Midbrain local circuits shape sound intensity codes

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INTRODUCTION

Sensory systems use distinct coding strategies to represent complex stimuli. Information contained within the intensity of a sensory stimulus, for example, is coded in different ways to extract multiple features of the input. Intensity-variant codes provide information about the context of a sensory stimulus, such as previous history or regional interaction (Albright and Stoner, 2002; Bartlett and Wang, 2005). Intensity tuning allows object recognition (Riesenhuber and Poggio, 1999; Barbour and Wang, 2003b; Freiwald and Tsao, 2010) and preserves input sensitivity (Watkins and Wang, 2003), implying that intensity codes and receptive fields adapt to changing sound stimuli, shifting their operating points toward preferred sound levels (Bialek and Schnitzer, 2004).

Firing rate codes of stimulus intensity require extensive central transformation to become efficient (Boeze et al., 2009; Arnal and Giraud, 2012; Zelano and Gottfried, 2012). In this respect, a hierarchical processing of sensory information requires interaction at multiple levels along the peripheral to central pathway. Recent evidence suggests that interaction between driving and modulating components can shape both top down and bottom up processing of sensory information. Here we show that a component inherited from extrinsic sources combines with local components to code sound intensity. By applying high concentrations of divalent cations to neurons in the nucleus of the inferior colliculus in the auditory midbrain, we show that as sound intensity increases, the source of synaptic efficacy changes from inherited inputs to local circuits. In neurons with a wide dynamic range response to intensity, inherited inputs increase firing rates at low sound intensities but saturate at mid-to-high intensities. Local circuits activate at high sound intensities and widen dynamic range by continuously increasing their output gain with intensity. Inherited inputs are necessary and sufficient to evoke tuned responses, however local circuits change peak output. Push–pull driving inhibition and excitation create net excitatory drive to intensity-variant neurons and tune neurons to intensity. Our results reveal that dynamic range and tuning re-emerge in the auditory midbrain through local circuits that are themselves variable or tuned.

Keywords: high divalents, inferior colliculus, monosynaptic, local circuits, sound intensity

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A high concentration of divalent cations (HiDi; raised Ca$^{2+}$) role of local circuits in forming codes of sound intensity, we applied NaH$_2$PO$_4$, 26 NaHCO$_3$; pH 7.35, or 1M a C l$(15–20$ M).

When the two synaptic pools activated in staggered regions of the intensity spectrum, they preserved tuning.

As sound intensity increased, the source of recruited synapses changed from monosynaptic to local. When the two synaptic pools activated at overlapping intensities they widened dynamic range. When the two synaptic pools activated at overlapping intensities they preserved tuning.

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MATERIALS AND METHODS

CBACa mice were obtained from Jackson Labs, Bar Harbor, Maine, or from our in-house breeding colonies. All animal procedures were approved by the Committee for Animal Care and Use at the Northeast Ohio Medical University and conformed to the guidelines for laboratory animal care and use published by the National Institutes for Health.

Single unit recordings were made in the IC of unanesthetized 1- to 2-month-old CBACa mice using methods previously described (Sivaramakrishnan et al., 2013). Data are reported from 109 cells separated by 30–60 ms. Well-isolated single units had stable spike amplitudes and shapes, and a signal-to-noise ratio $>5.1$. After a single unit was isolated, its characteristic frequency (CF) was determined. The CF was defined as the frequency at which the lowest sound pressure level consistently elicited stimulus-locked action potentials.

We constructed tuning curves by varying frequencies in 1 kHz intervals over a frequency range that spanned the low and high cut-off points for responses at the sound level used to identify the CF. In several cells, tuning curves were also constructed over a 4–60 kHz range.

CONSTRUCTION AND ANALYSIS OF RATE-INTENSITY FUNCTIONS

Sound pressure level was increased systematically from 0 to 96 dB SPL in 5 or 10 dB increments at 1 per second to prevent non-linearities in firing rate due to possible synaptic plasticity (Sivaramakrishnan et al., 2004), or peripheral non-linearities, which, for this study, might have complicated interpretation of the intensity-dependent activation of monosynaptic and polysynaptic inputs. Tone onset was delayed for 300 ms following the tone onset.
We recorded neuronal discharge patterns in vivo using the paired factor applied. Normality was confirmed (Origin software) before used as a criterion for significance and the Bonferroni correction effects of external and within-IC local inputs in structuring the head-fixed unanesthetized mice. Our aim was to examine the are indicated in the text or figure legends. Standard deviation, when used, is indicated in the text. Signifi-
cance was determined using paired $t$-test or ANOVA. Actual $p$ and $F(df_1,df_2)$ values are indicated in the text or figure legends.

