Antidromic Activation Reveals Tonotopically Organized Projections From Primary Auditory Cortex to the Central Nucleus of the Inferior Colliculus in Guinea Pig

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Antidromic activation reveals tonotopically organized projections from primary auditory cortex to the central nucleus of the inferior colliculus in guinea pig. J Neurophysiol 97: 1413–1427, 2007. First published December 6, 2006; doi:10.1152/jn.00384.2006. The inferior colliculus (IC) is highly modulated by descending projections from higher auditory and non-auditory centers. Traditionally, corticofugal fibers were believed to project mainly to the extralemniscal IC regions. However, there is some anatomical evidence suggesting that a substantial number of fibers from the primary auditory cortex (A1) project into the IC central nucleus (ICC) and appear to be tonotopically organized. In this study, we used antidromic stimulation combined with other electrophysiological techniques to further investigate the spatial organization of descending fibers from A1 to the ICC in ketamine-anesthetized guinea pigs. Based on our findings, corticofugal fibers originate predominantly from layer V of A1, are amply scattered throughout the ICC and only project to ICC neurons with a similar best frequency (BF). This strict tonotopic pattern suggests that these corticofugal projections are involved in modulating spectral features of sound. Along the isofrequency dimension of the ICC, there appears to be some differences in projection patterns that depend on BF region and possibly isofrequency location within A1 and may be indicative of different descending coding strategies. Furthermore, the success of the antidromic stimulation method in our study demonstrates that it can be used to investigate some of the functional properties associated with corticofugal projections to the ICC as well as to other regions (e.g., medial geniculate body, cochlear nucleus). Such a method can address some of the limitations with current anatomical techniques for studying the auditory corticofugal system.

INTRODUCTION

The inferior colliculus (IC) is an obligatory center of convergence for most ascending auditory information from lower brain stem nuclei (Aitkin and Phillips 1984; Casseday et al. 2002; Ehret 1997b; Malmierca et al. 2002). The IC also receives a large number of descending projections from higher auditory and nonauditory centers (Coomes et al. 2005; Winer 2005b; Winer et al. 1998, 2002), indicating that the IC is highly modulated by feedback mechanisms that may select specific features of sound. Traditionally, it was thought that these descending projections terminated mainly in the extralemniscal regions of the IC (Faye-Lund 1985; Herbert et al. 1991; Huffman and Henson 1990; Winer et al. 1998). However, there is some anatomical evidence suggesting that a substantial number of direct projections from the primary auditory cortex (A1) to the ICC exist (Saldana et al. 1996) and appear to be glutamatergic (Feliciano and Potashner 1995).

An interesting question is how these A1 to ICC projections are spatially organized. Saldana et al. (1996) showed in rats that anterograde tracers injected into certain regions within the primary auditory cortex (Te1) stained a large number of terminal fibers in some regions of the ipsilateral IC in a tonographic manner. They proposed that this topographic organization was tonotopic based on the frequency organization of Te1 and ICC in rats that appeared to align with their injection sites and corresponding stained regions, respectively. Andersen et al. (1980b) injected anterograde tracers into cat A1 and observed stained terminal fibers within the ICC in a tonographic pattern. They inferred that this topographic projection pattern was tonotopic based on electrophysiological mappings of the injected regions. Bajo and Moore (2005) demonstrated in gerbil that retrograde tracers injected into the ICC resulted in labeled cells within the ipsilateral A1 that appeared to be organized in a tonotopic pattern. They confirmed that the injected cells were contained within the ICC based on retrograde labeling of cells within brain stem nuclei that do not project to the other IC regions. More recently, Bajo et al. (2006) injected anterograde and retrograde tracers into the auditory cortex and IC, respectively, of the ferret and observed projections from the primary auditory fields to the ICC. The projections appeared to be tonotopically organized but were restricted to the dorsomedial region of the central nucleus, suggesting possible differences among species.

The preceding findings provide some evidence for a general tonotopic organization of corticofugal projections from A1 to the ICC. However, from these studies, it is not yet clear as to how individual A1 neurons spatially project to the ICC, both along the frequency and isofrequency dimensions. This is due to limitations in current methods used for studying auditory corticofugal projections. For anatomical studies, anterograde tracers can be injected into a small region within A1, and the corresponding stained terminal fibers within the ICC can be assessed (and vice versa with retrograde tracers). However, the extent of staining is not spatially localized enough to isolate the effects of individual or small groups of neurons. For smaller injections, there is always the possibility that cells are not stained, something that can lead to the erroneous inference that...
certain projections do not exist. Furthermore, it is difficult to
directly determine the functional properties associated with the
stained cells. For electrophysiological studies, A1 neurons can
be stimulated and the corresponding neural activity can be
recorded from individual ICC neurons. However, it is difficult
to confirm if those responses are caused purely by monosyn-
aptic rather than polysynaptic projections from A1. Thus a
more refined method for studying individual or small groups of
neural projections from A1 to ICC is needed to better under-
stand how cortical neurons spatially activate and modulate the
ICC.

In a previous study investigating the potential features of an
auditory midbrain implant (AMI) (Lim and Anderson 2006),
we electrically stimulated the ICC and recorded the corre-
sponding neural activity in A1 of guinea pigs. Considering that
we could record antidromically activated spikes in A1, it
suggests that direct projections from A1 to the ICC exist in the
guinea pig, which have been observed in tracer studies by
Coomes et al. (2005). It also suggests that it may be possible to
study the spatial organization of monosynaptic corticofugal
projections to the ICC using antidromic stimulation (Asanuma
et al. 1968; Bereshplova et al. 2006; Palmer and Rosenquist
1974; Schofield et al. 1987; Swadlow 1988). Therefore the goal
of this study was to test the hypothesis that A1 projections are
amply distributed and tonotopically organized throughout the
ICC, which thus far has been inferred from some anatomical
results but not yet confirmed with electrophysiological meth-
ods. To achieve our goal, we electrically stimulated different
regions along the frequency and isofrequency dimensions of
the ICC and recorded the antidromically activated spikes
across different layers and regions within A1 using multi-site
probes in ketamine-anesthetized guinea pigs. The ability to
acoustically stimulate and record neural activity across multi-
ple sites within the ICC and A1 enabled us to identify the
different cortical layers (using current source density analysis)
and best frequency (BF) regions. Histological techniques were
used to locate the placement of our sites along the isofrequency
dimension of the ICC. We then characterized the spatial
projection pattern of A1 fibers throughout the ICC.

