the frequency at which the lowest sound pressure level elicited tone-locked spikes during repeated presentations of an acoustic input, was determined before and after application of nimodipine for pressure injection made at the CF of each neuron and across the 4- to 64-KHz range of CFs in the ICC (Egorova et al. 2006; Grimsley et al. 2013).

Single-unit recordings were made through a differential amplifier (model 3000; A-M Systems). Spikes were considered well-isolated if they had stable amplitudes and shapes, and a signal-to-noise ratio >3:1. Only cells with first-spike latencies (FSLs) that locked to the tone during repeated presentations were used for analysis. The time of onset of tone-evoked responses was determined from the asymptote of FSL plots. FSLs were corrected for a 0.5-ms tone rise time and an acoustic travel time of 0.3 ms and were calculated from the minimum value of the median FSL (median value across the number of presentations of the tone at each sound level) across all sound levels.

Rate-level functions (RLFs) were constructed by using pure tones with sound pressure levels randomly increased in 5-dB increments between 0 and 96 dB SPL. Tones were 100 ms long, delivered at 1 s⁻¹, and presented 12 times at each sound level. Firing rates were averaged over the 12 sweeps at each sound level. Rates were calculated over the maximum duration of the response following criteria established in previous studies (Sivaramakrishnan et al. 2004). The duration of the response was measured between the onsets of the responses after the tone was delivered to the end of the spiking, whether or not spiking extended beyond the tone. To account for spontaneous spiking, the response onset was determined from the FSL, averaged over 12 sweeps. The maximum duration was then compared before and after nimodipine was applied. The higher value, which indicated the maximum time over which the neuron could sustain firing, was used as the duration over which firing rates were averaged for both control and nimodipine conditions. Because of the possibility that nimodipine could have distinct effects on spontaneous and evoked firing rates, we subtracted spontaneous firing rates from tone-evoked rates. Spontaneous rates were averaged over a 300-ms window preceding the tone, and this average value was subtracted from firing rates measured during the tone. Three to four RLFs were averaged in control conditions or following drug application to obtain steady-state RLFs, which were determined by the means and SDs over different trials. Nimodipine was applied at the start of collection of each RLF. Data collection for each RLF lasted for 240 s (4 min; 20 sound levels, 12 sweeps/level, 1 s/sweep). Approximately three to four pulses of nimodipine applied at the start of collection of each RLF were sufficient to produce steady-state effects, which were determined by the means and SDs of firing rates. RLF shapes were categorized as monotonic or nonmonotonic on the basis of changes in firing rate with sound level. Monotonic RLFs steeply saturated or gradually increased with sound level and had dynamic ranges between 10 and 70 dB. Nonmonotonic RLFs first increased and then decreased with sound level. In the mouse ICC, monotonic RLFs (narrow + wide dynamic range) comprised 56% and nonmonotonic RLFs 44% of the total population (Grimsley et al. 2013). For this study, we used similarly sized data sets (39 monotonic and 37 nonmonotonic RLFs).

Data analysis and statistical treatment. For measurements of K⁺ and Ca²⁺ currents in slices, trials were repeated five to eight times for each experimental protocol. The series of voltage steps were presented three to five times to each cell and then averaged for each cell and then across slices. Slope conductances for K⁺ currents were determined for the linear portion of the current-voltage curve, generally between −10 and +20 mV. Spontaneous synaptic potentials were identified with threshold detection, and amplitudes and durations were measured using MiniAnalysis (Synaptosoft) and peak detection.

To measure RLF thresholds, RLFs were normalized to their maximum values and thresholds were estimated from the lowest sound level that evoked locked spiking over repeated trials, with spike rates at 20% of the maximum spike rate. Since RLFs in control and drug conditions were measured at CF, absolute thresholds were compared in different conditions without being compensated for frequency-dependent threshold shifts. To measure the effect of nimodipine application on changes in the RLF gain, the RLF in the control condition was divided by the RLF following nimodipine application. For monotonic RLFs, the resulting RLF was fit with a sigmoidal function (r² = 0.977). For nonmonotonic RLFs, the resultant RLF was fit with a Gaussian function that was adequate (r² ≥ 0.96612) for measuring the gain at the peak of the RLF.

The operating range and dynamic range of monotonic RLFs were differentiated in the following way. Each neuron’s operating range was defined as the maximum firing rate reached during the series of 0–96 dB SPL tones and was measured as the firing rate averaged over
the three highest sound levels used (81, 86, and 91 dB SPL). Dynamic range was defined as the difference between the lowest and highest sound pressure level over which firing rates increased (May and Sachs 1992; Rees and Palmer 1988). Because different monotonic RLFs have different slopes in the regions of high sound levels, we measured dynamic ranges between 10 and 90% of the maximum firing rates (May and Sachs 1992) after fitting RLFs with a sigmoid function ($r^2 \geq 0.97967$ for all cells) using a Boltzmann fit: $y = A_2 + (A_1 - A_2)/[1 + \exp((x - x_0)/dx)]$, where $A_1$ and $A_2$ are the minimum and maximum values, $x_0$ is the center of the sigmoid function, and $dx$ is the $x$-differential corresponding to the largest change in $y$.

