Neural integration and enhancement from the inferior colliculus up to different layers of auditory cortex

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Straka MM, Schendel D, Lim HH. Neural integration and enhancement from the inferior colliculus up to different layers of auditory cortex. J Neurophysiol 110: 1009–1020, 2013. First published May 29, 2013; doi:10.1152/jn.00022.2013.—While the cochlear implant has successfully restored hearing to many deaf patients, it cannot benefit those without a functional auditory nerve or an implantable cochlea. As an alternative, the auditory midbrain implant (AMI) has been developed and implanted into deaf patients. Consisting of a single-shank array, the AMI is designed for stimulation along the tonotopic gradient of the inferior colliculus (ICC). Although the AMI can provide frequency cues, it appears to insufficiently transmit temporal cues for speech understanding because repeated stimulation of a single site causes strong suppressive and refractory effects. Applying the electrical stimulation to at least two sites within an isofrequency lamina can circumvent these refractory processes. Moreover, coactivation with short intersite delays (<5 ms) can elicit cortical activation which is enhanced beyond the summation of activity induced by the individual sites. The goal of our study was to further investigate the role of the auditory cortex in this enhancement effect. In guinea pigs, we electrically stimulated two locations within an ICC lamina or along different laminae with varying interpulse intervals (0–10 ms) and recorded activity in different locations and layers of primary auditory cortex (A1). Our findings reveal a neural mechanism that integrates activity only from neurons located within the same ICC lamina for short spiking intervals (<6 ms). This mechanism leads to enhanced activity into layers III–V of A1 that is further magnified in supragranular layers. This integration mechanism may contribute to perceptual coding of different sound features that are relevant for improving AMI performance.

auditory cortex; deep brain stimulation; enhancement; inferior colliculus; temporal integration

AUDITORY PROSTHESES HAVE BEEN treating hearing loss for decades, with the cochlear implant (CI) being able to provide sufficient temporal and spectral cues required for speech understanding (Kral and O’Donoghue 2010; Shannon et al. 1995; Zeng 2004). For people without a functional auditory nerve or an implantable cochlea, central prostheses have been implanted in the brain stem since 1979. Unfortunately, the performance of the auditory brain stem implant (ABI) has generally been less successful than the CI, particularly for patients with neurofibromatosis type II, one of the key target populations for the implant (Colletti et al. 2009; Lim et al. 2009; Schwartz et al. 2008). While some ABI patients have shown performance levels approaching that of CI patients (Colletti et al. 2012; Sennaroglu et al. 2011), continued research into central auditory implants needs to be performed to improve hearing perception for the majority of patients who cannot benefit from a CI.

As an alternative approach to the ABI, particularly for the neurofibromatosis type II population, deaf patients have been implanted with a new auditory midbrain implant (AMI) consisting of a single-shank array (20 sites) designed for stimulation along the tonotopic gradient of the central nucleus of the inferior colliculus (ICC) (Lim et al. 2007, 2009). While AMI stimulation can activate frequency-specific neurons and transmit spectral cues to higher auditory centers, it appears to exhibit limited temporal coding abilities (Lenarz et al. 2006; Lim and Anderson 2006; Lim et al. 2008, 2013; McKay et al. 2013).

Calixto et al. (2012) investigated this limited performance of the AMI by stimulating the ICC of guinea pigs with the AMI array and recording the evoked local field potentials (LFPs) in the primary auditory cortex (A1). Repeated stimulation of a single site caused refractory and suppressive neural effects, which likely resulted in the inadequate temporal coding. These negative effects could be overcome by stimulating at multiple sites within an isofrequency lamina. In addition, coactivation of sites with short delays (<5 ms) resulted in cortical activity that was enhanced beyond a linear sum of activation elicited by the individual sites. However, their interpretation of A1’s role in this enhancement effect was limited because they only analyzed LFPs in the main input layer III/IV (i.e., corresponding to thalamic input into A1). They also did not analyze spike activity in A1 because of the prevalence of antidromic field potentials from ICC stimulation that could partially mask the orthodromically activated spikes across cortical layers. As a result, they were unable to determine whether this enhancement effect was solely produced within the tectothalamic pathway, or if it also occurred within the thalamocortical and corticocortical pathways. Moreover, they were unable to determine whether this enhancement effect could be observed when stimulating across different ICC laminae, or if it solely occurred when stimulating within a lamina.

The purpose of this study was to further investigate the role of A1 in the enhancement effect by expanding the study performed by Calixto et al. (2012). We presented two electrical pulses with varying interpulse intervals (0–10 ms) to one or two sites within an isofrequency lamina or in different laminae of the ICC. By stimulating the ICC with much smaller electrode sites than the AMI sites (413 μm2 vs. 126,000 μm2), we could stimulate more locally within the ICC, which resulted in less antidromic activation of corticocollicular neurons, and
thus the antidromic field potential was isolated to layer V, as reported in a previous study (Lim and Anderson 2007a). Using 32-site electrode arrays, we recorded LFPs and multiunit spike activity simultaneously within different cortical layers to determine whether activity is further enhanced or altered by neuronal mechanisms within A1. We also investigated how the enhanced cortical activity varied across different locations in A1. Our results confirm that a neural mechanism exists within the ICC-to-A1 pathway for eliciting enhanced activity in A1. This neural mechanism integrates activity from different ICC neurons, which reside only within the same lamina and are coactivated within a brief time window (<6 ms). Our results also indicate that this integration effect is further enhanced up to supragranular layers of A1. Moreover, this integration effect exhibits similar properties to that of perceptual data obtained for short-interval stimuli in humans. These findings reveal a neural mechanism that may contribute to perceptual processing of different sound features, based on an interspike interval or synchrony code across ICC neurons, and needs to be considered for improving AMI stimulation strategies.