METHODS

Basic surgical procedures and methods for stimulation and record-
ing were similar to those presented in previous studies (Lim and
Anderson 2003, 2006). Experiments were performed on young pig-
m ented guinea pigs (Elm Hill Breeding Labs, Chelmsford, MA) in
accordance with policies of the University of Michigan Committee on
the Care and Use of Laboratory Animals. These experiments were
performed in two parts to investigate how A1 fibers are organized
along the frequency and isofrequency dimensions of the ICC: exper-
iment 1 (8 animals, 402–448 g) in which we electrically stimulated
different regions within an ICC lamina and recorded from antidi-
rally activated cells in different layers of the ipsilateral A1 (similar BF
as the ICC lamina); and in experiment 2 (5 animals, 320–588 g), in
which we electrically stimulated different BF regions within the ICC
and recorded from antidromically activated cells in different BF
regions of the ipsilateral A1.

Anesthesia and surgery

The animals were initially anesthetized with an intramuscular
injection of ketamine hydrochloride (40 mg/kg) and xylazine (5
mg/kg) with additional supplements to maintain an areflexive state.
After placing each animal into a stereotoxic device (David Kopf
Instruments, Tujunga, CA), we stereotaxically inserted silicon-sub-
strate multi-site Michigan probes (Center for Neural Communication
Technology, University of Michigan, Ann Arbor, MI) (Anderson et al.
1989; Drake et al. 1988; Najafi et al. 1985) into the right ICC and A1
(Fig. 1). The ICC probe (Fig. 1A) had two shanks separated by 500
µm (all distances are center to center) where each shank had eight
iridium sites linearly spaced at intervals of 200 µm. Each shank was
15 µm thick, 8 mm long, and tapered in width from 400 µm to a few
micrometers at the tip. Each site had an area of ~400 µm² and was
activated to an impedance of <100 kΩ (at 1 kHz). Activating the sites
from iridium to iridium oxide (via cyclic voltammetry) enabled us to
use them for both recording and stimulation (up to a conservative limit
of 60 µA) (Anderson et al. 1989; Beebe and Rose 1988; Weiland and
Anderson 2000). The ICC probe was inserted 45° off the sagittal plane
through the occipital cortex (not shown) and into the ICC where each
shank was aligned along the tonotopic axis of the ICC (Malmierca et
al. 1995; Merzenich and Reid 1974; Schreiner and Langner 1997;
Snyder et al. 2004). For A1, we used two different probes depending
on the experimental group (experiment 1 or 2). In Fig. 1B (experiment
1), the A1 probe consisted of a single shank with 16 sites linearly
spaced at intervals of 150 µm. Each shank was 15 µm thick, 5 mm
long, and tapered in width from 200 µm to a few micrometers at the
tip. The probe was inserted approximately perpendicular to the
cortical surface along a specific BF column of A1 (Abeles and Goldstein
This enabled us to simultaneously record from different cortical
layers. To record along the tonotopic gradient of A1, we used the
probe shown in Fig. 1C (experiment 2). This probe had eight shanks
each separated by 200 µm and each shank had two electrode sites
separated by 50 µm. Each shank was 15 µm thick, 1.6 mm long, and
tapered in width from 50 µm to a few micrometers at the tip. The
probe was inserted approximately perpendicular to the cortical
surface where each shank was positioned into a different frequency
region in A1 (Redies et al. 1989; Wallace et al. 2000). The sites were
inserted to a depth approximately corresponding to the output layer (V) of A1
(see Placement of probes). For experiment 2, the ICC and A1 probes
usually recorded from neurons sensitive to frequencies across a
similar three- to four-octave range between ~2 and 30 kHz. We were
unable to access the entire frequency range in guinea pigs (~0.05–45
kHz, roughly spanning 2.5 mm in ICC and 3.5 mm in A1) due to the

FIG. 1. Drawings of the multi-site probes
positioned along the tonotopic axis of inferior
colliculus central nucleus (ICC, A), a best fre-
quency (BF) column of primary auditory cor-
tex (A1, B), and the tonotopic gradient of A1
(C). Anatomy in A was derived from an image
presented in Malmierca et al. (1995) while that
in B and C was from Wallace et al. (2000) for
the guinea pig (simplified and not drawn to scale).
Electrode sites are represented by black dots on
each shank, *, blood vessels. DC, dorsocaudal
cortex; F, frequency; ICD, IC dorsal cortex; ICX,
IC external cortex; SC, superior colliculus.
limited distance spanned by our probe sites (1.4 mm). Thus we spanned a frequency range that allowed us to simultaneously sample reasonably low and high frequencies. All sites for both A1 probes had an area of ~400 μm² and unactivated impedances of 1–2 MΩ. After placement of the probes, the brain was covered with agarose to reduce swelling, pulsations, and drying during the recording sessions. For experiment 1, two to five ICC probe placements and only one A1 probe placement were made. For experiment 2, only one ICC probe placement and one A1 probe placement were made.