RESULTS

We recorded neuronal discharge patterns in vivo in the IC of head-fixed unanesthetized mice. Our aim was to examine the effects of external and within-IC local inputs in structuring the responsiveness of neurons to the range of sound intensities that span normal hearing. We isolated responses to extrinsic inputs from those evoked by local circuits by blocking polysynaptic activity locally in the IC by applying ACSF containing a raised concentration of $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$ (high-divalents, HiDi).

Electrical activation of laminical inputs in IC brain slices or acoustic stimulation using tones in vivo evokes a HiDi-insensitive and -sensitive component. The HiDi-insensitive component is a primarily monosynaptic input with a short onset latency. It shows little jitter during repeated laminical activation in slices and gives rise to most first spike latencies in vivo. The monosynaptic component is the only component activated at very low levels of afferent recruitment. With increased recruitment of laminical afferents, a second, HiDi-sensitive component prolongs the synaptic response. This second synaptic component has a longer latency than the monosynaptic component and reflects the integration of multiple polysynaptic inputs. HiDi blocks responses to these local polysynaptic inputs by raising the postsynaptic threshold for firing. 76% of IC neurons receive both monosynaptic and local inputs, 6% receive only monosynaptic inputs, and 17% receive only polysynaptic inputs. Effects of HiDi are restricted to the side of the IC from which recordings are made. Recovery from HiDi application is rapid ($<4.5$ min) and recordings can be made at successive depths within the same IC during a single recording session. HiDi concentrations must be titrated to an optimal value that raises postsynaptic firing threshold slightly, but does not affect single unit isolation, spike heights, or durations in vivo. For IC neurons, this concentration is achieved by raising $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$ 2.5-fold (2.5 HiDi; Sivaramakrishnan et al., 2013).

HiDi PRESERVES FREQUENCY TUNING CURVES

To measure the effects of HiDi on RIFs, we measured firing rates in response to tones before and after HiDi application. Because we constructed RIFs using tones at the neuron’s CF, we first examined the effects of HiDi on CF. Recordings were made from neurons with CFs between 4 and 64 kHz, which spanned the range of CFs we were able to obtain in the IC (Egonova et al., 2008). Responses at CF were unaffected by HiDi (109 cells analyzed, $p = 1$). Frequency tuning curves were also unaffected (Figure 1). The different frequencies in each tuning curve overlapped (ANOVA, $p > 0.5, n = 32$ cells) and half-widths of tuning curves were not significantly different ($t_{60} = 0.44; p = 0.66, 32 cells$ measured). HiDi therefore appeared to isolate CF and off-CF inputs that created IC tuning curves, suggesting that inputs at- and off-CF that form a neuron’s tuning curve comprise a group of external monosynaptic inputs to the IC.

EFFECTS OF HiDi ON RATE-INTENSITY FUNCTIONS

We focused on two issues. First, we asked whether a wide dynamic range of sound intensity was inherited from ascending inputs or re-emerged in the IC. The mismatch between narrow dynamic range peripheral responses to pure tones and wider dynamic range responses in central neurons could conceivably occur through a smooth “stitching” (Barbour, 2011) of multiple sources that arise from the activation of the predominantly narrow dynamic range ($<35$ dB) peripheral excitatory inputs in different regions of the
The tone.
the monosynaptic input continued to provide excitation during we used, responses in HiDi occurred throughout the tone, thus $n$ gradual spike loss toward high intensities ($60\,\text{dB}$). In neurons with wide dynamic range responses to sound intensity $>60\,\text{dB}$, the average maximum gain was $3.6\pm1.2$ at $90\,\text{dB SPL}$, an increase of $2.6$ over its unitary gain at low intensities. The local circuit therefore multiplied neuronal output. Because the multiplicative factor itself increased with intensity, the reduced spike rate was due to a threshold increase in HiDi, then spike rates should have been preferentially reduced at low sound intensities, when excitatory input is presumably low. Firing rates at low sound intensities, however, overlapped before and after HiDi application ($20\sim40\,\text{dB}$ above threshold; ANOVA, $p=0.37$; $n=28$ cells analyzed). Because we did not find evidence of non-linearities in postsynaptic spike characteristics in vivo (Sivaramakrishnan et al., 2013) we assume that the RIF in HiDi was due to the monosynaptic input and associated postsynaptic integration (RIF$_{\text{MS}}$). The difference between the firing rates before and after HiDi would arise from extrinsic sources or formed in the IC.