Origin software was used to fit data and calculate statistical variation. Normality was confirmed with the Shapiro-Wilk test, and significance was determined using a two-tailed $t$-test, ANOVA, or within-sample sum-of-squares variance. $P < 0.05$ with the Bonferroni correction factor applied. Results are means ± SD. Cluster analysis on monotonic RLFs was performed using the $K$-means clustering algorithm (where $K$ is the number of mutually exclusive clusters). The number of clusters was first specified, and an iterative algorithm was used by changing the cluster to which each data point was assigned. The process converged on the lowest squared Euclidean distance used by changing the cluster to which each data point was assigned. Silhouette tests provided a measure of the tightness of clustering of each monotonic RLF to the other RLFs in the same cluster. Data were grouped into varying numbers of clusters ($>2$), and the average silhouette value in each cluster was used to determine, for example, whether a 2-cluster or 3-cluster grouping was more suitable (Statistics Toolbox in MATLAB 2012b; The MathWorks).

RESULTS

$Ca_L$ in ICC neurons. Recordings in brain slices were used to measure the contribution of $Ca_L$ ($Ca_{av}$, 1.1–1.4) to the total $Ca^{2+}$ current ($I_{Ca}$) (16 neurons). Peak amplitudes of $I_{Ca}$ were $427 \pm 21.3$ pA (Fig. 1, A and C). Blocking T- and N-type $Ca^{2+}$ channels by combined application of NNC-HCl and $\omega$CTx-GVIA revealed a component of $I_{Ca}$ that was abolished by 10 $\mu M$ nimodipine (Fig. 1B). The NNC-HCl-sensitive $I_{Ca}$ component activated at $-50$ mV (13.45 ± 0.67 pA; holding potential $-90$ mV; Fig. 1C). Blocking this component shifted activation thresholds to $-30$ mV (14.01 ± 4.2 pA; $t_{30} = 5.3$, $P < 0.0001$), indicating a higher threshold of activation for the $\omega$CTx-GVIA- and nimodipine-sensitive components. Further addition of $\omega$CTx-GVIA reduced the peak magnitude of the total current to $209.6 \pm 22.3$ pA ($t_{30} = 6.43$, $P < 0.0001$; 49.06%) and isolated the nimodipine-sensitive $I_{Ca}$ ($Ca_{L}$). $Ca_{L}$ peaked at the same voltage as the other $I_{Ca}$ components ($-10$ mV). Nimodipine (10 $\mu M$) abolished most of the remaining $I_{Ca}$ (20.6 ± 14.6 pA; 4.8% of current remained). $Ca_{L}$ therefore formed the major portion of the $I_{Ca}$ that remained after T- and N-type $Ca^{2+}$ channels were blocked, contributing 44.3% to the total $I_{Ca}$. The nimodipine concentration of 10 $\mu M$ used, obtained from a dose-response curve performed with different concentrations of nimodipine on the $I_{Ca}$ that remained after the addition of NNC-HCl and $\omega$CTx-GVIA ($n = 5$ cells), produced a maximum effect on $Ca_{L}$ (10, 50, and 100 $\mu M$; $F_{2,12} = 0.8$, $P = 0.47$; Fig. 1D; $IC_{50} = 4.68$ $\mu M$).

$Ca_{L}$ effects on firing rates in vivo. To determine whether $Ca_{L}$ regulated neuronal responses to changes in sound level, recordings were made in vivo in awake mice. Single-unit recordings of firing rates were made with an extracellular electrode, and nimodipine was pressure applied through an attached barrel (Sivaramakrishnan et al. 2013). Rate-level functions (RLFs) were constructed before and after nimodipine application from responses to 100-ms-long CF tones presented to the contralateral ear at 1 s⁻¹. Sound pressure levels were varied between 0 and 96 dB SPL. Data are reported from 76 cells.