Stimulation and recording setup

Experiments were conducted in a sound-attenuating chamber and controlled by a computer interfaced with TDT hardware (Tucker-Davis Technology, Alachua, FL) using custom software written in MATLAB script (Mathworks, Natick, MA). To aid in the positioning of the probes, we presented varying levels of pure tones and broadband noise (50 ms in duration) to elicit acoustic driven activity in the contralateral (right) ICC and A1. The acoustic stimuli were presented via a speaker coupled to the left ear through a hollow ear bar. This speaker-ear bar system was calibrated using a 0.25-in Bruel & Kjær condenser microphone (Naerum, Denmark) where the tip of the ear bar was inserted into a short plastic tube with the microphone inserted into the other end. The tube represented the ear canal. All neural signals were passed through analog DC-blocking and anti-aliasing filters from 1.6 Hz to 7.5 kHz. For spike analysis, these signals were then digitally filtered between 0.3 and 3 kHz. After placement of the probes, we connected the ICC probe to a custom-made optically isolated current stimulator for electrical stimulation. Each ICC site was stimulated as an active electrode where the return was through a wire positioned in a neck muscle (monopolar configuration). Electrical stimuli were presented via a speaker coupled to the left ear through a hollow ear bar. This speaker-ear bar system was calibrated using a 0.25-in Bruel & Kjaer condenser microphone (Naerum, Denmark) where the tip of the ear bar was inserted into a short plastic tube with the microphone inserted into the other end. The tube represented the ear canal. All neural signals were passed through analog DC-blocking and anti-aliasing filters from 1.6 Hz to 7.5 kHz. For spike analysis, these signals were then digitally filtered between 0.3 and 3 kHz. After placement of the probes, we connected the ICC probe to a custom-made optically isolated current stimulator for electrical stimulation. Each ICC site was stimulated as an active electrode where the return was through a wire positioned in a neck muscle (monopolar configuration). Electrical stimuli consisted of single biphasic, charge-balanced pulses (200 μs/phase, cathodic-leading) from 1 to 56.2 μA in dB steps. Forty trials of each stimulus at 2/s were presented in a randomized sequence across all sites and levels to reduce adaptive effects. Neural data were recorded on all A1 probe sites in response to stimulation of each ICC site. The recording ground wire was positioned under the skin ~2 cm rostral to Bregma.

Placement of probes

Poststimulus time histograms (PSTHs) and frequency response maps (FRMs) were plotted to confirm that our sites were positioned within the desired BF regions within the ICC and A1. Methods for probe placement, and obtaining FRMs and BFs are presented in detail in our previous study (Lim and Anderson 2006). For experiment 1 (using Fig. 1B probe), we needed to identify the different cortical layers, especially the main output layer (V) of A1. This was possible by using current source density (CSD) analysis (Mitzdorf 1985; Muller-Preuss and Mitzdorf 1984). Evoked potentials were first recorded in response to 60 dB SPL broadband noise (40 trials). CSD profiles (Fig. 2B) were then calculated by taking the discrete approximation of the second spatial derivative of the averaged evoked potentials (Fig. 2A) recorded on our A1 sites using the finite difference formula

$$CSD(z) = \sigma \left( \phi(z + \Delta z) - 2\phi(z) + \phi(z - \Delta z) \right) / (\Delta z)^2$$

where $\phi$ is the evoked potential, $z$ is the depth location of each site along the A1 probe, $\Delta z$ is the differentiation step size, and $\sigma$ is the component of conductivity in the $z$ direction. $\Delta z$ was equal to our A1 site spacing of 150 μm and $\sigma$ was set to 1 because we were not concerned with absolute CSD values. CSD profiles for the two edge sites could not be calculated using the preceding equation because it requires evoked potential profiles from two neighboring sites. This one-dimensional CSD approximation has shown to provide a consistent representation for the current sinks and sources associated with columnar synaptic activity in the auditory cortex (Muller-Preuss and Mitzdorf 1984). The main input layer of A1, which approximately corresponds to layer III/IV (Huang and Winer 2000; Smith and Populin 2001), was taken as the site with the shortest latency current sink (Fig. 2B) and PSTH response (Fig. 2C). We selected this site as the reference depth because it could be consistently identified across animals. All other sites were labeled in reference to this site where a more positive depth corresponded to a deeper site. Layer V could then be identified with respect to the main input layer site. Based on depth measurements made in guinea pig A1, the main input layers of III/IV tend to span ~300–400 μm and layer V tends to span ~500–600 μm (M. N. Wallace, personal communication). Although the thicknesses of the layers may slightly vary across BF regions and animals, we still observed almost all the lowest antidromic threshold spikes within a localized range of depths (see Experiment 1: antidromic properties) consistent with anatomical findings (Coomes et al. 2005; Winer and Prieto 2001) and demonstrating the reliability of using CSD analysis for identifying different cortical layers. For experiment 2, we used the eight-shank probe shown in Fig. 1C to record from different frequency

![Figure 2](http://jn.physiology.org/DownloadedFrom/10.1152/jn.00516.2006)
regions of A1. We did not have a high-density probe available that could simultaneously record across multiple layers and BF regions. Our probe only had two sites per shank, so we could not perform CSD analysis to identify layer V. Instead, we inserted the probe into A1 until we recorded antidromically activated spikes in response to ICC stimulation. We positioned the probe to maximize the number of sites that recorded the lowest threshold antidromic spikes, which corresponded to layer V based on our results from experiment 1. Some sites that were not optimally positioned in layer V exhibited higher than normal antidromic spike thresholds that could artificially reduce the true spread of antidromic activation across the tonotopic gradient of A1 to what is recorded by our A1 probe. However, such effects do not alter our overall findings and are further discussed in our results sections for experiment 2.

