Tonotopic and localized pathways from primary auditory cortex to the central nucleus of the inferior colliculus

Craig D. Markovitz1, 4*, Tien T. Tang1 and Hubert H. Lim1, 2, 3

1 Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN, USA
2 Department of Otolaryngology, University of Minnesota, Minneapolis, MN, USA
3 Institute for Translational Neuroscience, University of Minnesota, Minneapolis, MN, USA
4 Correspondence: cdm.markovitz@gmail.com

INTRODUCTION

Physiological studies have demonstrated the role of corticofugal projections for various forms of auditory plasticity. For instance, descending pathways can alter midbrain coding for sound localization (Nakamoto et al., 2008; Bajo et al., 2010) and frequency (Zhang et al., 2005; Suga, 2008). A large extent of research on corticofugal effects on auditory plasticity has focused on the interactions between primary auditory cortex (A1) and the central nucleus of the inferior colliculus (CNIC; Xiong et al., 2009), the main ascending and tonotopic region of the auditory midbrain. In particular, activation of A1 neurons most sensitive to a specific frequency can shift CNIC neurons to become more responsive to that frequency. This can be achieved through repetitive A1 stimulation combined with pure tone stimulation (Yan and Suga, 1998; Yan et al., 2005), by pairing A1 stimulation with activation of the nucleus basalis or other neuromodulatory pathways (Ma and Suga, 2003; Zhang et al., 2005), or using fear conditioning paradigms (Gao and Suga, 1998, 2000; Ji et al., 2001). Furthermore, inactivation of A1 has shown to prevent or limit frequency shifts in the CNIC (B et al., 2001; Zhang et al., 2005), further signifying the substantial role of the corticofugal system in inducing subcortical auditory plasticity.

The ability to induce fine frequency plasticity within the CNIC through activation of A1 descending pathways argues for the existence of a well-defined tonotopic corticocerebellar organization. However, based on anatomical studies, descending cortical projections from layer V (and layer VI to a lesser extent; Schofield, 2009; Bajo and King, 2013) of A1 terminate predominantly in non-lemniscal midbrain regions, including the dorsal (DNIC) and external (ENIC) nuclei of the inferior colliculus (IC), which correspond to poor or non-existent tonotopy (Airkin et al., 1975; Faye-Lund, 1985; Huffman and Henson, 1990; Herbert et al., 1991; Winer et al., 1998; Winer, 2006; Malmierca and Ryugo, 2011). Traditionally, it was thought that there were no or minimal corticofugal projections to the CNIC. However, there has been increasing anatomical evidence that there are a reasonable number of projections from A1 to CNIC that are topographically organized (Andersen et al., 1980; Feliciano and Potashner, 1995; Saldana et al., 1996; Bajo and Moore, 2005; Coomes et al., 2005; Bajo et al., 2007; Xiong et al., 2009; Malmierca and Ryugo, 2011). One study using electrical stimulation of the CNIC and recording the antidromically activated neurons within A1 in guinea pig confirmed that the corticofugal projections to CNIC are precisely tonotopically organized in which A1 neurons only project to CNIC neurons within a similar frequency region (Lim and Andersen, 2007a). Considering that the corticocellular projections are glutamatergic (Feliciano and Potashner, 1995), these findings across studies provide one way in which the corticofugal projections can potentially elicit excitatory and tonotopic effects within the CNIC and contribute to the fine frequency plasticity shown in previous studies. However, questions remain as to how this descending activation can cause neurons located in neighboring frequency regions of the CNIC to shift their tuning toward the frequency of the stimulated A1 neuron if the corticofugal projections are organized in a point-to-point tonotopic pattern. In addition, most of the corticocellular neurons project to non-lemniscal midbrain regions with poor or non-existent tonotopy, which in turn can activate neurons across CNIC (Huffman and Henson, 1990; Jin et al., 2001). Thus, it is unknown from these previous studies if the descending neurons from A1 can actually...
elicit an excitatory and tonotopic activation pattern within the CNIC.

There have been several studies showing the effects of A1 electrical stimulation on neural firing in the IC in bats (Sun et al., 1989; Yan and Suga, 1996; Zhang and Suga, 1997, 2000; Jen et al., 1998, 2001) and, to a lesser extent, in cats (Massopust and Ordy, 1962; Mitani et al., 1983), rats (Suya and Popelar, 1984), mice (Sun and Ehret, 2001, 2002; Yan et al., 2005), and guinea pigs (Torterolo et al., 1998). These studies have demonstrated that cortical activation can result in excitatory and/or inhibitory effects within the IC. However, these studies either looked at residual effects (i.e., changes in tuning or responses to acoustic stimuli after electrical stimulation had ceased) or were not designed to systematically investigate the cortically induced activation patterns along the tonotopic and isofrequency dimensions of the CNIC. Based on one previous study in guinea pigs (Bledsoe et al., 2003), there appears to exist differences in excitatory and inhibitory patterns across the CNIC, but it not yet clear how these differences vary along and across the frequency laminae. Therefore, in this study, we investigated if electrical stimulation of A1 could induce responses systematically across the tonotopic axis of the CNIC, exciting not only neurons sensitive to the same frequency but also those in neighboring frequency regions that could enable subcortical shifts in frequency tuning. We also investigated if there was any spatial organization of A1 descending pathways along the isofrequency laminae of the CNIC by creating three-dimensional histological reconstructions of the midbrain.

MATERIALS AND METHODS

ANIMAL SURGERIES AND ELECTRODE IMPLANTATION

Experiments were performed on 20 young Hartley guinea pigs (295–410 g, Elm Hill Breeding Labs, Chelmsford, MA, USA) in accordance with policies of the University of Minnesota Institutional Animal Care and Use Committee. Each animal was anesthetized with an intramuscular mixture of ketamine (40 mg/kg) and xylazine (10 mg/kg) with 0.1 mL supplements every 45–60 min to maintain an areflexive state. Atropine sulfate (0.05 mg/kg) was administered periodically to reduce mucous secretions in the airway. Heart rate and blood oxygenation were continuously monitored via a pulse oximeter and body temperature was maintained at 38.0 ± 0.5°C using a heating blanket and rectal thermometer.