![Fig. 1](image-url) Calcium currents in ICC neurons. A: calcium currents ($I_{Ca}$) are illustrated for a series of voltage steps to $+50$ mV. Holding potential is $-90$ mV. B: the current remaining after addition of NNC-HCl (20 $\mu M$) and $\omega$CTx-GVIA (100 $nM$) is abolished by nimodipine (10 $\mu M$). C: $I_{Ca}$ current-voltage (I-V) curves illustrate differences in activation thresholds as well as maximum currents of the different components. Currents plotted are the maximum inward current at each membrane potential. All I-V curves are from currents evoked from a $-90$-mV holding potential (16 neurons). D: nimodipine dose-response curve shows an $IC_{50}$ of 4.68 $\mu M$ and saturation at $-10$ $\mu M$. To obtain dose-response curves, different concentrations of nimodipine were tested on the $I_{Ca}$ that remained in NNC-HCl and $\omega$CTx-GVIA (5 cells).
Because of the small volumes injected in vivo and large diffusion distances, effective drug concentrations in vivo are higher than with bath application in slices (Grimsley et al. 2013; Sivaramakrishnan et al. 2013). A dose-response curve on a subset of neurons \((n = 11)\), constructed from the effects of nimodipine on firing rates, indicated a saturating concentration of 50 \(\mu\text{M}\) \((50:100 \mu\text{M}: t_{29} = 0.16, P = 0.89; \text{Fig. 2}A\)). In contrast to the saturating effects of 10 \(\mu\text{M}\) nimodipine in slices, pressure application of 10 \(\mu\text{M}\) in vivo had a 73.7 \(\pm\) 3.6% effect on firing rates in vivo, suggesting diffusive loss as one possible reason that the effects of 10 \(\mu\text{M}\) nimodipine did not reach saturation. Since high concentrations of nimodipine have been shown to affect voltage-gated \(K^+\) conductances (Caro et al. 2011; Zhang and Gold 2009), we measured spike heights and widths. Both parameters were unchanged by 50 \(\mu\text{M}\) nimodipine (100 spikes analyzed; spike heights: \(t_{198} = 0.47, P = 0.64;\) spike widths: \(t_{198} = 0.39, P = 0.73; \text{Fig. 2}A\)). These results suggested that 50 \(\mu\text{M}\) nimodipine did not affect the voltage-gated conductances that shaped spikes in vivo. Since 10 \(\mu\text{M}\) nimodipine did not cause saturating effects on firing rates in vivo, we used the higher concentration of 50 \(\mu\text{M}\) to account for diffusive loss of the drug following pressure injection.

To distinguish drug effects on firing from artifacts of pressure injection, we looked for interruptions in firing and changes in first-spike latencies (FSLs) by using low concentrations of nimodipine (5 \(\mu\text{M}\)), which had little effect on firing rates. Pressure pulses of 5 \(\mu\text{M}\) nimodipine, applied while responses to tone sweeps were being recorded, did not interrupt firing (Fig. 3A). FSLs were also unaffected \((n = 8\) cells; \(\Delta\text{FSL} = 2.45 \pm 2.61 \text{ ms}; P > 0.76;\) FSLs were averaged for 40 sweeps before and 40 sweeps after the pressure pulses; \text{Fig. 3}A). Drug effects were checked for consistency by alternating nimodipine application with recovery periods. After the first application of nimodipine, the second and third applications did not cause additional changes (Fig. 3B, \(i–iv; P > 0.62\)).

**\(Ca_{\text{ii}}\) effects on level-variant responses.** The excitatory-inhibitory balance that shapes level-variant RLFs in the ICC (Sivaramakrishnan et al. 2004; Zhang and Kelly 2003) arises partly through local ICC connections, which activate at mid-to-high sound levels and affect the dynamic range of monotonic RLFs (Grimsley et al. 2013). The range of sound levels between 10 and 90% of the maximum firing rate was used as a measure of dynamic range \((n = 39\) cells; \text{Fig. 4}A; see METHODS). Figure 4B illustrates the effects of nimodipine on five cells with monotonic RLFs. The top three cells, with dynamic ranges of 65, 50, and 40 dB, were sloping monotonic functions, which did not saturate abruptly at high sound levels, but slowly increased firing rates (Stabler et al. 1996). In these cells, firing rates decreased in nimodipine. The bottom two cells, with 14- and 10-dB dynamic ranges, had steeply saturating RLFs. In these cells, firing rates increased after nimodipine application.

The differential effects of nimodipine on firing rates in wide- and narrow-dynamic range RLFs suggested that the effect of \(Ca_{\text{ii}}\) on firing rate might depend on the dynamic range of the control RLF. To determine whether the effects of nimodipine on firing rate correlated with dynamic range, we first examined the distribution of dynamic ranges among control RLFs in the monotonic population (Fig. 4C). The most frequently observed dynamic ranges were 10–15 dB (12.5-dB bin midpoint; 8/39 cells), 50–55 dB (52.5-dB bin midpoint; 9/39 cells), and 55–60 dB (57.5-dB bin midpoint; 11/39 cells). Dynamic ranges were distributed bimodally \((n = 39\) cells; \(r^2 = 0.99982\)). The two groups of dynamic ranges became distinct at \(\sim 35–45\) dB, with only 5% of neurons (2/39) lying in this separating region. Sixty-nine percent of RLFs (27/39 neurons) had dynamic ranges >45 dB, and 26% (10/39 neurons) had dynamic ranges <25 dB. This distribution of dynamic ranges is similar to those reported in the unanesthetized rabbit ICC (58% wide and 17% narrow dynamic range; Sivaramakrishnan et al. 2004) but differs from reported values in the unanesthetized bat ICC.