METHODS
Overview

Basic surgical procedures and methods for neural recording and stimulation were similar to those presented in previous work (Lim and Anderson 2007a; 2006; Neuheiser et al. 2010). This study was approved by the University of Minnesota's Institutional Animal Care and Use Committee. Silicon-substrate, 32-site Michigan electrode arrays (NeuroNexus Technologies, Ann Arbor, MI) were used to electrically stimulate the ICC and record the corresponding neuronal responses within A1 of ketamine-anesthetized guinea pigs. Appropriate placements of the array sites within the ICC and A1 were guided by acoustic-driven responses (Lim and Anderson 2007b; Snyder et al. 2004; Wallace et al. 2000). Neuronal action potentials (spikes) and LFPs recorded in response to application of two pulses at one ICC site were compared with neuronal activity induced by application of a single pulse at each of two sites, with varying delays between the two pulses. The two stimulated sites were either within the same isofrequency lamina, or across different laminae. These experiments investigated how activation of similar vs. different neurons within and across frequency laminae activated different regions and layers of A1.

Surgery

Experiments were performed on 14 male and female Hartley guinea pigs (373 ± 46 g, Elm Hill Breeding Laboratories, Chelmsford, MA). Animals were initially anesthetized with an intramuscular injection of ketamine (40 mg/kg) and xylazine (10 mg/kg) and were given periodic supplements to maintain a nonreflexive state. After fixing the animal into a stereotaxic frame (David Kopf Instruments, Tujunga, CA), we exposed the right side of the cortex from the caudal end of the occipital lobe to the middle cerebral artery of the temporal lobe. The dura was removed, micromanipulators were used to insert the arrays into the ICC and A1, and the exposed brain was covered with agarose gel.

Stimulation and Recording Setup

All experiments were performed in an acoustically and electrically shielded chamber and controlled by a computer interfaced with TDT System 3 hardware (Tucker-Davis Technology, Alachua, FL) using custom software written in Matlab (MathWorks, Natick, MA). For acoustic stimulation, sound was presented via a speaker coupled to the left ear through a hollow ear bar. The speaker-bar system was calibrated using a 0.25-in. condenser microphone (ACO Pacific, Belmont, CA) connected to the ear bar via a short plastic tube representing the ear canal.

All neural signals (i.e., LFPs and spiking activity) were passed through analog DC-blocking and anti-aliasing filters from 1.6 Hz to 7.5 kHz. The sampling frequency used for acoustic stimulation was 195 kHz and for neural recording was 24 kHz. Electrical stimulation up to 0.10 µA was presented on different sites on the ICC array in a monopolar configuration with a ground return in the neck muscles. The pulses were biphasic, charge balanced, cathodic leading and 205 µs/phase. The recording ground needle was positioned either under the skin ~2 cm rostral to bregma or directly in the brain in the parietal lobe. No obvious differences in results were observed when using the different recording grounds.

Placement of Arrays

Poststimulus time histograms (PSTHs) and frequency response maps were plotted online to confirm the array’s position within the ICC or A1. Details on these analysis methods and example plots for similar types of arrays are presented in previous publications (Lenz et al. 2006; Lim and Anderson 2006; Neuheiser et al. 2010). Briefly, we band-pass filtered the neural signals (300–3,000 Hz) on each site and labeled a spike as any negative peak that exceeded a threshold of three standard deviations above the background activity. Thus all analysis was performed on multunit spike data. For frequency response maps, four trials were presented for each pure tone (1–40 kHz, 8 steps/octave) and level (0–70 dB, 10 dB steps) stimulus. The best frequency (BF) was taken as the centroid of frequencies which elicited spiking responses at 10 dB above the visually determined threshold.

The A1 array consisted of four 5-mm-long shanks separated by 400 µm. Sites were linearly spaced at 200 µm along the shank, and each had an area of 177 µm². The array was inserted approximately perpendicular to the cortical surface in an attempt to align each shank along a column in A1 (Abeles and Goldstein 1970; Redies et al. 1989; Wallace et al. 2000). The average difference of BFs (∆BF) between sites in layers III/II, III/IV, and V along each shank was 0.09 ± 0.08 octaves (mean ± SD). Placement into A1 was confirmed when tonotopic shifts of low to high BFs were observed for ventral-rostral to dorsal-caudal locations (Wallace et al. 2000). Only A1 sites with similar BFs to the stimulated ICC sites were analyzed. To investigate differences across A1 locations, we reconstructed the site locations on the A1 surface based on microscope images (OPMI 1 FR pro, Zeiss, Dublin, CA) taken of our array placements and normalized based on various landmarks (e.g., middle cerebral artery, bregma and lateral suture lines, major blood vessels) successfully used in previous studies (Eggermont and Roberts 2004; Schreiner et al. 2000; Wallace et al. 2000). These normalized locations and their respective BFs can be seen overlaid on the auditory cortex in Fig. 1.