After the animals were fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) and a craniotomy was performed to expose the right auditory and visual cortices, two X-site electrode arrays (NeuroNexus Technologies, Ann Arbor, MI, USA) were inserted via hydraulic micro-manipulators into the right A1 and CNIC. The A1 array consists of four 5 mm long shanks separated by 500 μm with 16 iridium sites linearly spaced 200 μm (center-to-center) along each shank. The array was inserted 45° off the sagittal plane through the occipital cortex into the CNIC to align it along the tonotopic gradient of the CNIC (Snyder et al., 2004; Lim and Anderson, 2006). CNIC site impedances ranged between 0.8 and 3.0 MΩ. After placement of the probes, the brain was covered with agarose to reduce swelling, pulsations, and drying during the recording sessions.

RECORDING AND STIMULATION

Experiments were performed within a sound attenuating, electrically shielded room using custom software and TDT hardware (Tucker-Davis Technology, Alachua, FL, USA). All acoustic stimulation was presented to the animal’s left ear canal via a speaker coupled to a custom-made hollow ear bar. The speaker-car bar system was calibrated using a 0.25” condenser microphone (ACO Pacific, Belmont, CA, USA).

Multi-unit neural data was recorded and sampled at a rate of 25 kHz, passed through analog DC-blocking and anti-aliasing filters up to 7.5 kHz, and digitally filtered between 0.5 and 3.0 kHz for analysis of neural spikes. Spikes were determined as voltages exceeding 3.5 times the standard deviation of the noise floor.

Electrical stimulation of A1 consisted of single biphasic, charge-balanced pulses (205 μs/phase, cathodic-leading) ranging from 4 to 32 μA in 2 dB steps at a rate of 2/s. All 32 A1 sites were stimulated at each level in a randomized pattern for 20 trials for each stimulus condition. Poststimulus time histograms (PSTHs) of the responses recorded at 32 CNIC sites following A1 stimulation were plotted for further analysis. When excitation was shown in Figure 1. The CNIC array consists of two 10 mm long shanks separated by 500 μm with 16 iridium sites linearly spaced 100 μm along each shank. The array was inserted 45° off the sagittal plane through the occipital cortex into the CNIC to align it along the tonotopic gradient of the CNIC (Snyder et al., 2004; Lim and Anderson, 2006). CNIC site impedances ranged between 0.8 and 3.0 MΩ. After placement of the probes, the brain was covered with agarose to reduce swelling, pulsations, and drying during the recording sessions.
found in the CNIC in response to A1 stimulation, all analyses were performed using the lowest threshold cortical site along a given cortical shank, which was generally located at a depth of approxi-
mately 900–1500 μm and corresponds to layer V in the guinea
pig cortex (Wallace et al., 2001; Lim and Anderson, 2007a). Typi-
cally, one array placement (i.e., four shank placements) was made
in A1 and multiple array placements were made throughout the
CNIC during each experiment. Each CNIC array placement (i.e.,
two shank placements) resulted in sites along each shank that were
aligned along the tonotopic gradient of the CNIC. The CNIC array
was then moved to multiple locations across the laminae during
each experiment. The recording ground wire was positioned in the
neck muscles and the stimulation ground needle was implanted
into the brain tissue near the intersection of the midline and
bregma.

HISTOLOGY AND ELECTRODE SITE RECONSTRUCTIONS

A full explanation of the computer reconstructions of the mid-
brain for identifying the locations of CNIC sites was presented
in a previous publication (Markovitz et al., 2012) and is only
briefly described here. The CNIC array was dipped in a red fluo-
rescent dye (3 mg Di-I per 100 μL aceton; Sigma-Aldrich, St.
Louis, MO, USA) prior to its insertion into the brain. Immediately
following each experiment, the animal was euthanized with an
overdose (0.22 mL/kg) of Beuthanasia-D Special (active ingredi-
ents: pentobarbital sodium (390 mg/mL) and phenytoin sodium
(50 mg/mL); Merck, Summit, NJ, USA) into the heart and decap-
tated. The brain was immersed in 3.7% paraformaldehyde for
approximately 10 days. The midbrain was then block-cut, cryosec-
tioned into 60 μm thick sagittal slices, and fully reconstructed
along with the electrode shank tracks (marked with the red Di-I
stain) using computer software (Rhinoceros, Seattle, WA, USA).
To create computer simulations of isofrequency laminae, the mid-
brains were three-dimensionally normalized to each other based
on the size and orientation of the IC surface across animals, and
the electrode tracks were superimposed within one standard mid-
brain. Three planes were identified perpendicular to the shank
tracks and approximately correspond to low (2.0–3.2 kHz), mid-
dle (3.8–8.0 kHz), and high (10.0–16.0 kHz) frequency laminas.
These laminae were chosen to give us a representative view of the
isofrequency axis of the CNIC and were made to approxi-
mately correspond to two critical bands in thickness (Schreiner
and Langner, 1997; Egorova et al., 2006; Malmierca et al., 2008).
All neurophysiological data corresponding to a given frequency
range was superimposed onto a “pooled” lamina, and the distance
in the caudal-rostral and medial-lateral directions were normal-
ized based on the most proximal site location in each direction.
Though the actual laminae are curved and occupy an orientation
that is somewhere between the medial-lateral and dorsal-ventral
axes, we will use the “medial-lateral” notation for this dimension
since this is what is commonly used in other physiological stud-
ies that have mapped properties across the isofrequency laminae
of the CNIC (Schreiner and Langner, 1988; Ehret, 1997; Langner
et al., 2002; Hage and Ehret, 2003).

Site locations in A1 were identified by imaging the exposed
cortical surface with the inserted array shanks using a microscope-
mounted camera (OPMI 1 FR pro, Zeiss, Dublin, CA). The shank
locations across animals were then normalized based on their rel-
ative distances from the pseudosylvian sulcus, bregma, and the
lateral suture line, as successfully performed in previous stud-
ies (Scherer et al., 2000; Wallace et al., 2000; Eggermont and
Roberts, 2004).