Rate-intensity functions were constructed with 100 ms pure tones separated by $1\,\text{s}$ to prevent adaptive effects on firing caused by high tone repetition rates (Sivaramakrishnan et al., 2004). HiDi was then applied with pressure pulses for several minutes through one barrel of a multi-barrel electrode (Figure 1) and RIFs constructed again. The RIF that remained in HiDi was due to the monosynaptic input and associated postsynaptic integration (RIF$_{\text{MS}}$). The gain exerted by the local circuit, $\text{Gain}_L$, is the ratio of the control RIF to RIF$_{\text{MS}}$ and represents the multiplicative effect of the local circuit on output firing rate.

**LOCAL CIRCUITS WIDEN DYNAMIC RANGE**

In neurons with wide dynamic range responses to sound intensity $>60\,\text{dB}$ ($n=39$), firing rates decreased in HiDi. This decrease did not depend on the neuron's CF. Spike rasters showed clear breakdowns at mid-sound levels ($50\pm16\,\text{dB SPL}$; $n=15$) followed by a second wave of less intense firing (Figure 2A, neurons 1-3; $n=24$). In neurons with a sustained response to the 100 ms tones we used, responses in HiDi occurred throughout the tone, thus the monosynaptic input continued to provide excitation during the tone.

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local circuits must be dynamically regulated by a changing sound intensity.

In the population of wide dynamic range (>60 dB) neurons, the output RIF, RIFM, and GainL were sigmoidal ($r^2 > 0.98$; Figure 2D). RIFM activated at the same low threshold as RIF (19.2 ± 0.9, 18.6 ± 1.1 dB SPL; $t_{27} = 0.78$, $p = 0.44$), and saturated at 48 ± 7 dB, 36 dB lower than the saturation of RIF (84 ± 8 dB SPL). The local circuit activated at mid-sound intensities (46.1 ± 9 dB) and, as an average in the population, did not saturate strongly within the range of intensities tested. Dynamic ranges of the output RIF, and the monosynaptic and local circuit components were significantly different (73.8 ± 10.7; 32.6 ± 6.3; 53.4 ± 8.4; $F_{2,36} = 6.47$, $p < 0.002$; n = 33 cells). The dynamic range of the output RIF was ~13 dB narrower than the combined dynamic ranges of RIFM and the local circuit, and was likely due to increased K+ conductances (Sivaramakrishnan and Oliver, 2006). Thus monosynaptic inputs to the IC and local circuits combined to widen dynamic range.

**LOCAL CIRCUITS PRESERVE INTENSITY-TUNING**

Sound intensity tuning is a narrow dynamic range response (Barbour, 2011), and is highly sensitive to synaptic balance (Wehr and Zador, 2003; Sivaramakrishnan et al., 2004; Wu et al., 2006; Tan et al., 2007). In neurons that were strongly tuned to intensity (>50% reduction in firing rate at high sound intensities; Sivaramakrishnan et al., 2004; Barbour, 2011), HiDi changed firing rates in 46/52 cells. Peak firing rates in HiDi were less than the peak of the output RIF in most cells (31/46 cells; $t_{29} = 3.07$, $p = 0.003$; Figures 3A,B, left panels). In other cells, peak firing rates in HiDi were more than peak RIF (13/46 cells; $t_{30} = 3.12$, $p = 0.004$; Figures 3A,B, right panels). The net (excitatory + inhibitory) monosynaptic input was therefore sufficient to generate intensity-tuned responses and was tuned to the same intensity range as the output RIF.

In neurons in which peak firing rates in HiDi were lower than those in control conditions, the net local input increased responsiveness around tuned intensities to produce the higher firing rates of the output RIF. The gain of this net excitatory local input, GainL, increased with intensity, peaked and then decreased with further intensity increases (Figure 3B, bottom panel; neuron 1) with a gain of 1.66 ± 0.37 ($n = 31$). In neurons in which peak firing rates in HiDi were higher than in the control, the net local input decreased responsiveness around tuned intensities to lower the firing rates of the output RIF. GainL decreased with intensity, reached a trough, and then increased again (Figure 3B, bottom panel; neuron 2), with a gain of $-0.42 ± 0.38$ ($n = 15$). Local inhibition therefore exerts a divisive effect on the output RIF. This divisive effect increases with sound intensity, consistent with the recruitment of inhibitory local inputs and/or a larger driving force on inhibitory synaptic conductances. In the excitatory and inhibitory classes of local inputs, GainL was tuned to the same range of intensities as the output RIF. The local input therefore either boosted or suppressed peak firing, but preserved the tuned region.