![Fig. 2. Establishment of an effective nimodipine concentration for in vivo recordings. A: i, nimodipine dose-response curve shows responses saturating at 50 \(\mu\text{M}\). Firing rates were measured at sound levels that evoked maximum firing in each neuron (11 cells: 5 monotonic, 6 nonmonotonic). ii, Nimodipine (50 \(\mu\text{M}\)) does not change spike heights or widths. Spikes were evoked by a 100-ms CF tone at 40 dB SPL. Center insets: expanded traces of spikes before (top) and after (bottom) nimodipine was added. Population average: 100 spikes, 8 cells. B: slice recordings show effects of 50 \(\mu\text{M}\) nimodipine on \(K^+\) currents evoked in an external calcium concentration of 0.1 mM. i, \(K^+\) currents evoked by a step to +30 mV from a holding potential of −60 mV show no change in peak current or inactivation in nimodipine. ii, \(I\text{-V}\) curves shown before and after nimodipine overlap and are not significantly different.](image-url)
(26% wide and 39% narrow dynamic range; Zhou and Jen 2000).

We next measured the effects of nimodipine on firing rates in two regions of the RLF. The first region was restricted to the highest sound levels (81–91 dB SPL) and was chosen because firing rates at these sound levels form the upper limit of the neuron’s operating range (for the sound levels used). When the change in firing rate in the presence of nimodipine [(nimodipine − control)/(control)] was plotted as a function of the control dynamic range, monotonic RLFs fell into distinct groups. A group with dynamic ranges <25 dB (10/39 cells) consisted of neurons that increased their firing rates in nimodipine. The ratio of control-to-nimodipine firing rates was therefore negative (Fig. 4Di, blue circles). The larger number of cells (29/39) decreased their firing rates in nimodipine. The ratio of control-to-nimodipine firing rates was positive in this group (Fig. 4Di, red circles). K-cluster analysis provided supporting evidence that RLFs clustered into two groups (C1 and C2; Fig. 4D, i and ii, top) rather than three groups (c1, c2, and c3; Fig. 4D, i and ii, bottom). The mean silhouette coefficients were larger for the 2-cluster grouping (0.937 ± 0.21) than for the 3-cluster grouping (0.755 ± 0.28).

Because RLFs clustered into two distinct groups, the variation in firing rate with dynamic range was examined separately in each group. In C1, changes in firing rate did not vary systematically with dynamic range (linear fit; \( r^2 = 0.0699 \)). However, in C2, as dynamic range increased, firing rates in nimodipine decreased to a greater extent, and the change was linear (\( r^2 = 0.81297 \); Fig. 4Di, top). These results indicated that \( C_{A_{L}} \) increased neuronal operating range (firing rates at highest sound levels) proportionally with dynamic range, but only when dynamic ranges exceeded 30 dB. Thus, in the larger population of monotonic RLFs with the wider dynamic ranges, the change in firing rate caused by blocking \( C_{A_{L}} \) is a function of the RLF dynamic range.

The second region over which firing rates were measured included the entire RLF. This analysis measured the gain exerted by \( C_{A_{L}} \) and its dependence on sound level. As expected from the presence of two distinct clusters of monotonic RLFs (Fig. 4D), \( C_{A_{L}} \) exerted a negative gain on RLFs in C1 and a positive gain on RLFs in C2 (Fig. 4Ei). A more critical difference between C1 and C2 was the change in \( C_{A_{L}} \) gain with sound level (Fig. 4Eii). In a few neurons (3/10), in C1, \( C_{A_{L}} \) gain became increasingly negative as sound level increased. However, the population average indicated no change in \( C_{A_{L}} \) gain with sound level (e.g., gains at 3 points along the curve were not significantly different; \( F_{3,29} = 0.28, P = 0.75 \)). In contrast, in C2, \( C_{A_{L}} \) gain increased with sound level in all the neurons. Averaged gains at four points along the curve showed significant increases (\( F_{3,115} = 18.64, P < 10^{-5} \)). The maximum average gain in C2 was 1.83 ± 0.08 at 91 dB SPL.

To estimate the relationship between \( C_{A_{L}} \) gain and RLF dynamic range, the values of \( C_{A_{L}} \) gain at the three highest sound levels (81, 86, and 91dB SPL) were averaged. In C1, the maximum \( C_{A_{L}} \) gain showed no systematic dependence on dynamic range (linear fit; \( r^2 = 0.01564 \)). Thus, although \( C_{A_{L}} \) suppresses the sensitivity to level when dynamic ranges fall below 25 dB, the dynamic range itself does not predict the extent of suppression. However, in C2, the maximum \( C_{A_{L}} \) gain increased exponentially with dynamic range (first-order exponential fit; \( r^2 = 0.97254 \); Fig. 4F). Thus \( C_{A_{L}} \) gain doubled for every 1-dB increase in dynamic range. Nimodipine did not alter the absolute value of the dynamic range (control: 43.27 ± 19.93 SD; nimodipine: 43.001 ± 20.16 SD; \( t_{7} = 0.003, P = 0.99 \); Fig. 4G). \( C_{A_{L}} \) therefore appears to be selective for the operating range of ICC neurons during changes in sound level.

The dynamic range is retained whether or not \( C_{A_{L}} \) is active.