The identification of A1 layers was accomplished by performing current source density (CSD) analysis (Král et al. 2000; Mitzdorf 1985; Muller-Preuss and Mitzdorf 1984) in response to 70-dB SPL broadband noise (100 trials) using the finite difference formula:

\[
\text{CSD}(z) = \frac{\phi(z + \Delta z) - 2\phi(z) + \phi(z - \Delta z)}{\Delta z^2}
\]

where \(\phi\) is the averaged LFP across trials, \(z\) is the depth location of each site along an A1 array shank, \(\Delta z\) is the differentiation step size, and \(\sigma_r\) is the component of conductivity in the z-direction. \(\Delta z\) was equal to the A1 site spacing of 200 µm and \(\sigma_r\) was set to one, since absolute CSD values were not required for analysis. The one-dimen- sional CSD approximation provides a consistent representation for the current sinks and sources associated with columnar synaptic activity in the guinea pig auditory cortex (Lim and Anderson 2007a; Middlebrooks 2008). The main input layer of A1, which is layer III/IV in

\[\text{CSD}(z) = \frac{\phi(z + \Delta z) - 2\phi(z) + \phi(z - \Delta z)}{\Delta z^2}\]

\[\phi\approx \text{averaged LFP across trials}, z\approx \text{depth location of each site along an A1 array shank}, \Delta z\approx \text{differentiation step size,} \sigma_r\approx \text{component of conductivity in the z-direction.}\]

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division of the medial geniculate body (MGBv) and A1 in a tonotopic pattern (Lim and Anderson 2006; Malmierca et al. 1997; McMullen et al. 2005; Velenovsky et al. 2003). The average ΔBF between the two ICC sites was 0.2 ± 0.1 octaves. While 0.2 octaves is smaller than the bandwidth of an iso-frequency lamina, which is ~150 μm at 0.3 octaves (Malmierca et al. 2008), this separation still allowed us to stimulate different populations of neurons residing in neighboring laminae to achieve the goal of this study. The Off-BF DSS protocol was used in three animals, in which two of the animals also received the SSS and DSS protocol. For the DSS protocol, a total of 25 ICC site pairs were stimulated, and responses were recorded in 57 A1 locations from 13 animals. Every DSS stimulation site was also used in the SSS protocol for a total of 50 stimulation sites. For the Off-BF DSS protocol, a total of 9 ICC site pairs were stimulated, and responses were recorded in 36 A1 locations.

Electrical stimulation was presented with different levels and varying interpulse intervals (IPIs), with each stimulus being randomly presented for a total of 20 trials. For the SSS protocol, the IPIs included 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 ms, and both pulses were always presented at the same stimulus level. For the DSS and Off-BF DSS protocols, an IPI of 0 ms (simultaneous stimulation) was also used. The DSS stimulation levels varied between the two pulses on different sites because stimulation of different ICC locations elicits A1 activity with different thresholds (Lim and Anderson 2007b; Neuheiser et al. 2010). Due to restrictions in time, we identified only two or three levels above threshold for one of the sites, while stimulating the other site with up to 10 levels above its threshold across a 12-dB range. Only levels eliciting A1 activity above threshold but below saturation levels were analyzed. An average of six levels for SSS, 12 levels for DSS, and 11 levels for Off-BF DSS were used for each stimulation case (i.e., ICC-A1 stimulation-recording site pair). For each level, we plotted IPI curves in which the A1 response was shown as a function of IPI. Note that, for a given pair of ICC sites for the DSS protocol, we could obtain two different IPI curves by reversing the order of which ICC site was stimulated first. Due to inherent differences in neural activation patterns, we considered each of these two scenarios as different stimulation cases when later presenting the summary data and statistical analyses. This was further validated when fitting neural responses to a linear mixed model, where the predictors were IPI and stimulation order, and the random variable

Electrical Stimulation Parameters

As depicted in Fig. 2, we used three protocols to electrically stimulate one or two sites within the ICC and characterize the corresponding LFP and spiking activity within A1 supragranular layers I/II, main input layers III/IV, and output layer V. In the first two protocols, we stimulated within an ICC iso-frequency lamina either by delivering two pulses on the same site (single-site stimulation, SSS) or one pulse on each of two sites with similar BFs (dual-site stimulation, DSS). For the DSS protocol, the average ΔBF between the ICC sites was 0.05 ± 0.06 octaves, which is below the bandwidth of ~0.3 octaves seen in an iso-frequency lamina of cat (Schreiner and Langner 1997) and rat (Malmierca et al. 2008). The analysis was performed on the A1 sites with similar BFs to the stimulated ICC laminae (ICC-to-A1 ΔBF: 0.2 ± 0.2 octaves). We also presented two pulses in the third protocol, with one on an ICC site BF-matched to the A1 site, and the other on a neighboring ICC site 100 μm away along the same shank for the Off-BF DSS protocol. Stimulating sites further than 100 μm along the tonotopic gradient required current levels typically exceeding our 100 μA limit to effectively activate the A1 site, which is consistent with different ICC regions projecting to and activating different regions of the ventral

![Middle Cerebral Artery](image-url)
was the location of the electrode array. Stimulation order and IPI proved to be significant predictors ($P < 2 \times 10^{-16}$).

**Data Analysis**

**Evoked potential activity.** For IPIs up to 10 ms, only one negative LFP peak was present (e.g., Fig. 3A), and the area of the negative peak was calculated. The LFP response, which corresponds to a voltage recording over time, is proportional to current over time based on Ohm’s Law (assuming neural tissue can be approximated as a linear medium). Therefore, the LFP area reflects the total charge (i.e., area under a current vs. time curve), which is related to the amount of synaptic input into A1 surrounding the recording site (Eggermont and Smith 1995; Mitzdorf 1985). In this study, the LFPs shown were not filtered beyond the filters used in data collection to avoid altering the LFP shape, which can vary dramatically, depending on the filter range and type. However, averaging the LFP traces across trials in effect is a low-pass filter that smoothed the curves, resulting in a response that is an order of magnitude longer than the time scale of spikes (Fig. 3A; tens of milliseconds vs. milliseconds).