DATA ANALYSIS

Acoustic-driven responses

Acoustic stimuli were presented to the animal’s left ear canal and
acoustic-driven responses were recorded in A1 and the CNIC to
determine the functional location of each electrode site. Pure
tones (50 ms duration, 5 ms ramp/decay) of varying frequen-
cies (0.6–38 kHz, 8 steps/octave) and levels (0–70 dB in 10 dB
steps) were randomly presented (4 trials/parameter). The acoustic-
driven spike rates were calculated for responses recorded in the
CNIC (taken 5–60 ms after tone onset) and A1 (5–20 ms after
tone onset) to create frequency response maps (FRMs) for each
site. Best frequencies (BFs) were calculated from the FRMs as
the frequency centroid at 10 dB above the visually determined
threshold.

To verify the functional placement of our A1 array, FRMs with
approximately equal BFs for each site along a single cortical shank
confirmed that the array was inserted perpendicular to the cor-
tical surface along a cortical column. Across shanks, increasing
BFs along the rostrolateral to caudomedial direction and short
response latencies of approximately 15 ms verified that our array
was within A1, as shown in Figure 1 (Wallace et al., 2000; Lim
and Anderson, 2007b). High frequency (>20 kHz) A1 locations
were generally avoided to prevent confusion with the shared high
frequency border between A1 and the dorsocaudal cortical area
(Wallace et al., 2000). To ensure that we positioned sites fully span-
nning the isofrequency dimension of A1, we initially mapped the
cortical surface at the medial and lateral edges of A1 by recording
and assessing FRMs and acoustic-driven properties that distin-
guish A1 from the non-A1 regions as described in previous studies
(Redies et al., 1989; Wallace et al., 2000; Grimes et al., 2008). The pseu-
dosylvian sulcus (white stars in Figure 1) generally corresponds to
the medial edge along the isofrequency dimension of A1. The lat-
eral edge along the isofrequency dimension of A1 was identified by
observing neural responses that were poorly tuned to pure tones or
had long acoustic-driven latencies for locations beyond that edge.
Array placements within the CNIC were confirmed by observing
FRMs that systematically increased in BF with increasing depth
(Lim and Anderson, 2007b; Markovitz et al., 2012). FRMs for sites
outside of the CNIC in external regions of the IC typically exhib-
ited broad and weak tuning and/or multiple FRM peaks and were
excluded for the analysis in this paper.

Electrical stimulation threshold

The threshold level for CNIC activation in response to A1 stim-
ulation was determined using signal detection theory (Green and
Swets, 1966; Lim and Anderson, 2007b). Spike rate distributions
for a given CNIC site in response to 20 trials of A1 stimulation were
plotted for the “signal” condition (using a 30 ms window starting
4 ms after the electrical artifact) and the “noise” condition (using a
30 ms window before the electrical artifact) on the same axes.
The signal time window was selected based on visual identification of the stimulus-driven activity across all PSTH responses. By adjusting a criterion spike rate level across the signal and noise distributions, the percentage of signal trials exceeding that criterion (correct hits) and that of noise trials (false alarms) were calculated and plotted for varying criterion levels to obtain a receiver operating characteristic (ROC) curve. The area under the ROC curve corresponds to the performance level for an ideal observer detecting a stimulus based on the signal and noise distributions in a two-alternative, forced-choice task. Using the area under the ROC curve for each stimulus level, a neurometric curve was plotted with performance levels ranging from 0.5 (chance) to 1.0 (perfect detection). Activation threshold was defined as the lowest current level that achieves at least a 76% performance level. This performance value was chosen because it sits on the steepest portion of the neurometric curve, making it a robust measure.

First-spike latencies
First-spike latencies for CNIC sites in response to A1 stimulation were calculated from the PSTHs by taking the first time bin to exceed 3.5 standard deviations above the pre-stimulus noise floor, and were visually confirmed to avoid any spurious fluctuations in the PSTHs. All CNIC latencies were determined at a suprathreshold current level of 2 dB above threshold.

For cases with more than one activated site along a CNIC shank in response to stimulation of an A1 site, two groups were used for latency comparison: (1) BF-aligned, consisting of the CNIC site with the closest BF to the stimulated A1 site, and (2) BF-unaligned, consisting of all other CNIC sites along the same shank showing a response. We then directly compared latencies between these two groups after a normalization procedure. We stimulated one site on a given A1 shank and recorded the responses on the sites across the CNIC shank, which we define as an A1–CNIC shank pair. Normalization was performed for each A1–CNIC shank pair in which the shortest latency across all sites along the CNIC shank was labeled as time 0 while the remaining latency values along that same shank were normalized relative to that time. This normalization procedure enabled us to combine latency values across different placements and animals and directly compare those values between the BF-aligned and the BF-unaligned groups. The performance levels ranging from 0.5 (chance) to 1.0 (perfect detection). Activation threshold was defined as the lowest current level that achieves at least a 76% performance level. This performance value was chosen because it sits on the steepest portion of the neurometric curve, making it a robust measure.

RESULTS
CNIC EXCITATION IS INDUCED VIA STIMULATION THROUGHOUT A1
Multi-unit neural activity across the tonotopic axis and along isofrequency laminae of the CNIC was measured in response to focal electrical stimulation (single pulses, 4–32 μA, 205 μs/phase) of deeper output layers of A1 using 32-site electrode arrays. For each experiment, the A1 array (4 shanks, 8 sites/shank) was inserted into one position, while the CNIC array (2 shanks, 16 sites/shank) was inserted into several positions with the shanks aligned along the tonotopic axis of the CNIC, providing an average of 4–5 sites along a given lamina per animal. A total of 2,746 CNIC sites were sampled with BFs ranging from 1.0 to 24.8 kHz.

Focal electrical stimulation of 57 out of 80 locations fully spanning across the isofrequency dimension of A1 elicited activation on at least one site along a CNIC lamina (Figure 1). These data demonstrate that CNIC excitation can be induced via stimulation throughout most of A1.