\( C_{A_{L}} \) effects on level-tuned responses. Level tuning, coded by nonmonotonic RLFs, is established in the cochlear nucleus and thought to be conserved in the ICC and auditory cortex (Barone et al. 1996; Davis and Young 2000; Ramachandran et al. 1996; Davis and Young 2000; Ramachandran et al.
Peak firing, an indicator of neuronal sensitivity to level tuning (Bartlett and Wang 2011), is modulated by local feedback circuits in the ICC (Grimsley et al. 2013) and might involve the effects of CaL on neuronal firing. To determine whether CaL affected nonmonotonic RLFs, we analyzed the effect of nimodipine on neurons with single-peaked RLFs with firing rates that declined to \(50\%\) of the peak firing rate during the falling phase (strongly nonmonotonic RLFs). Peak firing rates, RLF widths, and the rising and falling segments of nonmonotonic RLFs were measured before and after nimodipine application. Data are reported from 37 cells.

Nimodipine application did not change the sound level at which firing rates peaked (\(t_{23} = 0.55, P = 0.58\)). However, peak firing rates changed in nimodipine. Figure 5A illustrates the effects of nimodipine on six nonmonotonic RLFs with different widths (11–38 dB; measured at half-maximum firing). In the RLFs with widths between 11 and 19 dB, peak firing rates either increased or decreased after nimodipine was applied. In the RLFs with 33- and 38-dB widths, peak firing was unaffected. Of the total sample of nonmonotonic RLFs (\(n = 37\)), nimodipine changed peak firing rates in 31/37 cells (84\%). 22/31 cells (71\%): increased firing; 9/31 cells (29\%): decreased firing. The remaining 6/37 cells (16\%) did not change firing rates in nimodipine. Thus, in the majority of level-tuned neurons, CaL regulated peak firing at tuned sound levels. Because the 6 cells that were unaffected by nimodipine had
Fig. 5. Nimodipine effects on nonmonotonic RLFs vary inversely with their width. A: nonmonotonic RLFs before and after nimodipine application in 6 cells with increasing RLF width (top to bottom). CFs (top to bottom) are 12, 11, 41, 22, 27, and 31 kHz. Error bars are SE. B: unimodal distribution of control RLF widths. RLF widths were measured at half-maximal firing (37 cells; 5-dB bin width). C: relationship between changes in peak firing in nimodipine and RLF width. Red line indicates sigmoidal fit. D: CaL gain as a function of sound level for cells with RLF widths >30 dB (i) and <30 dB (ii). CaL gain was obtained by dividing the control RLF by the RLF in nimodipine. Individual functions were fitted with a Gaussian function ($r^2 \approx 0.96612$ for all cells). Gains are plotted with reference from a gain of 1, irrespective of whether nimodipine increased or decreased firing. E: CaL gain as a function of RLF width (37 cells). A divisive gain ($<1$) increases as RLF width narrows. Red line indicates double-exponential fit. F: RLF widths do not change in nimodipine (37 cells). Gray lines represent individual cells. Black line represents population mean. G: asymmetric effects of nimodipine on RLFs. i, Difference integrals of the rising and falling phases of the RLF for 1 cell. Integrals are the product spikes/s × dB. ii, Difference integrals in the nonmonotonic population. CaL gain is greater during the falling phase compared with the rising phase in all cells (31 cells).
RLF widths >30 dB, it is likely that the broadest nonmonotonic functions in the ICC were not affected by Ca_{L} activation. The width of nonmonotonic RLFs is an indicator of neuronal sensitivity to level tuning (Bartlett and Wang 2011), and sound levels at which firing rates peak signify a level that is "preferred" by the neuron (Sadagopan and Wang 2008). Because nimodipine altered peak firing rates in nonmonotonic RLFs, but only in those with the narrower widths, we asked whether the changes in peak firing in nimodipine correlated with the width of the RLF. Such a correlation would imply that the extent to which Ca_{L} regulates the response of a neuron to its preferred level(s) depends on its sensitivity to level tuning.

Analyses were constructed in parallel to the analyses on monotonic RLFs. We first examined the distribution of RLF widths in the nonmonotonic population. Nonmonotonic RLF widths were distributed normally, and the distribution was unimodal ($r^2 = 0.84762$). Most RLF widths were between 15 and 30 dB ($n = 29/37$ neurons; Fig. 5B). We next examined the relationship between Ca_{L} gain and RLF width. As RLF width increased, the magnitude of nimodipine’s effect on peak firing decreased. This inverse relationship was well fit by a sigmoid function (Fig. 5C; sigmoidal fit parameters: $r^2 = 0.99238$; ceiling, 2.2544 ± 0.055; floor, 0.08929 ± 0.0212; midpoint gradient: $-0.16515$). The effect of Ca_{L} in suppressing peak firing is therefore an inverse function of the width of level tuning. Nimodipine did not suppress peak firing in cells with RLF widths >30 dB ($n = 6$; Δpeak firing rate: 0.086 ± 0.01), suggesting that Ca_{L} did not affect the widest nonmonotonic functions.