The intensity range over which peak firing rates were spread was inherited from monosynaptic inputs. HiDi did not change the range of intensities covered by the population of intensity-tuned
FIGURE 3 | External and local influences on intensity-tuning. (A) Spike rasters of two intensity-tuned neurons (neuron 1, neuron 2) in control and HiDi. HiDi decreases peak firing rate in neuron 1 and increases it in neuron 2. (B) RIFs for each of the neurons in (A). Neuron 1: RIFM and GainL are both excitatory. Top: RIFM is tuned to the same range of intensities as the output RIF. Bottom: GainL, the ratio RIF/RIFM, is also tuned to the same intensities as the output RIF. In this neuron, the local circuit supplies a gain of 1.9 at peak tuned intensities. Dotted line: gain of 1 implies no net effect of the local circuit. Neuron 2: RIFM is excitatory, GainL is inhibitory. Both RIFM and GainL are tuned to the same intensity range as the output RIF. The local circuit exerts a negative gain on firing rate. (C) Distribution of RIFs in intensity-tuned neurons (gray lines). Normalized data. Number of cells illustrated: RIF: 16; RIFM: 19; GainL(E): 9; GainL(I): 8. Peaks for the output RIF, RIFM, and GainL(E) are distributed over a 25 dB range and, for GainL(I), over 35 dB in the population. Black lines: population averages. Mean and SD. (D) Left: average normalized RIFs. Mean and SD. Number of cells: RIF: 16; RIFM: 19; GainL(E): 9; GainL(I): 8. GainL(I) curves are normalized to the minima. Right: Gaussian fits. R² > 0.8793 for all curves.

peaks. Output RIF peaks were distributed narrowly, over 35 ± 5 dB (20–55 dB), as previously reported in the unanesthetized IC and auditory cortex (Sivaramakrishnan et al., 2004; Barbour, 2011, but see Sadagopan and Wang, 2008). RIFm (35 ± 6 dB) and GainL(E) and GainL(I) (excitatory and inhibitory local gain respectively; 35 ± 8 dB) peaks were distributed over similar dB ranges as the output RIF (Figure 3C, F4,204 = 0.44; p = 0.78; n = 52). Population averages of the output RIF, the monosynaptic and local components peaked at ~ 41 dB SPL (Figure 3D, F4,204 = 1.82; p = 0.13; n = 52).
TEMPORAL ACTIVATION OF LOCAL CIRCUITS IN INTENSITY-VARIANT AND TUNED NEURONS

The prolonged nature of polysynaptic responses to afferent lemniscal stimulation in IC brain slices (Sivaramakrishnan et al., 2013) suggested that local circuits would be preferentially activated at later times during a tone. Analysis of RIFs in distinct onset and sustained regions of the tone suggested that tone duration contributed to both dynamic range and tuning.

In intensity-variant neurons, RIF and RIFM were both steeply saturating functions during the onset portion (the first 20 ms following response onset) of the tone. At later times (25–100 ms), the output RIF increased monotonically with a wide dynamic range, whereas RIFM remained a short dynamic range, saturating function (Figures 4A,B). As an average in the population, in the onset and sustained portions of the tone, RIFM saturated at a similar sound intensity (41 ± 6 and 43 ± 5 dB SPL, respectively). GainL averaged across cells. Local circuit gain does not increase during the onset portion ($F_{4,44} = 1.22; p = 0.31$), but increases during the sustained response ($F_{4,44} = 9.72; p < 10^{-5}$). Since the HiDi and control functions were normalized, their peaks overlap. A slight increase in gain occurs during the onset portion of the tone ($F_{4,52} = 2.44; p = 0.038$). Strong local circuit activation during the sustained portion of the tone occurs during the tuned region (vertical dotted lines). $\text{Gain}_L$ was measured prior to normalization of the control and HiDi RIFs and averaged across cells.