**ICC probe histology**

For experiment 1, we needed to identify the location of our ICC shank placements. During the experiment, we dipped the ICC probe (with 2 shanks) into a red stain [10% 1,1-dioctadecyl-3,3,3,3'-tetramethylindocarbocyanine perchlorate (di-I); Molecular Probes, Eugene, OR] before each insertion into the brain. An effective protocol consisted of repeatedly (10 times) dipping the probe into the stain for 10 s and letting it dry in air for 10 s (DiCarlo et al. 1996). At the end of each experiment, the animal was decapitated, and the head was immersed into 10% formaldehyde for ~5 days. The brain was removed and placed in 20% sucrose solution until the tissue sank. Frozen sections 40 μm thick were cut in the sagittal plane. Figure 3 shows an example of a sagittal section with six red dots (labeled with asterisks). Because the ICC probe was inserted 45° off the sagittal plane, each shank tract appeared as a dot in the sagittal section. Once the dots were located, we were able to measure their distances from the caudal \(X_{CR}\) and dorsal \(X_{DV}\) edge of the IC. Because the ICC laminae are roughly aligned 45° from the horizontal plane as shown in Fig. 1A (Malmierca et al. 1995; Merzenich and Reid 1974; Schreiner and Langner 1997; Snyder et al. 2004), we multiplied \(X_{DV}\) by \(\cos(45°)\) to obtain the approximate dorsomedial-to-ventrolateral distance \(X_{DV,V}\) along the laminae. In reality, each ICC lamina deviates from this 45° orientation to varying extents along the caudorostral and dorsolateral dimensions and is positioned in different locations relative to the dorsal and caudal edges of the IC. Thus we cannot claim that a certain location in one BF lamina is in the same relative location in another BF lamina even though they may have the same \(X_{DV,V}\) \(X_{CR}\) coordinates. Also the dimensions of a lamina can vary for different BF regions and sized animals. However, this method allowed us to systematically identify the locations of our shank placements relative to the caudal and dorsal edges of the IC. Because we also acoustically identified the stimulation site within the target BF lamina for each shank, the measured coordinates indirectly correspond to the locations of our stimulation sites along that lamina. We measured the total distance, \(T_{CR}\), from the caudal edge to the IC-SC division so that we could gauge the relative location of our sites along the caudorostral dimension across animals (for examples, see Fig. 7). We were unable to identify any consistent landmarks to gauge our relative location along the dorsolateral (i.e., dorsomedial-to-ventrolateral) dimension. Because \(X_{CR}, X_{DV},\) and \(T_{CR}\) change along the mediolateral dimension (for different sagittal sections), we only measured these parameters along one sagittal section corresponding to a normalized distance of 0.31 from the lateral edge of the IC to the midline of the brain to be consistent across animals. Using acoustic stimulation, we identified the sites that were located within the ICC. For each animal, only sites located within one target BF lamina of the ICC were used for stimulation and data analysis.

**Data analysis**

**Spike activity.** Multi-unit activity was recorded in A1 in response to electrical stimulation of the ICC. This activity consisted of both antidromic and orthodromic spikes. Any value exceeding 3.5 times the SD of the background (without spikes) neural signal was detected as a spike and an algorithm was used to determine the time of occurrence of the largest peak (positive or negative) of that spike. We then displayed the spike activity as PSTHs across 40 trials. For electrical stimulation in the ICC, we observed stimulus artifacts that usually lasted less than a millisecond. Only at high levels close to 56.2 μA did we observe artifacts that lasted between 1 to 2 ms. Because we did not observe stimulus-driven spike activity with latencies <2 ms, we just zeroed out all the PSTHs from 0 to 2 ms from stimulus onset for analysis.

**Antidromic identification.** In Fig. 4, PSTHs recorded across 14 sites along the single shank A1 probe (Fig. 1B, experiment I) for varying stimulus levels are presented. Each column corresponds to a different stimulus level, whereas each row corresponds to a different depth along a BF column of A1. As shown in Fig. 2, we were able to identify the main input layer site in A1 using CSD analysis. In Fig. 4, each A1 site was labeled with a depth relative to the main input layer site. A depth of −300–0 μm approximately corresponds to layers III/IV and a depth of 150–600 μm approximately corresponds to layer V. As the stimulus level was increased, orthodromic activity increased on all sites especially for depths between −300 and 0 μm. In deeper regions (300–600 μm), antidromic activity (arrows) was also present. The short latency and precisely stimulus-locked nature of these antidromic PSTH peaks is characteristic of antidromic activity. To confirm that these responses were antidromic, we performed a pulse rate test. Figure 5A shows an example of an orthodromic A1 response preceded by an antidromic PSTH peak. Figure 5B shows the same data plotted on an expanded time scale. Studies have shown that orthodromic activity from the ICC to A1 (via the thalamus) cannot follow pulse rates >100 pulses/s, whereas antidromic activity can follow pulse rates significantly higher than 100 pulses/s (Mitani and Shimokouchi 1985; Rose and Metherate 2001). Figure 5C presents an example where the orthodromic onset response was rapidly diminished by the third pulse, whereas the antidromic spike activity robustly followed a pulse train of 120 pulses/s. Although the collision test is generally more reliable in identifying antidromically activated spikes...
(Fuller and Schlag 1976; Swadlow 1998), we could more efficiently identify antidromic activity for multi-unit recordings by stimulating with pulse rates >100 pulses/s (Rose and Metherate 2001) and isolating the remaining units. Furthermore, the precise latencies and sudden appearance of antidromic spikes with stimulation level (for example, see Fig. 4) made it readily possible to differentiate antidromic from orthodromic activity.

ANTIDROMIC THRESHOLDS. Antidromic threshold corresponded to the stimulus level that caused a sudden increase in antidromic activity from no activity. For example, in Fig. 4, the threshold for antidromic activity was 8.9 μA, and it appeared at a depth of 450 μm. As the stimulus level was increased, antidromic activity began to appear on other sites. Usually we observed these antidromic spikes in layer V of A1 (see Experiment 1: antidromic properties). For experiment 2, we were unable to perform CSD analysis because there were only two sites along each shank of the A1 probe (Fig. 1C). Instead, we inserted the probe into A1 until we observed antidromic activity on our recording sites generally with the lowest thresholds. For data analysis, we only used the lower antidromic threshold site along each shank, resulting in a total of eight sites for each A1 probe placement.

ANTIDROMIC ACTIVATION SPREAD. In experiment 2, we investigated the extent of activation spread across the tonotopic gradient of A1 to determine if corticofugal fibers from different BF regions project down to a specific BF region in the ICC or if a strict BF-aligned projection pattern exists. We also investigated the extent of activation spread within the ICC. In particular, we wanted to determine the amount of current required to activate corticofugal fibers located a certain distance away from the stimulation site. This

FIG. 4. PSTHs (0.2-ms bins) recorded on 14 sites of the A1 probe (in Fig. 1B) aligned along a cortical column (BF = 12 kHz). Each column corresponds to a different stimulus level, whereas each row corresponds to a different cortical depth relative to the main input layer site in which more positive values indicate deeper locations. Both orthodromic and antidromic activity are displayed; ←, antidromic PSTH peaks.