Calculation of a given LFP area consisted of three steps: removal of the electrical artifact from each recorded LFP trace, averaging the LFP traces across trials, and calculation of the LFP area from the averaged curve by determining the onset, the negative peak, and the end of the LFP. The onset of the LFP was the last transition point before the peak, which was the negative inflection point at least 4 ms after the electrical stimulus. Following the peak, the end of the LFP was the zero-crossing (a return to the y-value of the onset point). Thus a horizontal line was created from the baseline just before the peak to the rising portion of the LFP, and the LFP response was obtained from the LFP area just before the peak, which was the negative inflection point at least 4 ms after the electrical stimulus.

An issue with LFP area calculation was that the shape, particularly the start point, was sensitive to irregular trials. The auditory cortex is known to be highly sensitive to preceding activity, with periods of synchronized states exhibiting larger, lower frequency waves (Harris et al. 2011). Strong oscillatory responses have also been observed in ketamine-anesthetized preparations (Eggermont 1992; Kisley and Gerstein 1999; Rennaker et al. 2007) and are strongest in medium anesthesia depths (Kisley and Gerstein 1999). To reduce variability in the LFP area calculation, we removed trials that exhibited large sporadic activity preceding our stimulus-driven activity. A trial was removed if the difference between its slope and that of the average slope for a given 25-ms sliding window was greater than three standard deviations. An average of about 17 trials, with a minimum of 14 trials, was included in the calculation of the LFP area for each stimulation case and level.

**Spiking activity.** Spikes were detected offline using the same online method described above. The electrical artifact was already removed from the signal for each trial during LFP analysis and prior to filtering the signals from 300–3,000 Hz for spike detection. Spikes were summed across a 45-ms window following the onset of the electrical stimulus for all of the same trials included for the LFP analysis. This window was sufficiently long to fully capture the A1 activity elicited by ICC stimulation. The driven spike rate (DSR) per trial was calculated by subtracting the spike rate obtained for this 45-ms window by the spontaneous rate obtained for the 20-ms window preceding the electrical stimulus. Spiking activity in layer V often had putative antidromic activity, which was evident because the spikes exhibited short latencies, low temporal jitter, a sudden increase from no activity to robust spiking per trial with a slight increase in current level above threshold, and isolated activity predominantly in layer V, as characterized in a previous publication (Lim and Anderson 2007a). Due to the difficulty of accurately isolating antidromic spikes from the multunit orthodromic activity, LFP analysis for layer V is included in this paper, while DSR analysis for layer V is not.

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**Fig. 3.** Repeated stimulation of a single site in the ICC (SSS protocol, top half) elicited different response trends in layer III/IV of A1 than stimulating along an isofrequency lamina (DSS protocol, bottom half). In these case examples, the local field potential (LFP) and spiking activity were each recorded in layer III/IV for one stimulation case within one animal in response to one stimulation level. A: LFP and poststimulus time histogram (PSTH) responses to stimuli with IPIs from 0.5 to 10 ms (left to right). Responses to an individual stimulus are also shown, where only the first response was analyzed (see METHODS for further explanation). Calculated LFP areas and driven spike rate (DSRs; B) were then normalized in C by the sum of responses to the individual pulses (IPs) to create IPI curves. The normalization factor 1 is indicated by the dashed line. Cortical responses to the SSS protocol first increase as IPI decreases, and then sharply decrease at delays less than about 2–3 ms. In contrast, responses to DSS continue to increase with shorter IPIs and are even enhanced (>1) for very short IPIs. Electrical artifacts were removed in A. Time is relative to initial stimulus onset.

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Normalization. Responses to individual pulses from each stimulation site were also recorded concurrently with each protocol. Because hardware limitations required two stimuli to be presented, a second pulse was applied after the completion of the response to the first pulse (Fig. 3B, DSS protocol). Layer I/II exhibited the most robust response to the 10-ms IPI, with a response equal to that of the single pulse at an IPI of 0 ms. This indicated that the second pulse did not contribute to the evoked potential already elicited by the first pulse. In contrast, responses to DSS stimulation continued to increase for shorter IPIs, resulting in enhanced cortical activity above a linear sum of the individual responses, or a normalized value of 1.

Using our multisite arrays, we simultaneously recorded from neural populations across layer I/II, layer III/IV, and layer V of A1. As the IPI decreased, the cortical activity increased in all three layers (Fig. 4A). This trend was much greater for layer II compared with the other layers (Fig. 4A). A greater increase is evident in the raw data, for the LFP peak for layer II was about one-half the size of that for layer III/IV at 8 ms IPI, but became closer in size as the IPI decreased down to 0.5 and 0 ms. The normalized curves in Fig. 4B highlight this layer difference, showing stronger activity at shorter delays for the more superficial layers compared with the deeper layers. We also observed greater spiking activity at short delays for layer II compared with layer III/IV (Fig. 5). In the PSTH plots, spiking activity for both layer groups increased for shorter IPIs, even exceeding that of layer III/IV. Layer II exhibited a greater amount of enhancement, with levels of neural activity in layer II/III, layer III/IV, and layer V of a single A1 shank for one stimulation case. While all cortical layers show larger LFP areas with shorter IPIs when stimulating along an ICC lamina (DSS protocol), layer II/III exhibits the greatest amount of enhancement. As the IPI decreased, the cortical activity increased in all three layers in response to the DSS protocol, and the extent of increase was much greater for layer II compared with the other layers (Fig. 4A). This greater increase in cortical activity was evidenced in the raw data, for the LFP peak for layer II was about one-half the size of that for layer III/IV at 8 ms IPI, but became closer in size as the IPI decreased down to 0.5 and 0 ms. The normalized curves in Fig. 4B highlight this layer difference, showing stronger activity at shorter delays for the more superficial layers compared with the deeper layers. We also observed greater spiking activity at short delays for layer II compared with layer III/IV (Fig. 5). In the PSTH plots, spiking activity for both layer groups increased for shorter IPIs, even exceeding that of layer III/IV. While it was not typical to see greater levels of neural activity in the deeper layers, it was common to see a greater increase in normalized activity for layer II, as shown in Fig. 5B.