**Figure 4** Temporal activation of monosynaptic and local inputs. For both intensity-variant and tuned neurons, onset responses are averaged over the first 20 ms; sustained responses are averaged between 25 and 100 ms. Response onsets are measured from the mean first spike latency. RIFs are normalized. All data are from cells that exhibited a sustained response during a 100 ms tone. (A,B) Intensity-variant neurons. (A) Onset and sustained responses in control (top) and HiDi (bottom) for five cells with dynamic ranges >60 dB. (B) Population averages. Twelve cells. Mean and SD. The PIF due to the monosynaptic input has a short dynamic range during both the onset and sustained portions of the response to the tone. GainL was measured at 30, 60, 70, 80 dB SPL prior to normalization of the control and HiDi RIFs and averaged across cells. Local circuit gain does not increase during the onset portion ($F_{4,44} = 1.22; p = 0.31$), but increases during the sustained response ($F_{4,44} = 9.72; p < 10^{-5}$). (C,D) Intensity-tuned neurons. (C) Onset and sustained responses in control (top) and HiDi (bottom) for three cells with different tuning widths. (D) Population averages. 14 cells. Mean and SD. Since the HiDi and control functions were normalized, their peaks overlap. A slight increase in gain occurs during the onset portion of the tone ($F_{4,52} = 2.44; p = 0.038$). Strong local circuit activation during the sustained portion of the tone occurs during the tuned region (vertical dotted lines). $\text{Gain}_L$ was measured prior to normalization of the control and HiDi RIFs and averaged across cells.
remained at 1 for all intensities during the onset portion of the tone (tested at 30, 50, 70, 80 dB SPL; $F_{14,11} = 1.22$, $p = 0.31$), but increased during the sustained portion ($F_{14,4} = 9.72$, $p < 10^{-7}$), reaching a maximum gain of $>30$ by 80 dB SPL. Integration during the tone therefore appears to favor activation of local circuits. The increase in output gain (by a factor of 3 at 80 dB SPL) suggests that integration during the tone results in non-linear changes in local circuits.

In intensity-tuned neurons, the output RIF and RIFM remained similarly tuned during the onset and sustained portions of the tone (Figures 4CD). The monosynaptic input therefore remained consistent with integration. During the onset portion of the tone, GAIn, increased slightly at higher intensities (tested at 20, 30, 40, 50 dB SPL; $F_{14,7} = 2.74$, $p = 0.038$, $n = 14$ cells), corresponding to the falling limb of the output RIF. With integration over the later part of the tone, however, GAIn was strongly tuned, with a tuned region that corresponded with that of RIF-Gain, increased to $1.5$ during the tuned region (difference between baseline gain and maximum gain during the tuned region; $t_{27} = 3.19$, $p = 0.004$; $n = 14$ cells). Additional changes in gain occurred during the falling limb of RIF. Between 20 and 40 dB SPL, within the tuned region, the average change in Gain, was higher during the sustained portion of the tone (increase from baseline gain of $0.53 \pm 0.21$) compared with the onset portion (increase of $0.13 \pm 0.22$ from baseline gain, $t_{27} = 2.57$, $p = 0.01$; $n = 14$).

**PUSH–PULL GAIN CONTROL BY MONOSYNAPTIC INPUTS**

The inability of RIFm to reach the peak firing rates of the output RIF in intensity-variant and in intensity-tuned neurons where HI increased peak firing rates (as in Figures 2 and 3) suggested a saturation of the net monosynaptic input. This saturation might reflect saturation of ascending excitation, or might be due to the strong inhibition that the IC receives from brainstem sources (Cast and Benson, 2003). Decreased excitatory input accompanied by increased inhibitory input, or vice versa, produces a push–pull gain control of neuronal output by mutual reinforcement (Ferster, 1988) and typically occurs through increased conductance of the postsynaptic membrane due to the inhibitory input (Steriade, 2001; Destexhe et al., 2003). Push–pull gain control shapes sensory receptive fields (Perister and Miller, 2000; Hirsch and Martinez, 2006) and has been suggested to be a characteristic feature of driving inputs (Abbott and Chance, 2003).

To determine whether the saturation of RIFs2 reflected excitation saturation alone or included monosynaptic inhibition, we recorded firing rates first in HiDs, and then after blocking (monosynaptic) inhibition with antagonists of GABA_\text{A} (gabazine, G الزيد) and glycine (strychnine) receptors. We dissolved the antagonists in HiDs to prevent re-activation of local inputs. In neurons with wide dynamic ranges, spike rates dropped in HiDs and increased again in inhibitory antagonists (Figure 5A; $n = 16$ cells). The RIF in HiDs/strychnine was due to monosynaptic excitation. Monosynaptic excitation increased continuously with intensity (up to $\sim 90$ dB SPL; $n = 16$, Figure 5B). Because the excitatory component diverged from the net monosynaptic input (at $42 \pm 12$ dB above threshold, $n = 16$), the saturation of the monosynaptic input was due to inhibition. The gain of monosynaptic inhibition (net monosynaptic/excitatory component) decreased with intensity, while the excitatory component increased (Figure 5C). Monosynaptic excitation and inhibition thus produced push–pull gain control of total extrinsic input. This finding supports the suggestion that push–pull excitation–inhibition is a characteristic of driving inputs (Abbott and Chance, 2005).