CORTICOCOLICULAR PATHWAYS ARE TONOTOPIC
Across the 20 experiments, we recorded from 87 CNIC shank positions which, combined with the 80 A1 stimulation locations (i.e., shank locations), resulted in a total of 346 A1–CNIC shank pairs. Of these, we found 88 A1–CNIC shank pairs that exhibited an excitatory activation pattern. When excitation was observed in the CNIC in response to stimulation of an A1 site, there were two highly distinct response patterns that emerged. We observed a narrow tuning (NT) type, in which typically only a single recording site out of 16 along a CNIC shank responded at all levels from threshold up to our maximum current level (Figure 2A). We also observed a broad tuning (BT) type, in which activation of multiple CNIC sites occurred at threshold with neural activity spreading across an increasing number of recording sites in the CNIC as we increased the stimulation level (Figure 2B). A summary of the NT and BT activation patterns across positions and animals is shown in Figure 3. In Figure 3A, only one point along the ordinate (i.e., CNIC site) is plotted for a given location along the abscissa (i.e., A1 site) since the NT pattern did not exhibit activation across more than one site along a CNIC shank. This NT pattern was tonotopic in which the stimulated A1 sites and the activated CNIC sites had similar BFs. The BT pattern was also tonotopically organized. However, the BT pattern consisted of activation across multiple sites along a CNIC shank in which several points along the ordinate are plotted for a given location along the abscissa as shown in Figure 3B. The data in Figure 3 were plotted for a stimulation level of 2 dB above threshold. At this level, the BT pattern typically exhibited activity across 3–6 CNIC sites (frequency span – mean: 0.71, SD: 0.48 octaves).

NEIGHBORING CNIC FREQUENCY REGIONS ARE ACTIVATED BY A1 STIMULATION
For the BT response pattern, A1 stimulation activated several CNIC sites that had BFs different from the BF of the stimulated A1 sites. In a previous study in guinea pig that stimulated the CNIC and recorded the antidromically activated spikes within A1 (Lim and Anderson, 2007a), it was shown that A1 neurons only project to CNIC neurons with a similar BF. This monosynaptic projection from A1 to CNIC could explain the BF-aligned activation observed for both the NT and BT patterns. However, it cannot explain the BF-unaligned sites activated for the BT pattern.

To gain further insight into these different activation patterns, we analyzed the first-spike latencies of CNIC responses to A1 stimulation. Comparing the first-spike latencies for the BF-aligned NT pattern (mean: 8.1, SD: 2.0, range: 5–12 ms) with only the BF-aligned sites for the BT pattern (mean: 7.2, SD: 1.5, range: 4–10 ms) resulted in no statistical difference (p = 0.091). These latencies were consistent with the published antidromic latencies of 2–10 ms (Lim and Anderson, 2007a) when accounting for the additional synaptic delay within the CNIC to record the postsynaptic spikes.
The latencies, normalized to the fastest projection for each latencies between the BF-aligned and BF-unaligned sites for the BT projections from A1 to CNIC. We next compared the first-spike of sites as the stimulation level was increased. For the NT and BT patterns could be elicited through the monosynaptic part of the CNIC may serve a more modulatory role through information through the lemniscal pathway in different ways. In particular, the caudomedial portion compared to the rostrolateral (0.0–5.8%) portion of the CNIC (25.3–39.2%) of sites in the caudomedial portion less of the stimulated site location across the isofrequency dimension of A1. We did not observe any obvious differences in the location of NT (triangles) or BT (squares) activation across each of the laminae, and thus combined those data together for further analysis. From all sites superimposed onto a lamina, we calculated the percentage of those sites that showed excitation (either NT of BT types) in the caudomedial and rostrolateral portions of each lamina. We found that 25.3–39.2% of sites in the caudomedial portion of the CNIC laminae exhibited excitation, while only 2.2–6.5% of sites in the rostrolateral portion were activated.

The caudomedial activation pattern was also consistent regardless of the stimulated site location across the isofrequency dimension of A1. Figure 5A shows how we divided A1 into three regions corresponding to different locations along the isofrequency dimension of A1. We then calculated the percentages of sites that elicited excitation in the caudomedial versus the rostrolateral portion along the three laminae assessed in our study. Regardless of the A1 region, there was always a higher percentage of sites causing excitation in the caudomedial (22.1–44.6%) versus the rostrolateral (0.0–5.8%) portion of the CNIC (Figure 5B).

These findings suggest the existence of two subregions along the isofrequency dimension of the CNIC that may process sound information through the lemniscal pathway in different ways. In particular, the caudomedial portion compared to the rostrolateral portion of the CNIC may serve a more modulatory role through descending activation from the auditory cortex, involving neurons located throughout A1.
DISCUSSION

Our results indicate that focal cortical stimulation can induce excitatory responses in the CNIC, in accord with previous studies indicating an excitatory corticocollicular pathway (Feliciano and Potashner, 1995; Zhang and Suga, 1997; Totorolo et al., 1998; Yan and Suga, 1999). These responses can be elicited via stimulation throughout A1, in agreement with studies demonstrating that corticocollicular projections originate across A1 (Bajo and Moore, 2005; Coomes et al., 2005; Schofield, 2009). Also, the descending excitatory pathway, like the ascending lemniscal auditory system (Lorente De Nó, 1981; Malmierca, 2003), is arranged tonotopically and can influence neighboring frequency regions. This organization may provide a potential mechanism for enabling the subcortical frequency plasticity that has been extensively shown in previous studies (Suga, 2008; Xiong et al., 2009; Bajo and King, 2013).

TECHNICAL LIMITATIONS

The use of electrophysiology and invasive brain stimulation has several inherent limitations that need to be discussed for interpreting our results. First, our electrode arrays only allowed us to stimulate and record from a few discrete locations in A1 and the CNIC at any given time. Therefore, several of the values and percentages described in the results could be underestimations of true physiological values. For example, when we stated that 57 out of 80 A1 locations resulted in CNIC excitation, it is possible that a larger proportion of A1 sites would have caused excitation in the CNIC had we been able to more fully map the CNIC for each cortical location. We were only able to record from a few locations (4–5 per animal on average) along a lamina within the CNIC for each cortical location. Similarly, the percentages shown in Figures 4 and 5 could have been higher if we had been able to sample from a larger number of A1 and CNIC locations.