While Figs. 3–5 highlight responses at specific amplitudes of electrical stimulation, we recorded cortical activity across many stimulation levels to investigate potential trends. Figure 6 shows normalized IPI curves recorded in layer III/IV of A1 in response to ICC stimulation across valid levels (see Methods for criteria). The deepest layers were averaged to calculate the IPI-a curves (black lines) for a single stimulation case in Fig. 6.

**RESULTS**

Typical Responses in Different A1 Layers

Cortical responses to ICC stimulation greatly varied, depending on whether pulses were delivered to one neural population (SSS protocol) or two neural populations (DSS protocol) within the same ICC isofrequency lamina for short delays between the pulses. Figure 3A is a typical example of how cortical activity from one site in layer III/IV varied in response to the SSS or DSS protocols at one stimulation level. As delays decreased from about 10 to 3 ms, LFP areas and DSRs increased for both protocols. However, for IPIs less than about 2–3 ms, the activity decreased for the SSS protocol but continued to increase for the DSS protocol. Responses were quantified (Fig. 3B) and then normalized to the sum of individual responses (Fig. 3C) to show IPI curves. IPI curves show how normalized responses vary with IPI when stimulating at one level for a single stimulation case (i.e., one A1-ICC location pair from one animal). Across levels and layers, we systematically found that responses to the SSS protocol decreased at very low IPIs. This is likely due to refractory effects, for at very short pulse delays the responses equal that of a single pulse, or a normalized value of 0.5. This indicated that the second pulse did not contribute to the evoked potential already elicited by the first pulse. In contrast, responses to DSS stimulation continued to increase for shorter IPIs, resulting in enhanced cortical activity above a linear sum of the individual responses, or a normalized value of 1.

**Layer V**

While Figs. 3–5 highlight responses at specific amplitudes of electrical stimulation, we recorded cortical activity across many stimulation levels to investigate potential trends. Figure 6 shows normalized IPI curves recorded in layer III/IV of A1 in response to ICC stimulation across valid levels (see Methods for criteria). These levels were averaged to calculate the IPI-a curves (black lines) for a single stimulation case in Fig. 6.
While some differences were observed between levels, we did not identify any obvious trends. For both LFPs and DSRs, normalized activity due to DSS generally continued to increase with shorter IPIs, while activity in response to SSS typically decreased at shorter IPIs for all valid levels and stimulation cases across animals. Because the IPI-a curve accurately captured the typical cortical response for each stimulation case, we used the IPI-a curves to compare activity across A1 locations and layers in the following sections.

Responses in Different A1 and ICC Locations

We investigated whether the DSS curves varied between different cortical columns with similar BFs by comparing simultaneously recorded responses within a designated layer. Figure 7 shows three examples of LFP and DSR responses, such that each example (row) corresponds to layer I/II A1 activity simultaneously recorded in response to stimulation at one ICC location. Layer I/II was presented, since we observed the largest enhancement effects compared with those in deeper layers and therefore expect more pronounced differences. Similar trends were observed for the other layers and are not shown. Within each example, the general shape of the IPI-a curves was quite similar across A1 locations, although the magnitude of cortical activity could vary. The shape of the IPI-a curves appeared to exhibit greater differences between ICC locations, although this observation needs to be validated in further experiments.

To investigate differences in cortical activity for DSS across A1 locations, we plotted LFP or DSR activity as a function of medial-to-lateral and caudal-to-rostral cortical location for each of the IPI values and A1 layers across all animals. Figure 8 shows DSR and LFP activity for the 0.5-ms IPI, chosen because responses would be the most enhanced without being affected by current summation occurring at the 0-ms IPI case (see DISCUSSION for further explanation of current summation effects). We did not identify any A1 location trends either for the LFP and DSR data in Fig. 8 or for any other IPI value or A1 layer.

Summary Across Locations and Animals

Because of the similarity in patterns of IPI-a curves, we averaged IPI-a curves across all stimulation cases and animals to create mean IPI-a curves for a given layer, stimulation protocol, and data type (i.e., LFP or DSR) in Fig. 9. The LFP and DSR responses to DSS and SSS stimulation are shown in Fig. 9, A and B, for different cortical layers, confirming the results from the individual cases presented above. As shown in Fig. 9B, similar trends were observed for both the LFP and DSR protocols.
IPI responses to DSS were significantly larger than that at 10-ms IPI. Responses to SSS were also significantly larger for layer I/II compared with the deeper layers for IPIs between 2 and 6 ms for LFPs and 3 and 5 ms for DSRs ($P < 0.05$). These results suggest that coactivation of neurons within an ICC lamina elicits greater enhanced activity along the thalamo-cortical or corticocortical pathway within supragranular layers of A1 compared with the deeper layers.

One relevant question is if this enhancement effect can also be achieved by coactivating neurons across different frequency laminae. Figure 9C shows the mean IPI-a curves when stimulating two sites located 100 μm apart in different frequency regions of the ICC with the Off-BF DSS protocol. Overall, the Off-BF DSS curves looked similar to those for SSS in which there was a slight rise in activity as the IPIs decreased from 10 ms that then started to decrease at about 2–3 ms. However, the curves did not approach a normalized value of 0.5 for short IPIs, as occurred for SSS, possibly because stimulation may not induce complete refraction to the first pulse. The two stimulated sites for Off-BF DSS are far enough apart that non-overlapping and nonactivated neurons can still be activated by the second pulse (i.e., second site). At an IPI of 0 ms, there is a sudden increase in activity, which is likely caused by current summation that simultaneously activates a larger volume of neurons. An important observation from Fig. 9C is that current summation seems to predominantly occur for IPIs less than 0.5 ms. Especially since the sites are spaced much farther apart for DSS than for Off-BF DSS, this further supports that the increase in activity for shorter IPIs for DSS, at least down to 0.5 ms, is caused by a functional interaction between neurons within an ICC lamina rather than an artificial effect of current summation.