The threshold and dynamic range of monosynaptic excitation were similar to that of the output RIF ($t_{14} = 0.73$, $p = 0.47$; $n = 16$, Figure 5D). The excitation–inhibition balance determined first spike latencies, which were shortened in HiDs/Gz/strychnine (HiDs/Gz/strychnine $12.65 \pm 3.92$ SD; control $15.34 \pm 5.15$ SD; $t_{14} = 2.33$, $p = 0.01$) but not in HiDs alone ($t_{14} = 1.53$, $p = 0.13$; control $15.34 \pm 5.15$; HiDs $14.58 \pm 4.03$).

In intensity-tuned neurons ($n = 14$), the excitatory component increased firing rates over the net input (Figure 6A). Monosynaptic excitation remained tuned and peak firing rates occurred in the same intensity range as that of the net input (Figure 6B, green trace; $t_{14} = 0.25$, $p = 0.82$). Monosynaptic inhibition opposed excitation in the flank regions of the input (Figure 6C). Inhibition decreased ($by 68.6 \pm 7.39$; $n = 14$) during the rising limb of excitation and returned to a baseline gain of $\sim 1$ ($84.6 \pm 8.82$) during the falling limb. Between the flanks, inhibitory gain was co-tuned with excitation (Figure 6C; shaded areas). Excitation contributed symmetrically to the total monosynaptic input (Figure 6D, left); rising and falling slopes were symmetrically steeper than the net input, by approximately twofold ($n = 14$). The excitatory and inhibitory components both had wider tuning widths than the net monosynaptic component, suggesting a push–pull control of tuning width by monosynaptic excitation and inhibition (Figure 6D, right; $n = 14$ neurons, RIFas, RIFm: $t_{27} = 3.56$, $p = 0.0014$; RIFas, RIFm: $t_{27} = 3.55$, $p = 0.0001$).

**DISCUSSION**

The goal of our study was to determine the pattern of input convergence that would allow changes in sound intensity to be represented in parallel as intensity-variant and tuned codes. To characterize input pattern, we isolated synaptic inputs based on their source, inherited monosynaptic, or local, polysynaptic, while introducing sounds of different intensities. From the responses of neurons to these two synaptic compartments, we were able to predict regions of the sound intensity code that were more or less susceptible to adaptive gain control.

**MONO- AND POLYSYNAPTIC INPUTS CREATE INVARIANT AND VARIABLE CODING DOMAINS**

At low sound levels, the net monosynaptic input (excitatory + inhibitory) generated the steepest part of the RIF. It carried information about threshold, dynamic range, CF, and first spike latency. The $\sim 35$ dB dynamic range and saturating RIF suggest that monosynaptic inputs are part of the pathway that includes narrow dynamic range ($\sim 35$ dB) auditory afferents whose recruitment increases with sound level (Sachs and Abbas, 1974). These afferent contacts, if made through large glutamatergic terminals or dense terminal arbors (Winer, 2005; Nakamoto et al., 2013) on proximal dendrites, would have the properties of driving inputs (Sherman and Guillery, 1998). Our results suggest that these driving inputs
FIGURE 5 | Monosynaptic excitation and inhibition in wide dynamic range neurons. (A) Spike rasters. Firing rates decrease in HiDi (middle), but increase again in the inhibitory antagonists (bottom). Gabazine (Gz, 50 μM); strychnine (8 μM). The inhibition is a monosynaptic input. (B) RIFs for the cell in (A). The RIF in HiDi/Gz/strychnine, due to the excitatory component of the monosynaptic input increases throughout the range of intensities, unlike the net monosynaptic input. RIFM, which saturates. (C) RIFM consists of an excitatory component, RIFM(E), and an inhibitory monosynaptic component which exerts a gain, GainM(I) = RIFM/RIFM(E). Push–pull interaction between monosynaptic inhibition and excitation generates the net monosynaptic input. RIFM(E)/RIFM slope ratios: rising limb, 2.12 ± 0.085; falling limb, 2.24 ± 0.13. (D) average threshold and dynamic range. 16 cells. Mean and SEM.

include those that create tuning curves, which are frequency specific channels that persist through the auditory pathway (Lin et al., 2007; Kandler et al., 2009; Sumner et al., 2009). A primary role of narrow dynamic range peripheral afferents may therefore be to ensure throughput of the rate-level code through proximal monosynaptic inputs. Ascending brainstem inputs are spread over a wide area and likely drive a broad range of cells with different CFs (McAlpine et al., 1998). Driving inputs with diverse strengths interacting with different intrinsic operating ranges of IC neurons would cause dynamic changes (Hasenstaub et al., 2007) in local IC circuits, increasing or decreasing their gain with changes in sound intensity.