FIG. 5. A: example PSTH (0.5-ms bins) recorded in A1 that consisted of a short-latency, precisely stimulus-locked antidromic spike peak followed by more temporally diffuse orthodromic activity in response to single pulse ICC stimulation. B: same data on an expanded time scale. C: PSTH for same recording site in A but in response to a 200-ms pulse train (120 pulse/s) demonstrating the ability of the antidromic spike peak to follow high pulse rates, whereas the orthodromic activity rapidly diminishes by the 3rd pulse.
was important not only to gauge how close our activated fibers were from our stimulated sites, but also to provide insight into some of the biophysical and anatomical properties of these fibers. Measuring the activation spread in A1 was directly possible from our recording setup since we simultaneously recorded from eight sites positioned across the tonotopic gradient of A1 in response to stimulation of a certain BF ICC region. Measuring the activation spread within the ICC was obtained indirectly from the A1 spread data. As we will demonstrate in our experiment 2 results, corticofugal fibers from a certain BF region only project to a similar BF region in the ICC. This one-to-one tonotopic alignment allowed us to directly scale our A1 spread data to obtain the activation spread within the ICC. The following equation describes this scaling process

\[
\text{ICC Activated Distance} = \frac{1}{0.5} \times (\text{A1 BF Gradient})^{-1} \times \text{A1 Spread Distance}
\]

Units: [\mu m ICC] = \left[\text{oct}/\mu\text{m A1}\right]^{-1} \times \left[\text{oct}/\mu\text{m A1}\right] \times [\mu m A1]

The BF gradients with respect to distance along the tonotopic dimension have shown to be approximately linear on an octave scale for both the ICC (Snyder et al. 2004) and A1 (Arenberg et al. 2000; Bakin et al. 1996) in guinea pigs. Thus it is possible to scale the spread of antidromic activation along the tonotopic gradient of A1 (in \mu m) by the A1 BF gradient to get the total BF range of activated corticofugal fibers (in octaves). By multiplying this range by the inverse of the ICC BF gradient (and assuming a one-to-one tonotopic projection from A1 to ICC), we can obtain the activation spread of corticofugal fibers along the tonotopic gradient of the ICC. The 0.5 scale factor is applied to obtain the distance of activated fibers from the stimulation site assuming the site was located at the midpoint of the total activated region. This ICC activated distance provides a measure for how far fibers along the tonotopic gradient of the ICC can be activated from a stimulated site for a certain current level. Assuming an isotropic, homogeneous volume conductor model with passive current spread through the tissue medium, we can fit the ICC activated distance \(D\) versus current level \(I\) data to the theoretical equation

\[
I = I_o + kD^2
\]

where \(I_o\) is the current threshold for activation of a cell/fiber (at \(D = 0\)) and \(k\) is a constant that accounts for the resistivity of the medium (Asanuma and Sakata 1967; Bagshaw and Evans 1976). The ICC does have a well-defined layered structure that is anatomically similar along the tonotopic axis (Oliver 2005). Thus estimating the ICC as infinite dimensional homogeneous layers is a reasonable assumption.

RESULTS

All analyses are based on data from 13 animals. The results are separated into two groups corresponding to experiments 1 and 2. For experiment 1 (8 animals), we electrically stimulated three to eight different locations within an ICC lamina and recorded the antidromically activated spikes across different layers along a BF column of A1 (similar BF as ICC lamina). Only one BF region was investigated for each animal. The BFs corresponding to all eight animals were 9, 10, 12, 14, 20, 22, and 23 kHz. There were a total of 44 stimulated ICC sites. Stimulation of one site did not elicit any antidromic activity in A1 even at our maximum level of 56.2 \mu A. For experiment 2 (5 animals), we electrically stimulated different frequency regions within the ICC and recorded the antidromically activated spikes across different frequency regions within A1 (approximately in layer V). For each animal, there was only one ICC probe placement (with \(\pm 2\) valid shanks) and one A1 probe placement. Across animals we had a total of eight different ICC shank placements aligned along the tonotopic axis of the ICC in which 51 sites (of 64 because 8 sites aligned along a shank) could be used for stimulation. For each stimulated ICC site, we analyzed the spike activity recorded on eight sites aligned along the tonotopic gradient of A1. Stimulation of all 51 ICC sites elicited antidromic activity in A1.

Experiment 1: antidromic properties

Electrical stimulation of different regions within an ICC lamina antidromically activated fibers projecting from A1. The antidromic spikes were recorded from different cortical layers, usually in layer V but also within layers III/IV and VI. In attempt to identify the main output region of A1, we determined the depth corresponding to the lowest antidromic threshold for each stimulated ICC site. Figure 6A presents a histogram of these different depth values. Almost all depths were between 450 and 600 \mu m, which corresponds to the middle and deeper parts of layer V approximated from the histological depth measurements made in guinea pig A1 by M. N. Wallace (personal communication), and is consistent with anatomical results by Coomes et al. 2005. This is also consistent with findings in cat where Winer and Prieto (2001) showed that corticocollicular projections (A1 to the IC) originated from the bottom two (of 3) subdivisions of layer V. However, some of the lowest thresholds did occur in the upper layers (III/IV). It is possible that we may have recorded from dendritic processes extending from output neurons located in layer V that were closer to our recording sites than the actual layer V neurons. This is consistent with findings presented by Schofile et al. (1987), who observed, for projections from the visual cortex to the superior colliculus, that antidromically activated dendritic processes extending from corticocortical cells located in layer V could elicit spike activity in layers III/IV of the cortex. The one depth value at 1,350 \mu m (layer VI) may be a result of an antidromically activated fiber originating from layer V but that passes in close proximity to a deeper recording site. There is some evidence in guinea pigs that a small percentage (<10%) of the corticofugal neurons that project to the IC from the ipsilateral auditory cortex originate from layer VI (B. R. Schofield, personal communication), which could also explain why we occasionally observed antidromic spikes in deeper layers.