It is evident that the Off-BF DSS responses are unlike the DSS in that the curves do not continue to increase with shorter IPIs. This indicates that the same integration mechanism achieved by stimulating different neural populations within an ICC lamina cannot be effectively accessed by stimulating different neural populations across ICC laminae.

For both LFPs and DSRs ($P < 0.05$), the cortical activity at 0-ms IPI was significantly greater than that at the 10-ms IPI in layers I/II and III/IV in response to the Off-BF DSS protocol. Similar to the SSS data, the cortical activity for IPIs at 2–4 ms for LFPs and DSRs ($P < 0.05$) in layers I/II and III/IV was significantly greater than that at the 10-ms IPI. Consistent with SSS and DSS, the activity was also significantly larger in layer I/II than deeper layers for IPIs between 2 and 6 ms for LFPs and DSRs ($P < 0.05$).

**Discussion**

Repeated stimulation of a single ICC neural population induces strong refractory effects in A1 for IPIs shorter than 2–3 ms. In contrast, activation of two different ICC populations within the same lamina elicits increased A1 activity with shorter IPIs from 6 ms down to 0 ms. Increased A1 activity with shorter IPIs did not occur for stimulation of two sites in different ICC laminae, except at the 0-ms IPI associated with
current summation. These results suggest that an enhancement mechanism exists within the ICC-to-A1 pathway that can functionally integrate activation on a fast temporal scale (<6 ms window) from different neural populations mainly originating from the same ICC lamina. Enhanced activity was observed across layers I–V of A1, with the greatest increase in activity within layer I/II.

**Functional Role of the Enhancement Mechanism**

The differences observed between SSS and DSS activation for short IPIs are consistent with previous results presented by Calixto et al. (2012) in which the ICC was stimulated with an AMI array that consisted of much larger sites (126,000 μm² vs. 413 μm²), and only LFPs recorded in layer III/IV of A1 were used for analysis. In general, IPIs below 2–3 ms for SSS exhibit strong refractory effects, perhaps because ICC neurons are unable to be reactivated in such a short period of time (Sivaramakrishnan and Oliver 2006). However, as also reported by Calixto et al. (2012), we did not observe any obvious differences in this refractory-like effect across stimulation levels (e.g., SSS curves in Fig. 6). It would be expected that increasing the current level would activate a larger population of neurons within the ICC. Considering that the ICC consists of an inhomogeneous network of different types of neurons (Oliver 1984, 2005), different levels should activate neurons with varying activation and refractory effects. Because of this inhomogeneity, we expected much greater differences in IPI curves across levels than observed in our study. At IPIs above 2–3 ms, the responses to SSS follow the same general pattern as observed for DSS. This suggests that both SSS and DSS may have access to the same enhancement mechanism, but SS is limited by refractory effects.

We also showed that coactivation of neurons across different frequency regions was not to access this enhancement mechanism, which is consistent with studies showing that specific ICC regions project to and activate specific MGBv and A1 regions in a tonotopic pattern (Lim and Anderson 2006; Malmierca et al. 1997; McMullen et al. 2005). The enhancement mechanism across frequency regions may have been masked by lateral inhibition, for bandwidths in the MGBv and A1 have shown to be less than 0.2 octaves for sharply tuned units (Bartlett et al. 2011). However, excitatory bandwidths have shown to vary from 0.1 octaves to over 1 octave along an isofrequency band of A1 (Schreiner and Mendelson 1990; Schreiner et al. 2000; Schreiner and Sutter 1992), and we would expect this variation to cause large differences in the IPI-a curves across cortical locations. In contrast, we found similar profiles across medial-to-lateral locations along the isofrequency dimension of A1 that were recorded simultaneously in response to stimulation of the same ICC sites (data not shown). Moreover, the similarity in the shape of the IPI-a curves between the Off-BF DSS and SSS further suggests that the integration mechanism requires activation of neurons within multiple locations along an ICC lamina, which was not possible with both of these protocols. Regardless of the role of lateral inhibition, the integration mechanism could not be achieved by stimulating neurons in different ICC lamina.

We propose that the enhancement mechanism identified in this study is designed to integrate activation of different neurons within a given ICC isofrequency lamina and could contribute to the coding of various acoustic features that rely on a population code with short interspike intervals. One possible role of this enhancement mechanism relevant for the AMI is in coding for fast temporal information. Individual ICC neurons...
cannot follow fast temporal patterns beyond hundreds of Hertz (within ~5- to 10-ms range) (Joris et al. 2004; Krishna and Semple 2000). Instead, it seems plausible that populations of neurons along an ICC lamina are designed to code for fast features through a synchrony or spike-interval code that then results in converging activity onto different A1 neurons via the MGBv to elicit varying firing rates. Consistent with this explanation, a previous study showed that individual ICC neurons fire in a sparse and precisely timed pattern to stimuli, rarely fire more than once within a 10-ms window, and are temporally correlated with other neurons mainly within the same lamina and with interspike delays of less than roughly 5 ms (Chen et al. 2012). In other words, coding for sound features within the ICC on a time scale of less than 10 ms should involve neurons within a similar lamina that fire in a correlated pattern. We have identified an integration mechanism from ICC to A1 that can actually process this type of coactivity across multiple ICC neurons with spike intervals less than 6 ms (Fig. 9B) to elicit enhanced activity across A1, especially in supragranular layers.