The sensitivity of sound intensity codes to the pattern of sound stimuli provides clues to the changing nature of synaptic inputs to central neurons during a change in intensity. Changes in tone repetition rate, addition of tonic noise, modulation of sinusoidal amplitude, and selective stimuli for most probable sound levels alter dynamic ranges and receptive fields (Riesz and Palmer, 1988; Joris et al., 2004; Nelson et al., 2007; King et al., 2011). The intensity code is therefore highly plastic, and synaptic input must adjust dynamically to allow for the invariant and mutable regions of the level code, both of which are required to interpret changes in sound level. Convergence of narrow dynamic range (~35 dB) peripheral excitatory afferents appears more conducive to retaining the invariant than variant aspects of level codes (Carlyon and Moore, 1984; Gibson et al., 1985; Spirou et al., 1999). Afferent excitation is also strong and rises steeply, which non-intuitively narrows dynamic range in central neurons by pushing target cells to their operating limits, causing premature firing rate saturation (Sivaramakrishnan et al., 2004).

Our results show that local input fine-tunes and filters intense excitation, conferring plasticity to the system. Local recruitment would favor non-linear processes involving multiple excitatory and inhibitory sub-domains of local inputs in the IC and are likely to underlie much of the spectrotemporal complexity that appears at high sound intensities (Lesica and Grothe, 2008). Extensive connections within IC frequency laminae (Wallace et al., 2012) and axonal collateralizations (Oliver et al., 1991) are likely to recruit the majority of local neurons with increasing sound intensities. Frequency representation in the IC broadens with sound intensity, and while this is generally attributed to an increased inherited input strength, our results suggest that extrinsic input saturates at mid-sound intensities, and further increase in input recruitment occurs at the local level. Local recruitment could be triggered by commissural connections that serve as a means of di- or polysynaptic input (Moore et al., 1998). Cooling of the commissure has been recently shown to preserve short latency (<20 ms) responses to acoustic input while selectively blocking longer-latency (>20 ms) responses (Orton et al., 2012). This separation of early and late components by commissural blockage is...
FIGURE 6 | Monosynaptic excitation and inhibition in intensity-tuned neurons. (A) Spike rasters in control (top); HiDi (middle); HiDi + Gz + strychnine (bottom). Left: the cell in (A) middle, right: two other cells. (B) Normalized RFm, RFm (E), and GainM (I) for the three cells in (B). GainM (I) changes the direction of its gain control with sound intensity. Hatched region: GainM (I) exhibits a "tuned" gain. (C) Normalized RIFM, RIFm (E), and GainM (I) for the three cells in (B). GainM (I) changes the direction of its gain control with sound intensity. Hatched region: GainM (I) exhibits a "tuned" gain. (D) Left: linear fits (black lines) of the rising and falling limbs of RIFM (E) and RIFm (E) for Neuron 1 in (B). Rising limb: RIFM, \( r^2 = 0.90285 \); slope, 4.12821 spikes/s/dB SPL; RIFm (E), \( r^2 = 0.92772 \); slope, 8.2253; falling limb: RIFM, \( r^2 = 0.88935 \); slope, \(-3.30769\); RIFm (E), \( r^2 = 0.98401 \); slope, \(-6.3333\). Right: population averages of tuned widths. Tuned widths were measured at half the peak height of normalized functions. 14 cells. Mean and SEM.

similar to the time courses of the HiDi-insensitive and sensitive components of tone-evoked responses in our study and strengthens our hypothesis that the short latency response is evoked by a direct ascending monosynaptic lemniscal contact, while the longer latency components are driven by local-circuits. Optical imaging with voltage-sensitive dyes in IC slices suggests that commissural propagation is a high-threshold pathway, evoked either by increasing excitation or by reducing inhibition in the opposite IC (Chandrasekaran et al., 2013), and might partly account for our finding that the HiDi-sensitive local circuit is a high-threshold input.

The exact complement of inputs and postsynaptic membrane properties that influence changes in firing rate would be expected to vary with the complexity of sound stimuli. Our data suggest that the local circuit activates at high intensities, contributing a high-threshold component of sound intensity codes. NMDARs activate close to the resting potential in a substantial population of IC neurons (Wu et al., 2004; Sivaramakrishnan and Oliver, 2006) 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 the multiplicative gain, which we report is about 3. Thus a combination of several factors, including variations in the complexity and number of synapses involved in the circuit, intrinsic membrane conductances, and slow acting transmitter systems, likely play a role in the dynamic change in gain of the local circuit.