![Figure 6](http://jn.physiology.org/DownloadedFrom/)  
**FIG. 6.** Experiment 1: distributions for the depths (A), thresholds (B), and latencies (C) corresponding to the lowest threshold antidromic spike recorded in A1 for each stimulated ICC site \(n = 43, \text{experiment 1}\).
The antidromic thresholds corresponding to the depths in Fig. 6A are plotted in B. Although there were a few high thresholds and even one case where no antidromic activity was elicited, the majority (70%) of thresholds were <10 \ \mu A (almost all were <30 \ \mu A). Based on current spread data for mammalian CNS stimulation (Ranck 1975), 10 \ \mu A (200-\mu s monopolar cathodal pulse) would not be able to activate fibers more than ~100 \ \mu m away. A current level of 30 \ \mu A would not be able to activate fibers more than ~200 \ \mu m away. Considering that corticofugal fibers projecting from A1 to the ICC have shown to be much thinner (<1 \ \mu m in diameter) (Winer 2005b) than the fibers used to obtain those estimates suggests that ICC stimulation should exhibit even less spread of antidromic activation. Later, we present data supporting this inference (see Experiment 2: ICC spread of activation). Therefore to have antidromically activated corticofugal fibers with low stimulus levels for almost all our ICC locations throughout different BF regions (see next sections), fibers directly projecting from A1 must be amply scattered throughout the ICC. Otherwise, higher thresholds would have been observed.

Most of the antidromic latencies we observed (Fig. 6C) were ~2–3 ms with some as large as 10 ms. These values are consistent with results presented by Mitani et al. (Mitani and Shimokouchi 1985) where they found latencies between 1.1 and 6.7 ms in cat. Schofield et al. (1987) also found latencies between 2 and 10 ms for antidromically activated visual cortical fibers that projected down to the superior colliculus in rats. Considering that these antidromically activated fibers are only traveling between two regions rather than several synapses raises the question as to why these latencies are generally long. There is anatomical evidence that these corticofugal fibers are very thin (<1 \ \mu m in diameter) (Winer 2005b). Thus the antidromically activated spikes from the ICC to A1 would exhibit slow conduction velocities thus longer latencies due to the small diameters of these corticofugal fibers.

Experiment 1: spatial distribution within the ICC

To assess how A1 neurons project to different laminae within the ICC, we plotted the location of all our stimulation sites within the target ICC lamina for each animal (Fig. 7). For each plot, we used the total distance (\(T_{\text{CR}}\)) from the caudal edge of the IC to the IC-SC division as the maximum value along the caudorostral (\(X_{\text{CR}}\)) axis. Along the dorsomedial-to-ventrolateral (\(X_{\text{DmVl}}\)) axis, we did not have any consistent landmarks. So we used a maximum distance of 2.5 mm for all the plots. Each site location is defined by a shaded circle (except for 1 location labeled with an asterisk where no antidromic activity was elicited using our maximum level of 56.2 \ \mu A) in which its border corresponds to the maximum distance from the site (center) that a corticofugal fiber can be activated with a current level equal to the antidromic threshold for that site. The diameters of the circles were calculated using our ICC spread Eq. 2 obtained from experiment 2, which is presented in Fig. 14 (see Experiment 2: ICC spread of activation). That equation is consistent with the current spread data presented in Fig. 1 of Ranck (1975) and represents the upper spread limit for activating A1 fibers within the ICC. From our data, we can only claim that each shaded circle corresponds to either a single neuron or a small number of neurons projecting from a BF column of A1 to an ICC lamina. We used principal component analysis and spike waveform features (amplitude and duration) to extract single-unit data from our multi-unit A1 recordings. However, many antidromic spikes exhibited similar waveform shapes making it difficult to determine if our single-unit data corresponded to one neuron or multiple neurons. Furthermore, slight movements of our sites along a BF column as we stimulated different ICC locations could result in different waveform properties for the same A1 neuron.

In general, Fig. 7 demonstrates that fibers projecting from A1 are amply distributed throughout the ICC laminae. Based on stained sections of the guinea pig ICC (particularly in Figs. 1E, 2, and 6 presented by Malmierca et al. 1995), the iso-frequency layers corresponding to the BF regions presented in our Fig. 7 roughly span a caudorostral distance of 2 mm and a dorsomedial-to-ventrolateral distance of 2–2.5 mm. Across animals, our sites spanned a caudorostral distance (\(X_{\text{CR}}\)) of ~1.9 mm and a dorsomedial-to-ventrolateral distance (\(X_{\text{DmVl}}\)) of ~2.1 mm in which stimulation of all but one of these sites (asterisk) elicited antidromic activity in A1. Some of the circles did span larger areas with diameters up to several hundred micrometers. However, if we assume that only a few or no A1 neurons project to the ICC, then the circles should span almost the entire lamina and/or exhibit greater overlap. Furthermore, stimulation of most (70%) of our sites activated fibers within

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**FIG. 7.** Experiment 1: the locations of the sites along a target ICC lamina (BF labeled in bottom left corner) for each animal are displayed as shaded circles where the site is located at the center. The circle radius corresponds to the maximum distance a corticofugal fiber can be located from the site and still be activated with a current level equal to the antidromic threshold for that site. The maximum distances were calculated from the fitted equation presented in Fig. 14 (for more details on this equation, see Experiment 2: ICC spread of activation). \(X_{\text{DmVl}}\), dorsomedial-to-ventrolateral location; \(X_{\text{CR}}\), caudorostral location; \(T_{\text{CR}}\), total distance between caudal edge and the IC-SC division along \(X_{\text{CR}}\) axis (for more details on how these coordinates were measured, see ICC probe histology).
Based on our data, there does appear to be some differences based on BF region. Figure 7, A–D, corresponds to mid-BF regions (9–14 kHz) and generally consists of smaller circles than those in Fig. 7, E–H, which correspond to higher BF regions (20–23 kHz). A greater number of larger circles suggest that fewer corticofugal fibers span the ICC lamina and/or higher current levels are required to activate the fibers. Figure 8 better depicts this difference by displaying the antidromic threshold distributions for the mid BF (Fig. 8A) and higher BF (Fig. 8B) regions. All but one location for the mid BF regions exhibited antidromic thresholds that were <10 μA (50% were <5 μA), whereas thresholds were scattered across a larger range for the higher BF regions (12% were <5 μA). Thus, lower BF A1 neurons appear to project more densely to an ICC lamina than higher BF neurons. Another possibility is that higher BF ICC regions may consist of corticofugal fibers with thinner diameters (different biophysical properties), which would result in higher current levels for activation (BeMent and Ranck 1969b; Ritchie 1982; Rubinstein 1991; Waxman and Swadlow 1977).