Second, electrical stimulation can cause complex functional effects by activating a combination of cell bodies and passing fibers (Ranck, 1975; McIntyre and Grill, 2000; McIntyre et al., 2004), especially in highly interconnected regions such as the cortex. We attempted to mitigate these effects by analyzing stimulation levels at or close to activation threshold to limit current spreading across A1. It was typical for activation of CNIC sites to be induced by stimulation of multiple sites along a cortical shank. At our highest stimulation level of 52 μA and using a similar stimulation wave-form, current spreading from a stimulated site within brain tissue has shown to activate neurons at an average distance of approximately 100–150 μm (Ranck, 1975; McIntyre and Grill, 2000). Since our sites along a cortical shank were spaced at 200 μm, current spreading may have caused different cortical sites along a shank to activate overlapping neural populations. Also, layer V pyramidal cells, which are the neurons providing the majority of descending projections to the midbrain (Schofield, 2009), have extensive connections along a cortical column that were likely activated by our stimulation (Winer and Prieto, 2001). Therefore, our analysis focused on the cortical site inducing the lowest activation threshold in the CNIC and avoided making comparisons of activation patterns for stimulation of multiple sites along a single cortical shank. The four cortical shanks on each probe, on the other hand, were spaced 500 μm apart and stimulation of sites on different shanks were expected to activate distinct neural populations.

Third, our electrophysiological setup does not allow us to make claims regarding whether the cortically driven excitation in the CNIC was the result of direct or indirect pathways. However, we attempt to describe the potential neural pathways below and, based on our results and previous studies, postulate the likely sources of this cortically driven excitation in the CNIC.}

TONOTOPIC ACTIVATION OF CNIC NEURONS

Our results indicate that corticocollicular excitatory pathways are arranged in a tonotopic manner. This agrees with several
anatomical studies which, when compared with previously published frequency maps within the CNIC, have provided evidence for direct corticofugal projections from A1 to the CNIC that are topographically arranged (Anderson et al., 1980; Feliciano et al., 1975; Syka et al., 2000), it is possible that some underlying topographic descending organization exists that could enable frequency-specific activation of neighboring frequency laminae in the CNIC (Saldana et al., 1996). A study in bats showed that cortical activation could modulate activity within the CNIC via the ENIC, though this study did not investigate the tonotopic effects (Jen et al., 2001). (2) Another possibility is that A1 stimulation may activate other nuclei along the auditory pathway that then project to the CNIC. For instance, it is known that electrical stimulation of the auditory cortex can alter coding properties across different frequency regions in the ipsilateral cochlear nucleus (Luo et al., 2008; Liu et al., 2010) and that cortical projections synapse on neurons in the cochlear nucleus that then project to the IC (Schofield and Coomes, 2015). Furthermore, another study showed that two pathways exist from the cochlear nucleus to the IC, a narrow one and a wide one (Malmierca et al., 2003), which may be analogous to the NT and BT patterns described in this study. (3) It is also possible that A1 stimulation activates local cortical interconnections between different frequency regions that then project to the CNIC (Winer and Prieto, 2001) that could contribute to the BT pattern. (4) Based on our data, we suggest that intrinsic projections within the CNIC connecting different isofrequency laminae, as previously described by Malmierca et al. (1995), could explain our BT results. This organization would allow A1 stimulation to activate BF-aligned CNIC neurons that could then activate or modulate neurons within neighboring and even distant frequency regions. The longer latencies observed for the BF-aligned versus the BF-unaligned sites in the BT pattern, on the other hand, differ from the antidromic data and likely arise from polysynaptic pathways (Winer and Prieto, 2001) that could contribute to the BT pattern. (2) Another possibility is that A1 stimulation may activate other nuclei along the auditory pathway that then project to the CNIC. For instance, it is known that electrical stimulation of the auditory cortex can alter coding properties across different frequency regions in the ipsilateral cochlear nucleus (Luo et al., 2008; Liu et al., 2010) and that cortical projections synapse on neurons in the cochlear nucleus that then project to the IC (Schofield and Coomes, 2015). Furthermore, another study showed that two pathways exist from the cochlear nucleus to the IC, a narrow one and a wide one (Malmierca et al., 2003), which may be analogous to the NT and BT patterns described in this study. (3) It is also possible that A1 stimulation activates local cortical interconnections between different frequency regions that then project to the CNIC (Winer and Prieto, 2001) that could contribute to the BT pattern. (4) Based on our data, we suggest that intrinsic projections within the CNIC connecting different isofrequency laminae, as previously described by Malmierca et al. (1995), could explain our BT results. This organization would allow A1 stimulation to activate BF-aligned CNIC neurons that could then activate or modulate neurons within neighboring and even distant frequency regions. The longer latencies observed for the BF-aligned versus the BF-unaligned sites in the BT pattern and the broad but systematic tonotopic pattern observed for the BT pathways are consistent with
this proposed descending organization. It is important to note that although the NT pattern consisted of only a single activated BF-aligned site in CNIC, there could also be local projections to neighboring frequency regions that are inhibitory, and thus prevents activation across the tonotopic gradient of the CNIC (Oliver et al., 1994).