Ca_{L} gain, which was measured as the ratio of RLFs before and after nimodipine application, changed nonmonotonically with sound level in RLFs whose widths were <30 dB (Fig. 5Di). As expected, there was no change in Ca_{L} gain with sound level when RLF widths were >30 dB (Fig. 5Dii). To estimate the relationship between Ca_{L} gain and RLF width, we used the gain that corresponded to the sound level that evoked peak firing rates. Nimodipine changed peak firing rates by >100% in some cells (e.g., Fig. 5A, top 3 cells; Fig. 5C). A plot of Ca_{L} gain vs. RLF width revealed that Ca_{L} affected peak firing rates by >100% in RLFs with the narrowest widths (e.g., Ca_{L} changed firing rates between 119 and 85% for RLFs with widths between 7 and 14 dB, respectively). The total range of peak suppression in the nonmonotonic population was 119-0.01%.

The relationship between Ca_{L} gain and RLF width was exponential (Fig. 5E; second-order exponential fit; $r^2 = 0.96415$). RLF width was unaffected by nimodipine (Fig. 5F; control: 22.525 ± 10.810 SD; nimodipine: 21.1415 ± 10.28761 SD; $t_{37} = 0.43$, $P = 0.67$). Thus, although RLF width itself is not affected by Ca_{L}, the modulation of peak firing rates by Ca_{L} varies systematically, and inversely, with RLF width.

The results above indicate that the predominant effect of Ca_{L} in the level-tuned population in the ICC is to regulate firing rates at peak or “tuned” sound levels. To test whether the effects of nimodipine were symmetrical about the sound level that generated peak firing, nonmonotonic RLFs were divided into rising and falling areas, and the area under the curve (spikes/s × dB) was measured in each section. The area measured for each half of the control RLF was subtracted from the corresponding area obtained after nimodipine application to obtain the area due to Ca_{L} activation. For the RLF illustrated in Fig. 5Gi, areas (spikes/s × dB) for the rising phase were 58.34 for control and 179.17 for nimodipine, and control − nimodipine = Ca_{L}: 120.83; areas for the falling phase were 183.33 for control and 450 for nimodipine, and control − nimodipine = Ca_{L}: 266.67. Ratio of the fall-to-rise area was 2.21. Thus, for this cell, Ca_{L} was twice as effective during the falling phase of the RLF. In the nonmonotonic population affected by nimodipine (31/37 cells), the fall-to-rise ratio was >1 (3.197 ± 1.35 SD; Fig. 5Gi). Ca_{L} therefore affects level tuning asymmetrically, suppressing firing more in the high-sound level region that corresponds to the falling phase of nonmonotonic RLFs.

Ca_{L} does not change threshold and first-spike latency. The effects of nimodipine on RLFs indicates that Ca_{L} activates at sound levels that evoke high firing rates in both level-variant and level-tuned neurons. As expected from the lack of effect of Ca_{L} at low sound levels, nimodipine did not change thresholds or FSLs in cells with monotonic or nonmonotonic RLFs. Thresholds, determined from normalized RLFs, varied by 1 ± 4.59 dB in monotonic neurons ($n = 39$; $t_{37} = 0.3$, $P = 0.76$) and 0.32 ± 4.71 dB in nonmonotonic RLFs ($n = 37$; $t_{37} = 0.23$, $P = 0.81$). In control conditions, monotonic neurons had lower FSLs than nonmonotonic neurons (monotonic: 13 ± 1.73 ms, $n = 39$; nonmonotonic: 21.17 ± 1.26 ms, $n = 37$; $P < 0.05$), consistent with similarly reported differences in the auditory thalamus (Bartlett and Wang 2011). In both monotonic and nonmonotonic neurons, FSLs did not change in the presence of nimodipine (monotonic: 13.67 ± 1.53 ms; nonmonotonic: 20.3 ± 2.1 ms; $P > 0.54$ for both populations).

Temporal profile of Ca_{L} activation. The preservation of FSLs in nimodipine implied that Ca_{L} activated with a delay following tone onset. We examined whether the effects of Ca_{L} on firing rates would increase with temporal integration during the tone.

In the cell with a monotonic RLF (Fig. 6, A–C), spike rasters of single-tone sweeps showed less frequent firing when nimodipine was applied (compare Fig. 6A, top and middle). A reduction in the number of spikes was more evident later during the tone (e.g., >50 ms after tone onset), particularly during sweeps at higher sound levels (e.g., 76, 81, and 86 dB SPL; Fig. 6A, bottom). Consistent with previous studies (Feldman and Knudsen 1994; Grimsley et al. 2013; Zhang and Kelly 2003), in control conditions, RLF shapes changed with time during the tone (Fig. 6B). When windowed over the whole tone (cumulative, 5–105 ms; Fig. 6B, top left), RLFs had shallow slopes. At early times (e.g., 5–25 ms after tone onset), RLFs were steeply saturating, becoming shallower later during the tone (e.g., 47–67 ms). In nimodipine, RLF shapes followed the general profile of control RLFs in successive time windows during the tone, but firing rates were reduced.