One interesting observation we observed was the difference in projection patterns between the laminae in Fig. 7, G and H. Considering that both correspond to similar BF regions (and dimensions) yet Fig. 7H exhibits a greater number of larger circles suggests that different ICC projection patterns may exist for neurons located within different locations along the isofrequency dimension of A1 for higher BF regions. Such an organization might reflect a topographic map of corticofugal projections along the isofrequency dimension that is associated with sound features other than frequency. However, it is also possible that for the 22–23-kHz BF ICC lamina there are just fewer corticofugal fibers in more caudal and ventrolateral locations where most of the placements were made in Fig. 7H. At least this would suggest that unlike the mid BF A1 neurons that appear to project amply throughout an ICC lamina, some higher BF A1 neurons may exhibit more spatially specific projection patterns within the ICC. In future studies, we will need to systematically map the antidromic responses along the isofrequency dimension of A1 for stimulation of different locations along an ICC lamina to confirm these observations.

**Experiment 2: tonotopic organization**

Results from experiment 1 demonstrated that corticofugal neurons project from A1, mainly from layer V, to different regions within an ICC lamina. These projections were assessed for BF-aligned regions between A1 and the ICC. For experiment 2, we were interested in characterizing the distribution of these corticofugal projections along the tonotopic dimension. Figure 9 presents multi-site PSTHs recorded across eight A1 sites located in different BF regions in response to stimulation of sites within different BF ICC regions for two stimulus levels. The colorscale was adjusted to provide better visualization of the antidromic activity. For ICC site 1, the orthodromic activity was still present and partially masked the antidromic component of the response for both levels. Generally, stimulation of a site within a low BF ICC region elicited antidromic activity in a low BF region of A1. Similarly, stimulation of a higher BF ICC site elicited antidromic activity in a higher BF A1 region. From these plots, it is evident that the projection pattern from A1 to the ICC is tonotopically organized such that A1 neurons project to ICC neurons with a similar BF. Even when the stimulus level is increased 5–10 dB above antidromic threshold, the responses maintain localization (see next section).

We quantified the tonotopic projection pattern from A1 to the ICC across all our animals by plotting the BF of the A1 site with the lowest antidromic threshold against the BF of the stimulated ICC site (Fig. 10). Perfect BF alignment (diagonal line) was not always possible due to the set geometry of our electrode sites. Thus we presented the closest possible BF-aligned sites with dots. Stimulation of 69% of our ICC sites elicited antidromic activity with the lowest threshold on the closest BF-aligned A1 sites. The fact that stimulation of almost all of our sites (48 of 51) elicited antidromic activity with the lowest threshold on an A1 site that was at most one site away from the closest BF site demonstrates that corticofugal projections from A1 to the ICC are tonotopically organized, at least for the BF regions we assessed (~3–30 kHz). Furthermore, stimulation of almost all the sites (47 of 51) elicited antidromic activity with a current level <30 μA with more than half of them (27 of 51) being <10 μA. Combined with the inferences made from experiment 1, these low thresholds across different BF regions suggest that corticofugal fibers are amply scattered throughout the ICC along the frequency and isofrequency dimensions.

**Experiment 2: A1 spread of activation**

We have shown that A1 neurons tonotopically project to different ICC neurons. However, the question arises as to how spatially specific these tonotopic projections are. To answer this question, we assessed the spread of antidromic activation...
along the tonotopic gradient of A1 in response to varying levels of stimulation within the ICC. Figure 11A presents a typical example where a black dot indicates that antidromic activity was present on an A1 site for a given stimulus level. At threshold and even up to ~8 dB above threshold, antidromic activity was present on only one A1 site. As the stimulus level was increased, neighboring sites gradually began to exhibit antidromic activity. Even for the case where we observed the greatest spread across all our sites and animals (Fig. 11B), antidromic activity was still spatially localized to two sites at threshold that then gradually spread to neighboring sites for higher levels. These results, along with the findings from Fig. 10, suggest that A1 neurons do not project to multiple BF regions within the ICC but rather project only to ICC neurons with a similar BF in a one-to-one pattern.

We did observe cases where stimulation of an ICC site elicited a discontinuous activation pattern in A1 (Fig. 11C). At threshold, localized antidromic activity was observed. However, as the stimulus level was increased, antidromic activity appeared on a more distant A1 site in a discontinuous pattern that did not usually spread to other sites. This pattern may be caused by activation of corticofugal fibers passing across different BF regions en route to their target ICC lamina. Stimulation of ~25% (13 of 51) of our ICC sites elicited discontinuous antidromic patterns, which included discontinuities over one site to as many as five sites. The example in Fig. 11C was discontinuous over three sites.

To summarize our spread results, we plotted the mean activation spread and SD for different stimulus levels above threshold in Fig. 12A. Data for the discontinuous cases were excluded from this analysis. Activation spread was plotted in terms of distance rather than site number, where the distance between two sites was 200 μm. On average, antidromic activity did not span a distance of >200 μm across A1 (appeared on only 1 site) even at 6 dB above threshold. As the stimulus level was increased, antidromic activity gradually appeared across a larger region of A1 but was still only ~400–500 μm at 24 dB above threshold. Figure 12B plots A1 spread distance as a function of absolute ICC current level to display the extent of antidromic activation across A1 independent of thresholds, and is used for further analysis in the next section.