ASCENDING AND DESCENDING LEMNISCAL PATHWAYS

In a previous study in the guinea pig, electrical stimulation of the rostroventral portion (or equivalently the rostrotoral portion) along a CNIC lamina achieved lower thresholds, smaller discriminable level steps, larger evoked potentials, and shorter first-spike latencies in A1 than stimulation of the caudodorsal (or caudomedial) portion (Lim and Anderson, 2007b). Based on those results, the authors suggested that there might exist at least two functional subregions along a given isofrequency lamina of the CNIC that projects in different ways up to the auditory cortex. Interestingly, there are several anatomical and functional studies across species that are consistent with this proposed sub-projection lemniscal pathway. In gerbil, it was shown that brainstem nuclei project in different ways to the caudomedial versus rostrolateral CNIC (Cant and Benson, 2006). In particular, the lateral lemniscus and cochlear nucleus project throughout the CNIC whereas the superior olivary nucleus projects predominantly to the rostrotoral region of the CNIC. The rostrotoral CNIC in gerbil was also shown to project predominantly to the rostral portion of the ventral division of the medial geniculate body (MGIV; approximately along the isofrequency dimension) whereas the caudomedial CNIC projects predominantly to the caudal portion of the MGII (Cant and Benson, 2007). Both in cat and rat, it was shown that the rostral MGIV projects throughout auditory cortex, including A1, but caudal MGIV projects predominantly to regions outside of A1 [e.g., ventral auditory field in cat or posterior auditory field in cat appear to receive more projections from the caudal than the rostral MGII (Mortel and Imig, 1987; Rodrigues-Dagaeff et al., 1988; Storace et al., 2010)]. There is also a functional study showing that in the thalamus of cats in response to acoustic stimulation, neurons in the rostral portion of the MGIV (approximately along the isofrequency dimension) have more precise tonotopy and sharper tuning, are more time-locked, and have shorter latencies than the caudal portion (Rodrigues-Dagaeff et al., 1989). Therefore, based on these results across species, there appears to be two segregated pathways that exist along the ascending lemniscal pathway from the CNIC up to the auditory cortex.

In the current study, we observed that stimulation of A1 resulted in activation predominantly in the caudomedial portion along the isofrequency lamina of the CNIC. Based on the ascending lemniscal organization described above, it is possible that the caudomedial pathway may serve a more modulatory role in the processing of ascending acoustic information while the rostrotoral pathway is involved with robust transmission of acoustic information to A1. It is important to note that different reconstruction techniques were performed across the studies described above, and thus further studies are needed to confirm if the caudomedial versus rostrolateral CNIC regions identified in this study are the same regions identified across those other studies and species (and consistent with the caudal versus rostral pathways through MGIV). Also, different frequency regions were investigated across studies [e.g., 2–16 kHz in this study versus 9–23 kHz in (Lim and Anderson, 2007b)]. However, the consistency in results observed across species in terms of this proposed sub-projection lemniscal organization raises the possibility that it may be a general feature of the mammalian brain. It is also important to note that previous studies in multiple species have shown a differential pattern of excitation and inhibition in various locations within the CNIC in response to cortical stimulation (Mittani et al., 1983; Syka and Popelar, 1984; Hid desperate et al., 2003). However, these studies did not reconstruct or identify their stimulation and recording sites along the frequency and isofrequency dimensions of the CNIC and A1, and thus further studies across species are still needed to confirm that the caudomedial activation pattern in the CNIC identified in our study is a general feature of the mammalian brain. In addition, the cortically induced suppressive effects across the frequency and isofrequency dimensions of the CNIC also need to be investigated.

**IMPLICATIONS FOR FREQUENCY PLASTICITY**

Our findings provide functional evidence for a precise tonotopic organization within the lemniscal corticothalamic system that could...
enable the fine frequency plasticity shown in the CNIC in previous neurophysiological studies (Xiong et al., 2009). Both the NT and BT response patterns show excitation of BF-aligned neurons, consistent with direct corticocollicular projections being glutamatergic (Feliciano and Potashner, 1995) and tonotopic (Saldana et al., 1996; Bajo and Moore, 2005; Lim and Anderson, 2007a). However, A1 neurons are not limited to interact with the neurons in different frequency regions of the CNIC. In this way, activation of A1 neurons tuned to a specific frequency could cause BF-unaligned CNIC neurons to become more sensitive to that frequency. Other inhibitory pathways into and within the CNIC (Jen et al., 2001; Kelly and Caspary, 2005; Pollak et al., 2011) would likely be involved in altering the tuning selectivity of BF-unaligned CNIC neurons in response to A1 activation. As proposed by several studies (Schofield, 2010; Hormigo et al., 2012; Hurley and Sullivan, 2012), cholinergic, serotonergic, or noradrenergic input from the pontomesencephalic tegmentum, raphe nuclei, or locus coeruleus, respectively, provide neuromodulatory reinforcement directly into the CNIC or indirectly through non-lemniscal midbrain pathways. These different pathways could in turn sustain the spectral changes induced by lemniscal corticalfugal activation. Together, these findings provide an initial functional framework for further investigating how modulation and plasticity of different sound features can occur within the central auditory system through spatially organized interactions among the ascending, descending, and neuromodulatory networks (Winner, 2006; Suga, 2008; Xiong et al., 2019).

ACKNOWLEDGMENTS

This work was supported by NIH NIDCD R03-DC011589, NIH NIDA T32-DA022616, the University of Minnesota Institute for Engineering in Medicine Walter Barnes Lange Memorial Award, the University of Minnesota Frida Martha Kunze Fellowship, and start-up funds from the University of Minnesota (Institute for Translational Neuroscience and the College of Science and Engineering). We would like to thank Jessica Pohl, Patrick Hogan, and Kyle Weisen for assistance with the three-dimensional midbrain reconstructions.