To quantify the temporal profile of Ca_{L} effectiveness, the ratio of RLFs before and after nimodipine application (Ca_{L} gain) was calculated in different time windows during the tone and plotted as a function of sound level. Figure 6C illustrates the relationship between Ca_{L} gain and sound level for the cell in Fig. 6, A and B. The maximum value of Ca_{L} gain increased from early to late times during the tone. For example, in the time windows 5–25 and 26–46 ms, Ca_{L} gain reached its maximum value at 91 dB SPL (gain of ~1.3 and 1.2, respec-
During the tone, beginning 5 ms after tone onset (Fig. 7A). RLFs measured over the whole tone (cumulative; 5–105 ms) and in successive 20-ms time windows. Peak firing rates remained at 51 dB SPL in all the time windows before and after nimodipine application ($F_{5,11} = 0.26$, $P = 0.92$).

To estimate $\text{Ca}_{L}$ gain in the different time windows, the control RLF was divided by the RLF in nimodipine. The resulting RLF was then fitted with a Gaussian function ($r^2 > 0.953$). A plot of $\text{Ca}_{L}$ gain as a function of sound level indicated that the maximum gain, integrated over the whole tone, was similar to that integrated within individual time windows ($0.2 \pm 0.06$; sum of squares; variance $= 0.005$; Fig. 7C). This temporal constancy in $\text{Ca}_{L}$ gain is unlike the temporal increase in gain in monotonous cells. As an average in the nonmonotonous population ($n = 31$), the maximum $\text{Ca}_{L}$ gain did not vary at different times during the tone and was similar to the cumulative gain ($F_{4,154} = 0.85$, $P = 0.49$; Fig. 7D). The maximum effect of $\text{Ca}_{L}$ on peak firing in level-tuned neurons therefore does not change with temporal integration during the tone.

**DISCUSSION**

We have examined the effects of blocking $\text{Ca}_{L}$ on neuronal responses in the ICC during changes in sound pressure level. We quantified the influence of $\text{Ca}_{L}$ on level-variant and level-tuned RLFs and correlated its effects with dynamic range and the width of level tuning. We found that $\text{Ca}_{L}$ boosts firing at mid-to-high sound levels in level-variant, monotonic RLFs and suppresses or enhances peak firing in level-tuned, nonmonotonic RLFs. Enhancement of firing in monotonic RLFs is proportional to dynamic range, whereas the changes in peak firing in nonmonotonous RLFs vary inversely with the width of level tuning. Temporal integration favors the enhancement of
firing rates by CaL in level-variant responses but does not influence the effects of CaL on level tuning. Our results suggest that CaL refines responses to sound level in the ICC by differentially regulating level-variant and level-tuned neuronal populations.

**Synaptic and nonsynaptic regulation of sound-level codes.** Both level-variant and level-tuned RLFs are shaped by CaL, which is an ion channel intrinsic to ICC neurons, suggesting that nonsynaptic sources influence sound level codes. The contribution of CaL to neuronal firing in the ICC becomes important when excitatory input produces a large enough depolarization to reach its activation threshold, which slice recordings show is approximately −30 mV. This implies that, in vivo, the combination of the net excitatory input and the resulting postsynaptic membrane depolarization of ICC neurons, which results in the activation of CaL, corresponds to sound levels that are ≥10 dB above threshold in both monotonic and nonmonotonic neurons (Figs. 4 and 5). Absolute sound level may therefore not be the determining factor in activating CaL; instead, a relative change from threshold is the more important criterion. Thus, although CaL does not activate at threshold, it requires a relatively small increase (~10 dB) above threshold to affect a neuron’s firing rate.

During changes in sound level, CaL exerts two broad effects on firing rates in the ICC: it enhances the operating range of a large population of monotonic RLFs and a small population of nonmonotonic RLFs, and suppresses the operating range of a smaller population of monotonic RLFs and the peak firing of a large population of nonmonotonic RLFs. The opposing enhancing and suppressing effects of CaL on RLFs suggest differences in the interaction between the intrinsic activation of CaL in ICC neurons and its downstream effects on local ICC circuitry. Possible mechanisms underlying these differences are discussed below.

The enhancement of firing rate by CaL in monotonic RLFs with wide dynamic ranges is furthered by temporal integration, suggesting mutual feedback between glutamatergic transmission and CaL activation. Monotonic RLF gain in the ICC is increased by NMDA receptor (NMDAR) activation, which increases with temporal integration (Feldman and Knudsen 1994; Zhang and Kelly 2003). Both CaL and NMDAR activation can increase RLF gain by activating in the same ICC neuron in response to ascending input. The effectiveness of NMDARs on firing rate increases with local feedback (Augustinaite et al. 2014), and CaL itself can enhance activity in local circuits (Topolnik et al. 2009), suggesting a nonsynaptic-synaptic feedback excitation that increases with sound level. The rising slope of narrow dynamic RLFs is unaffected by CaL, which contrasts with its sensitivity to CaL in wide-dynamic range RLFs. At a population level, this difference may imply compartmentalization of intrinsic effects on operating ranges that depend on the rising slope of RLFs. Thus more gradually rising RLFs may allow for greater influence of nonsynaptic mechanisms on the excitatory-inhibitory synaptic balance that drives increasing firing rates with sound level. In nonmonotonic RLFs with narrow dynamic ranges, CaL decreases or increases peak firing rates. Intrinsic calcium-dependent conductances, such as the large and small calcium-activated potassium conductances, which regulate firing frequency in ICC neurons (Sivaramakrishnan and Oliver 2001), might underlie the differential CaL-mediated changes in peak firing. Local circuits in the ICC exert both excitatory and inhibitory feedback control of responses to sound level (Grimsley et al. 2013), and wide- and narrow-dynamic range monotonic RLFs might...
be subject to different feedback circuits, some of which involve disinhibition. As with monotonic RLFs, CaL, as a regulator of intrinsic firing, can have downstream effects on synaptic balance, changing the relative strength of inhibitory connections in a local circuit.