Feedback gain enhancement by local circuits has to be balanced by the relatively short operating range of IC neurons, the majority of which go into depolarization block at membrane potentials as negative as -30 mV (Sivaramakrishnan et al., 2004). Local inputs at high sound levels could consist of mixed excitation and inhibition with a variable synaptic gain that prevents premature firing block of the postsynaptic cell.

TUNED AND WIDE-DYNAMIC RANGE NEURONS BELONG TO STEREOTYPIC MICROCIRCUITS

Tuned and wide-dynamic range responses to sound intensity are created and maintained by distinct synaptic infrastructures. Intensity tuning itself appears to be independent of synaptic source. The restricted spread of peaks to 35 dB, which is unaffected by input source, suggests that peak distribution does not emerge in the IC. Wide-dynamic range responses, on the other hand, are composed of sub-domains of narrow dynamic range inputs with high efficacies in non-overlapping intensity regions. Our results suggest that intensity-tuned and wide dynamic range neurons belong to different IC microcircuits that either linearly integrate inherited inputs, or bypass subsets of inherited inputs to create emerging non-linearity. The hierarchical organization between extrinsic...
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AUTHORS CONTRIBUTION

CAG, ITS, and SS collected data. CAG and SS analyzed data. SS designed the study and wrote the paper.

REFERENCES

Albright, T. D., and Chiao, F. S. (2005). Directed and modulators from push-pull and balanced synaptic input. Proc. Natl. Acad. Sci. USA 102, 157–162. doi: 10.1073/pnas.0410001102

Abbott, L. F., Marella, J. A., Sen, K., and Nelson, S. B. (1997). Spatial depression and cortical gain control. Science 275, 224–228. doi: 10.1126/science.275.5297.222

Albright, T. D., and Stoner, G. R. (2002). Cortical oscillations and sensory predictions. Trends Cogn. Sci. 6, 398–404. doi: 10.1016/S1364-6613(02)02557-9

Abbott, L. F., and Chance, F. S. (2005). Defining cortical frequency tuning in the auditory midbrain. J. Neurosci. 25, 441–447. doi: 10.1166/jneurosci.25.4.441

Albrecht, D. (1985). The existence of cortical mechanisms tuned to orientation. J. Neurosci. 5, 539–556. doi: 10.1523/JNEUROSCI.05.1985.05.01

Abbott, L. F., and Nelson, S. B. (1990). NEURON: a tool for modeling neuronal circuits. Nat. Neurosci. 398. doi: 10.1016/j.neurosci.2011.03.088

Bialek, M. N., and Schreiner, C. E. (2004). Short-term adaptation of auditory receptive fields to dynamic stimuli. J. Neurophysiol. 91, 804–812. doi: 10.1152/jn.00846.2003

Lei, J. K., Qiaoan, B. N., Rennhoff, M. A., Belmonte, R., Liu, S. T., Vicini, S., et al. (2013). Mosy filter CAG-CAY235 mediates homeostatic plasticity in mature hippocampal neurons. Neuron 77, 99–114. doi: 10.1016/j.neuron.2012.10.033

Lesica, N. A., and Gedeon, B. (2008). Dynamic spectrotuemporal filtering in the auditory midbrain. J. Neurosci. 28, 5412–5421. doi: 10.1523/JNEUROSCI.0785-08.2008

Liu, B. H., Wu, K. G., Arbuckle, B., Tao, H. W., and Zhang, J. (2006). The effects of Lesley filtering on auditory cortex survival in Dunning cortical frequency tuning with recurrent excitatory circuitry. Nat. Neurosci. 10, 1594–1599. doi: 10.1038/nn1912

McAlpine, D., Jing, X., Shackleford, T. M., and Palmer, A. R. (1998). Convergent input from brainstorm coincidence detectors unto delay-sensitive neurons in the inferior colliculus. J. Neurosci. 18, 4620–4629.

Morone, D. R., Kottak, V. C., and Sanes, D. J. (1998). Commensal and kinaesthetic input to the gerbil inferior colliculus. J. Neurophysiol. 80, 2228–2234.

Muggio, M., Perez-Garcia, E., Novair, T., Rock, T., Senn, W., and Larkum, M. E. (2009). Densest encoding of sensory stimuli controlled by deep internal neuronal pools. Nature 457, 1137–1141. doi: 10.1038/nature07568

Murphy, B. K., and Miller, K. D. (2009). Balanced amplification: a new mechanism of selective amplification of neural activity patterns. Neuron 61, 655–658. doi: 10.1016/j.neuron.2009.02.005

Nakanishi, K. T., Mellett, J. G., Kilima, J., Stony-Workley, M. E., Sowack, C. S., and Schallhorn, B. R. (2011). Analysis of excitatory epsps in the guinea pig inferior colliculus.
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