Psychophysical results in humans further suggest that this enhancement mechanism may be involved with transmitting fast temporal features to higher cortical centers. Both acoustic and CI stimulation studies have identified short-term temporal integration in which presentation of repeated clicks or electrical pulses with shorter interstimulus intervals (within ~5 ms) elicits a louder sensation or lower detection threshold (Flanagan 1961; McKay and McDermott 1998; Vieimeister and Wakefield 1991). Generally, short-term temporal integration can be viewed as a sliding neural window that integrates and tracks the rapid changes in the sound waveform (Forrest and Green 1987; Oxenham and Moore 1994; Vieimeister 1979). The shape and time scale of this perceptual short-term integrator as a function of IPI for acoustic clicks or CI pulses is quite similar to the IPI-a curves presented in Fig. 9B. Therefore, short-term temporal integration may be coded from ICC to A1 by integrating inputs from different neurons along an ICC lamina.

It is important to note that our results are based on electrical stimulation of the ICC, which can induce greater synchronized activation across neurons surrounding the stimulated sites than typically occurs to acoustic stimulation. The neural integration and enhancement effect observed from ICC to A1 in our study needs to be further investigated in acoustic stimulation experiments, especially in identifying its functional role in processing different sound features. Nevertheless, our results demonstrate the existence of a neural mechanism for different neurons along an ICC lamina to interact with each other on a fast time scale and alter activity in A1, which is absent or at least differs from what exists across different ICC laminae.

Potential Neural Mechanisms Underlying Cortical Enhancement

The DSS enhancement effects were unlikely to have been caused by current summation or antidromic activation. Current summation can occur for short IPIs for overlapping current fields, but any charge accumulation on the membranes of overlapping stimulated neurons should dissipate faster than 1 ms based on chronaxie values (Grill et al. 2005; Miocinovic and Grill 2004; Ranck 1975). Moreover, increased activity was not present at the 0.5-ms IPI for the SSS or Off-BF DSS protocols, and thus any increased activity at or above 0.5 ms IPI for the DSS protocol cannot be simply due to current summation. In terms of antidromic activation effects, we observed antidromic spikes in A1 layer V in response to stimulation of 62% of the ICC sites. It is possible that antidromically stimulating the corticothalamic axons in ICC could activate axon collaterals projecting from layer V up to more superficial layers (Winer and Prieto 2001) that would then prime A1 neurons to enhance the activity to a second pulse. However, stimulating 38% of ICC sites did not elicit any noticeable antidromic activity across A1 at levels at which orthodromic activity was prevalent, yet we still observed large increased or enhanced responses with almost every DSS case. Moreover, the cortical activity recorded across layers I-V in response to DSS was significantly larger when antidromic activity was not detected compared with when it was detected for IPIs between 0.5 and 1.5 ms for LFP (P < 0.03) and 0.5 and 1 ms for DSR (P < 0.02), with other IPIs not showing statistical differences. If antidromic activity was the primary cause of the increased cortical activity, we would expect that the activity would have been greater rather than smaller when antidromic activity was detected. Therefore, it is unlikely that antidromic activation is the primary cause of the enhancement effect.

The enhanced activity in A1 can be observed in layer III/IV LFPs, which reflect the synaptic input from MGBv, and layer III/IV DSRs, giving evidence that the tectothalamic, thalamocortical, or corticocortical pathways may be involved individually or in concert to integrate responses from an ICC lamina. In the tectothalamic pathway, ICC neurons diverge and synapse onto a larger span of isofrequency thalamic neurons, and a large span of ICC neurons converge onto the same thalamic neurons with excitatory synapses (Bartlett and Smith 1999; Cetas et al. 2003; Lee and Sherman 2010; McMullen et al. 2005). Therefore, stimulation of two different ICC populations with shorter intervals could sufficiently activate overlapping MGBv populations and transmit increased excitation to A1 (Broicher et al. 2010; Cruikshank et al. 2002). This increased excitation could be caused by several mechanisms within the thalamocortical pathway. A greater number of MGBv neurons could be activated that then converge onto and elicit enhanced activity in the same A1 neurons. It is also possible that the same MGBv neurons are repeatedly activated due to converging inputs from different ICC neurons, causing the thalamic neurons to fire in a burst mode (Sherman 2001; Swadlow and Gusev 2001). This high-frequency firing could elicit enhanced activity in the same A1 neurons. At least in the visual system, it has been shown that two thalamocortical neurons activated within ~7 ms window can more robustly elicit a cortical response (i.e., a supralinear effect that is stronger for shorter interspike intervals) compared with that of a single thalamocortical neuron (Usrey et al. 2000). Repeated activation of the same thalamocortical neuron can also elicit enhanced cortical activity but with a slower interspike interval scale (a window of ~15 ms). If similar synaptic effects exist between the visual and auditory systems and considering the similarity in the window time scale of ~7 ms with our results of 6 ms, the enhanced activity in layers III/IV of A1 could be largely attributed to activation of different MGBv neurons caused by DSS, which is not possible with SSS. Moreover, in the auditory system of ketamine-anesthetized guinea pigs, most MGBv neurons re-
spond with single or double spikes, while nonlemniscal thalamic neurons typically respond with bursting patterns (He and Hu 2002; Hu 1995). Further evidence that the enhancement effect may be associated with the thalamocortical pathway is provided by studies that have performed intracellular recordings in A1 neurons and revealed a strong inhibitory component that quenches preceding excitatory inputs within about 4 ms (Tan and Wehr 2009; Wehr and Zador 2003), which is consistent with the time scale of our 6-ms enhancement window. Perhaps the same temporal sequence for thalamocortical transmission in which cortical neurons receive excitatory inputs, followed by inhibitory inputs, is also active in narrowing the window for the integration in our study.