REFERENCES

Ahles, L. M., Webster, W. R., Voie, J. L., and Crusby, D. C. (1975). Inferior colliculus. I. Comparison of response properties of neurons in central, peri-central, and external nuclei of adult cat. J. Neurophysiol. 38, 1196–1207.
Andersen, R. A., Snyder, R. L., and Merzenich, M. M. (1980). The topographic organization of corticocollicular projections from phonologically identified loci in the A1, Ai, and anterior auditory cortical fields of the cat. J. Comp. Neurol. 191, 478–494.
Bajo, V. M., and King, A. J. (2007). Corticocollicular pathways: a possible role for frequency processing in the midbrain. Front. Neural Circuits 1, 614. doi: 10.3389/neuro.17.011.07
Bajo, V. M., and Moore, D. R. (2005). Descending projections from the auditory cortex to the inferior colliculus in the gelatin. Movements organelles of the auditory corticocollicular projections. J. Comp. Neurol. 495, 511–528.
Catt, N. R., and Benzon, C. G. (2007). Multiple topographically organized projections connect the central nucleus of the inferior colliculus to the ventral division of the medial geniculate nucleus in the cat. Movements organelles of the auditory corticocollicular projections. J. Comp. Neurol. 503, 452–463.
Costopoulos, D. L., Schofield, R. M., and Schofield, B. R. (2005). Unilateral and bilateral projections from cortical cells to the inferior colliculus in the cat. J. Comp. Neurol. 491, 562–720.
Eggermont, J. J., and Roberts, L. E. (2004). The neuroanatomy of tonotopy. Trends Neurosci. 27, 676–682.
Eggermont, M., Vietarianti, L., and Ebert, G. (2008). Frequency response areas of mouse inferior colliculus neurons II: Central bands. Neuroreport 19, 1471–1476.
Ebert, G. (1997). “The auditory midbrain, a “shunting yard” of acoustic information processing,” in The Central Auditory System, eds G. Ebert and R. Romand (New York: Oxford University Press, Inc.), 259–316.
Fury-Land, H. (1985). The nontonotopic projections to the inferior colliculus in the albino rat. Annu. Embryol. (Berl.) 175, 53–70.
Feliciano, M., and Potashner, S. I. (1995). Evidence for a glutamatergic pathway from the guinea pig auditory cortex to the inferior colliculus. J. Neurosci. 15, 1386–1397.
Gao, E., and Suga, N. (2008). Experience-dependent plasticity in the auditory cortex and the inferior colliculus of bat: role of the corticofugal system. Proc. Natl. Acad. Sci. U.S.A. 97, 8081–8086.
Green, D., and Swets, J. (1966). Signal Detection Theory and Psychophysics. New York: Wiley.
Gr anskey, J. M. S. (2008). Electrophysiological Response Characteristics of Guinea Pig Auditory Cortex in Simple Normal and Compensatory Communication Cats. Ph.D. thesis, University of Nottingham, Nottingham.
Hage, S. R., and Ebert, G. (2003). Mapping responses to frequency sweeps and tones in the inferior colliculus of house mice. Eur. J. Neurosci. 18, 2291–2312.
Herbert, H., Aschoff, A., and Oswald, J. A. (1991). Topography of projections from the auditory cortex to the inferior colliculus in the rat. J. Comp. Neurol. 304, 103–122.
Hormigo, S., Horta Junior Iac, A., Gomez-Nieto, R., and Lepet, D. E. (2012). The selective serotoninergic mRNA4 impairs the nontonotopic projections from the locus coeruleus to the inferior colliculus in rats. Front. Neural Circuits 6:4. doi: 10.3389/fncir.2012.00041
Jen, P. H., Sun, X., and Chen, Q. C. (2001). An electrophysiological study of neural pathways for corticocollicularinhibited neurons in the central nucleus of the inferior colliculus of the frog-longs-head. Front. Neurosci. Exp. Brain Res. 157, 292–302.
Kim, B. W., Gao, E., and Suga, N. (2001). Effects of acetylcholine and atropine on plasticity of central auditory neurons caused by conditioning in bats. J. Neurophysiol. 86, 211–225.
Kolb, J. R., and Caspary, D. M. (2007). “The pharmacology of the inferior colliculus,” in The Inferior Colliculus, eds J. A. Winer and C. E. Schneider (New York: Springer Science-Business Media, Inc.), 481–295.
Langner, G., Albert, M., and Brade, T. (2002). Temporal and spatial coding of periodicity information in the inferior colliculus of awake chinchilla (Chinchilla lamb). Hear. Res. 168, 110–130.
Lim, H. H., and Anderson, D. J. (2006). Auditory cortical responses to electrical stimulation of the inferior colliculus: implications for an auditory midbrain implant. J. Neuroscience 26, 975–988.
Lim, H. H., and Anderson, D. J. (2007a). Auditory cortical responses to electrical stimulation of the inferior colliculus: implications for an auditory midbrain implant. J. Neuroscience 27, 905–923.

*fnccir-07-00077* — 2013/4/28 — 19:04 — page 9 — #9
in guinea pig. J. Neurophysiol. 97, 1413–1427.

Lin, H. S., and Anderson, D. I. (2007). Spatially distinct functional output regions within the central nucleus of the inferior colliculus: implications for an auditory midbrain implant. J. Neurosci. 27, 8733–8743.

Liu, X., Sun, Y., Wang, Y., and Yan, J. (2010). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

Ma, X., and Suga, N. (2003). Aug-Luo, F., Wang, Q., Kashani, A., and Yan, X., Yan, Y., Wang, Y., and Yan, J. (2005). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

McIntyre, C. C., and Grill, W. M. (2005). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

Massopust, L. C. Jr., and Ordy, J. (2005). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

Merchan, M. A., and Oliver, D. L. (2008). Descending connections from primary auditory cortex to the midbrain and Brain Res. 1240, 158–161.

Markovitz et al. (2011). “Descending Connections of Corticofugal Feedback in Herring.” J. Comp. Physiol. A Neuronal Syst. Behav. Physiol. 194, 169–181.

Markovitz, M. S., and Layton, R. M. (2005). The unequal variability of central auditory transduction in the guinea pig. J. Comp. Neurol. 482, 475–489.

Rodrigues-Dagaeff, C., Simm, G., De Ribaupeyre, E., and Rouiller, E. M. (1994). Functional specialization of neurons in the central nucleus of the macaque monkey. J. Neurophysiol. 72, 1578–1592.

Quian Quiroga, R., and2003. Selective microstimulation of cells of auditory cortex. Neuroscience 159, 246–258.

Schefrin, C. E., and Langner, G. (1998). Corticofugal modulation of subcortical responses to tonotopic and localized corticocollicular pathways. J. Neurophysiol. 62, 185–189.