Inhibitory circuits in the ICC are also likely to underlie the suppression of peak firing rates in level-tuned RLFs by CaL. Feedback inhibition in the ICC is indicated in level tuning in neurons that respond to ipsilateral stimulation with monotonic RLFs and to contralateral and binaural stimulation with nonmonotonic functions (Wallace et al. 2012). Excitatory contralateral input (Hernandez et al. 2006; Moore et al. 1998) onto inhibitory ICC neurons is one pathway by which the activation of CaL can decrease firing rates. The involvement of contralaterally evoked CaL activation in the ICC is supported by observations that the nimodipine-sensitive CaL is an intrinsically high-threshold channel (Fig. 1) and that contralateral feedback is a delayed, high-threshold pathway (Chandrasekaran et al. 2013; Orton et al. 2012). Blocking CaL activated by contralateral input to the ICC would therefore increase firing rates in nonmonotonic RLFs, especially at high sound levels. The roles of nonmonotonicity, to remain level-invariant to complex sounds, detect tones in noise, and discriminate between sound levels (Polley et al. 2004; Rees and Palmer 1988; Sadagopan and Wang 2008), suggest a need for network stability. CaL may contribute to this stability by damping nonmonotonic excitation through downstream effects on inhibitory neurons, which have stabilizing effects on networks (Hulme and Connelly 2014; Lee and Chung 2014; Paille et al. 2013).

Inhibition in nonmonotonic RLFs in the auditory system can be asymmetric (Tan et al. 2007) and strongest at the peak and at high sound levels. As our results indicate, firing rates at both the peak and during the downward limb of level-tuned RLFs are decreased by CaL. Thus asymmetric inhibition of level-tuned RLFs appears to be partly determined by CaL activation. A basic absence of NMDAR-mediated facilitation, perhaps due to suppression of excitation by inhibition during the sustained portion of the tone (Wang et al. 1996), is likely to underlie the lack of effect of temporal integration on CaL-mediated firing rates in nonmonotonic RLFs. Our results do not, however, suggest that all nonmonotonic RLFs in the ICC are generated in inhibitory neurons; rather, CaL has a strong effect on the inhibitory component of nonmonotonicity.

Effects of CaL on a population code of sound level. Three effects of CaL on firing rates suggest that each broad monotonic and nonmonotonic population in the ICC contains subpopulations. First, in both monotonic and monotonic populations, CaL exerts both a multiplicative and divisive gain on different neurons. Second, if steeply saturating RLFs with narrow dynamic ranges reflect dynamic ranges in the auditory periphery (Sachs and Abbas 1974), whereas the wider dynamic ranges reflect local ICC circuits (Grimsley et al. 2013), then CaL selectively suppresses maximum firing in “inherited” RLFs and enhances maximum firing in RLFs shaped by local components. A similar argument might explain the lack of effect of CaL on broadly level-tuned RLFs, which might reflect broad nonmonotonic RLFs in the cochlear nucleus (Rhode 1999). Third, on a finer scale, the relationships between CaL effects on firing rates and the dynamic range of level variance or the width of level tuning is a strong indicator of a graded effect on neurons within each population. Whether neurons group into functional populations by the extent and spatial profile of input convergence, intrinsic channel densities, or other variables, remains to be tested.

Figure 8 summarizes the key feature of the gain exerted by CaL on neurons that are level variant or level tuned. Modulation of firing rates by CaL in each neuronal group depends on the response range of the feature associated with sensitivity to sound level in that group. In the level-variant group, the effectiveness of CaL associates with dynamic range. In the level-tuned group, its effectiveness associates with the width of level tuning. The modulation of firing rate by CaL changes in opposing directions with dynamic range and tuned width. Modulation or downregulation of CaL will therefore decrease sensitivity to level variance and, at the same time, increase sensitivity to level tuning. As an intrinsic, nonsynaptic mechanism, CaL appears to be level selective. Its maximum effects occur at levels that evoke the highest firing rates in ICC neurons, irrespective of whether neurons are level variant or tuned. Level variance and tuning may therefore be linked, and a population code of sound level in the midbrain may arise by mutual information processing between neuronal populations with their own distinct coding domains.

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AUTHOR CONTRIBUTIONS
C.A.G. and S.S. performed experiments; C.A.G., D.B.G., and S.S. analyzed data; S.S. conception and design of research; S.S. interpreted results of
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