We observed the greatest enhancement in supragranular layers of A1, which may be due to lemniscal or non-lemniscal thalamocortical projections, as well as corticocortical projections, as proposed in Fig. 10. Within lemniscal projections, supragranular layers could integrate responses across granular layers or be primed by tonotopic thalamocortical projections, which primarily target layer III/IV, but also have collaterals that extend to layer I (branching arrow in pathway 1, Fig. 10) (Cetas et al. 1999; Huang and Winer 2000; Mitani et al. 1985). These collaterals are thought to synchronize temporal feedback and feedforward processes between corticocortical and corticofugal circuits (Huang and Winer 2000; Lee 2012; Winer et al. 2005). Non-lemniscal projections could also prime supragranular layers (pathway 2, Fig. 10), in which the earliest thalamic signals may arrive at the cortex in layer I via giant axons that predominantly originate in the medial division of the MGB (Huang and Winer 2000; Lee 2012). Finally, the horizontal projections in supragranular layers (Winer 2010) may contribute to lateral inhibition even at long ranges (Moeller et al. 2010) that could alter spike timing and integration of the neurons (pathway 3, Fig. 10). In addition, supragranular layers may utilize several pathways, for layer II is thought to act as a hub which integrates responses from layer I and layer III cells before sending information to layer V and VI cells (Mitani et al. 1985). In other words, supragranular layers may be responsible for integrating thalamocortical and corticocortical information before sending it to corticofugal projections.

Anesthesia Effects

Ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, is known to alter cortical activity by reducing both NMDA receptor currents and postsynaptic potential components mediated by non-NMDA receptors (Leong et al. 2004; Orser et al. 1997). In addition to reducing cortical spontaneous activity (Syka et al. 2005; Zurita et al. 1994), ketamine typically inhibits sound-evoked activity in the auditory cortex, although this effect is not uniform and ketamine may occasionally increase or alter the shape of the response (Syka et al. 2005; Wang et al. 2005; Zurita et al. 1994). In addition, ketamine can affect the temporal processing of the cortex to the response to electrical stimulation of the cochlea (Kirby and Middlebrooks 2012). Ketamine can also alter neural activity within the auditory thalamus with minimal effects within the ICC (Huetz et al. 2009; Suta et al. 2003; Ter-Mikaelian et al. 2007). In general, ketamine is thought to be less suppressive than other anesthetics, particularly pentobarbital (Astl et al. 1996; Wehr and Zador 2005).

In the SSS protocol, it is unlikely that ketamine is responsible for the strong refractory effects observed for IPIs less than 2–3 ms, since similar effects have been observed in AMI patients in which perceptual thresholds were measured for two electrical stimuli with varying IPIs (McKay et al. 2013). It is also unlikely that ketamine caused the enhancement effects observed for DSS, since it was not observed for SSS and Off-BF DSS under the same anesthetic conditions. However, the effects of ketamine could have reduced the amount of enhancement or altered the shape of the IPI curves.

In addition to increasing cortical inhibition, ketamine is known for inducing strong oscillatory responses (Eggermont 1992; Kisley and Gerstein 1999; Rennaker et al. 2007) and causing greater variability in sound-evoked responses (Kisley and Gerstein 1999). To minimize the impact of these bursts, we removed sweeps with aberrant activity. However, the increased variability in cortical responses may still have confounded some of our location results, and perhaps different cortical locations may prove to show differences in IPI-a curves in an awake preparation.

Future Work and Clinical Relevance

The presented results are relevant for improving stimulation paradigms for the AMI. In particular, to circumvent the refractory effects caused by rapid and repeated stimulation of one ICC site and to access the enhancement mechanism, multiple neural populations within an ICC lamina need to be stimulated. For safety reasons, an AMI array with only two shanks is initially being developed for human implantation, which will still allow differential activation not only across frequency laminae, but also along an iso-frequency lamina using current steering approaches across the shanks. We are continuing to investigate if different stimulation locations along an ICC lamina elicit different DSS IPI-a curves that could code for and transmit different types of acoustic information, especially temporal features important for speech perception. We are also investigating how stimulating three or more sites affects the

![Fig. 10. Pathways that may contribute to the enhanced activity within supragranular layers in response to coactivation of ICC neurons. Initial enhancement may occur via the lemniscal pathway (pathway 1). The ventral division of the medial geniculate body (MGBv) could integrate responses from different neurons along an ICC lamina and then project to layer I/II in A1 through layer III/IV and/or through direct feedforward mechanisms (Cetas et al. 1999; Huang and Winer 2000; Mitani et al. 1985). The feedforward axon collaterals originating from layer III/IV may prime responses in layer I/II. The supragranular layers could also be integrating converging responses from layer III/IV. In addition, layer I/II could be primed by giant axons from the medial geniculate body (MGB) (pathway 2) (Huang and Winer 2000; Lee 2012; Winer et al. 2005), or via horizontal connections across supragranular layers (pathway 3) (Lee 2012; Winer 2010; Winer 1985).](http://jn.physiology.org/doi/10.1152/jn.00022.2013)
enhanced activity in A1, which might further reveal a synchrony and/or spike-interval code along an ICC lamina that converges in A1.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.S. and H.H.L. performed experiments; M.S. analyzed data; M.S. and H.H.L. interpreted results of experiments; M.S. and D.S. prepared figures; M.S. drafted manuscript; M.S. and H.H.L. edited and revised manuscript; M.S., D.S., and H.H.L. approved final version of manuscript; H.H.L. conception and design of research.

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