Merchan, M. A., and Oliver, D. L. (2005). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

Markovitz et al. (2011). “Descending Connections of Corticofugal Feedback in Herring.” J. Comp. Physiol. A Neuronal Syst. Behav. Physiol. 194, 169–181.

Markovitz, M. S., and Layton, R. M. (2005). The unequal variability of central auditory transduction in the guinea pig. J. Comp. Neurol. 482, 475–489.

Rodrigues-Dagaeff, C., Simm, G., De Ribaupeyre, E., and Rouiller, E. M. (1994). Functional specialization of neurons in the central nucleus of the macaque monkey. J. Neurophysiol. 72, 1578–1592.

Quian Quiroga, R., and2003. Selective microstimulation of cells of auditory cortex. Neuroscience 159, 246–258.

Schefrin, C. E., and Langner, G. (1998). Corticofugal modulation of subcortical responses to tonotopic and localized corticocollicular pathways. J. Neurophysiol. 62, 185–189.

Merchan, M. A., and Oliver, D. L. (2005). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

Markovitz et al. (2011). “Descending Connections of Corticofugal Feedback in Herring.” J. Comp. Physiol. A Neuronal Syst. Behav. Physiol. 194, 169–181.

Markovitz, M. S., and Layton, R. M. (2005). The unequal variability of central auditory transduction in the guinea pig. J. Comp. Neurol. 482, 475–489.

Rodrigues-Dagaeff, C., Simm, G., De Ribaupeyre, E., and Rouiller, E. M. (1994). Functional specialization of neurons in the central nucleus of the macaque monkey. J. Neurophysiol. 72, 1578–1592.

Quian Quiroga, R., and2003. Selective microstimulation of cells of auditory cortex. Neuroscience 159, 246–258.

Schefrin, C. E., and Langner, G. (1998). Corticofugal modulation of subcortical responses to tonotopic and localized corticocollicular pathways. J. Neurophysiol. 62, 185–189.

Merchan, M. A., and Oliver, D. L. (2005). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

Markovitz et al. (2011). “Descending Connections of Corticofugal Feedback in Herring.” J. Comp. Physiol. A Neuronal Syst. Behav. Physiol. 194, 169–181.

Markovitz, M. S., and Layton, R. M. (2005). The unequal variability of central auditory transduction in the guinea pig. J. Comp. Neurol. 482, 475–489.

Rodrigues-Dagaeff, C., Simm, G., De Ribaupeyre, E., and Rouiller, E. M. (1994). Functional specialization of neurons in the central nucleus of the macaque monkey. J. Neurophysiol. 72, 1578–1592.

Quian Quiroga, R., and2003. Selective microstimulation of cells of auditory cortex. Neuroscience 159, 246–258.

Schefrin, C. E., and Langner, G. (1998). Corticofugal modulation of subcortical responses to tonotopic and localized corticocollicular pathways. J. Neurophysiol. 62, 185–189.

Merchan, M. A., and Oliver, D. L. (2005). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

Markovitz et al. (2011). “Descending Connections of Corticofugal Feedback in Herring.” J. Comp. Physiol. A Neuronal Syst. Behav. Physiol. 194, 169–181.

Markovitz, M. S., and Layton, R. M. (2005). The unequal variability of central auditory transduction in the guinea pig. J. Comp. Neurol. 482, 475–489.

Rodrigues-Dagaeff, C., Simm, G., De Ribaupeyre, E., and Rouiller, E. M. (1994). Functional specialization of neurons in the central nucleus of the macaque monkey. J. Neurophysiol. 72, 1578–1592.

Quian Quiroga, R., and2003. Selective microstimulation of cells of auditory cortex. Neuroscience 159, 246–258.

Schefrin, C. E., and Langner, G. (1998). Corticofugal modulation of subcortical responses to tonotopic and localized corticocollicular pathways. J. Neurophysiol. 62, 185–189.

Merchan, M. A., and Oliver, D. L. (2005). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

Markovitz et al. (2011). “Descending Connections of Corticofugal Feedback in Herring.” J. Comp. Physiol. A Neuronal Syst. Behav. Physiol. 194, 169–181.

Markovitz, M. S., and Layton, R. M. (2005). The unequal variability of central auditory transduction in the guinea pig. J. Comp. Neurol. 482, 475–489.

Rodrigues-Dagaeff, C., Simm, G., De Ribaupeyre, E., and Rouiller, E. M. (1994). Functional specialization of neurons in the central nucleus of the macaque monkey. J. Neurophysiol. 72, 1578–1592.

Quian Quiroga, R., and2003. Selective microstimulation of cells of auditory cortex. Neuroscience 159, 246–258.

Schefrin, C. E., and Langner, G. (1998). Corticofugal modulation of subcortical responses to tonotopic and localized corticocollicular pathways. J. Neurophysiol. 62, 185–189.

Merchan, M. A., and Oliver, D. L. (2005). Corticofugal modulation of initial sound processing in the brain. J. Neurosci. 28, 11615–11621.

Markovitz et al. (2011). “Descending Connections of Corticofugal Feedback in Herring.” J. Comp. Physiol. A Neuronal Syst. Behav. Physiol. 194, 169–181.

Markovitz, M. S., and Layton, R. M. (2005). The unequal variability of central auditory transduction in the guinea pig. J. Comp. Neurol. 482, 475–489.

Rodrigues-Dagaeff, C., Simm, G., De Ribaupeyre, E., and Rouiller, E. M. (1994). Functional specialization of neurons in the central nucleus of the macaque monkey. J. Neurophysiol. 72, 1578–1592.

Quian Quiroga, R., and2003. Selective microstimulation of cells of auditory cortex. Neuroscience 159, 246–258.
Markovitz et al. (2013) Tonotopic and localized corticocollicular pathways from primary auditory cortex to the central nucleus of the inferior colliculus. Front. Neural Circuits 7:77. doi: 10.3389/fncir.2013.00077

Copyright © 2013 Markovitz, Tang and Lim. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and any copyright notices concerning any third-party graphics etc.