In Fig. 12 there was some variation in activation spread across stimulation sites for each level. This may be attributed to differences in biophysical properties of A1 neurons projecting into the ICC. However, some technical limitations likely contributed to this variation. Corticofugal fibers projecting from the vicinity of our A1 recording sites may not have always been in direct contact with our stimulation sites as was evident from Fig. 7. If an ICC site was not in direct contact with its target fiber (within a given BF lamina), then higher
levels of stimulation would be required to activate that fiber. Furthermore, depending on how close fibers in neighboring laminae were to this stimulation site would affect the extent of activation spread at higher stimulus levels. Also we could not guarantee that all our A1 sites were optimally positioned near neurons with the lowest antidromic thresholds due to the set geometry of our recording probe. Depending on how much additional current within the ICC was required to antidromically activate neurons close to those A1 sites could further alter the spread of antidromic activation across A1.

Experiment 2: ICC spread of activation

As explained in METHODS, assuming a one-to-one tonotopic projection pattern from A1 to the ICC and the existence of linear BF gradients (on an octave scale) enables us to directly scale our A1 spread data to obtain current-distance values for activation of corticofugal fibers across the tonotopic gradient of the ICC using Eq. 1. We showed in the previous section that a one-to-one tonotopic pattern exists. Figure 13 demonstrates that the BF gradients in A1 and the ICC are approximately linear. In Fig. 13, A and C, the inclusion of sites with BFs <5 kHz resulted in nonlinear curves. Therefore we omitted those sites and recalculated the BF gradients in Fig. 13, B and D, by fitting straight lines to all the curves. For A1, we obtained a gradient (slope inverse value) of 711.9 μm/octave. This is consistent with results presented by Aренберг et al. (2000) where they obtained values ranging from 203 to 712 μm/octave. The fact that our value equaled their upper limit may reflect differences in calculation methods for these BF shifts. Our results are closer to the value of ~650 μm/octave that can be estimated from the optical imaging results presented by Bakin et al. (1996). For the ICC, we obtained a gradient of 583.3 μm/octave, which is consistent with the values (380–716 μm/octave) presented by Snyder et al. (2004).

The data in Fig. 14 is just a scaled version of the data presented in Fig. 12B excluding points corresponding to BFs <5 kHz. The dark line represents a least-squares fit (R² = 0.9, P < 0.001) of Eq. 2 to the maximum spread value for each current level resulting in a k equal to 0.000504 μA/μm² and I₀ equal to 1.6 μA. As discussed in the previous section, there was some variation in A1 activation spread due to misalignments between our sites and the corresponding neurons within the ICC and A1. These misalignments tend to artificially reduce the true spread of activation within the ICC. So we fit Eq. 2 to only the maximum spread value for each level. If we assume that at least some of our spread values for each level corresponded to optimally aligned sites within the ICC and A1 and to a representative sample of fibers, then the fitted equation should provide an approximate upper limit for the current-distance relationship of activation of corticofugal fibers across the tonotopic axis of the ICC. Furthermore, it appears that even across different tissue regions and anisotropic conditions, the current-distance relationships tend to be fairly similar and depend more on properties associated with conduction velocity and fiber diameter (BeMent 1969a,b; Ranck 1975; Rubinstein 1991). Thus the fitted curve in Fig. 14 should also provide a reasonable upper estimate for current spread along the isofrequency laminae of the ICC. Our curve is consistent with data presented in Fig. 1 of Ranck (1975) in which current-distance curves across different tissue regions and preparations for 200-μs/phase cathodal pulses were compiled into one plot. Although we used biphasic cathodic-leading 200-μs/phase pulses, studies have shown that threshold increases should be minimal (<3%) at that pulse width (McIntyre and Grill 2000; Rubinstein et al. 2001). Ranck’s lower bound of data points (i.e., shortest activated distance for a given current level) corresponds to fibers with slower conduction velocities as low as 9 m/s, which in turn corresponds to fibers with smaller diameters as low as 1.5 μm based on the well-known scaling factor of 6 (Hurst 1939; Ritchie 1982; Waxman and Swadlow 1977). Considering that corticofugal fibers within the ICC appear to be very thin (<1 μm in diameter) (Winer 2005b) and our fitted curve aligns with Ranck’s lower bound of data points, our inference that our
DISCUSSION

Overall, our results demonstrate that corticofugal fibers from A1 to the ICC are amply scattered throughout the ICC and tonotopically organized in a one-to-one pattern. However, there does appear to be some differences in spatial distribution (or even biophysical properties) of the fibers across an ICC lamina that depends on BF region and possibly isofrequency location in A1. These findings also provide insight as to some of the functional effects observed in other electrophysiological studies that orthodromically stimulated pathways projecting into the ICC. Although there are limitations associated with the antidromic stimulation method, we have demonstrated the potential of combining this method with other electrophysiological techniques and electrode technologies for studying the descending auditory system that can complement anatomical studies. These findings are further discussed in the following text.

Antidromic stimulation method

As is evident from our results, antidromic stimulation combined with other electrophysiological techniques can be used as a method for studying the spatial distribution of descending monosynaptic projections from A1 to the ICC. The success of this method was attributed to the multi-site Michigan probe technology, which enables us to stimulate and record from the same electrode sites and simultaneously across multiple regions in the ICC and A1. Thus it is possible to first characterize the neurons surrounding each site within the ICC and A1 based on acoustic response properties (i.e., BF, threshold, PSTH response, binaurality, frequency bandwidth, etc.). Then different regions of the ICC can be electrically stimulated, and the antidromically activated spikes can be recorded in different layers and regions of A1. CSD analysis, histological techniques, and acoustic-driven responses can be used to identify the location of each site within the ICC and A1. As a result, the spatial projection pattern of different A1 neurons to the ICC and the electrophysiological properties associated with these projections can be identified using antidromic stimulation. This method can also be used to study monosynaptic projections from the auditory cortex to other regions, such as the medial geniculate body and cochlear nucleus.

There were a few limitations with this method we observed in this study. With respect to our probe designs, we were not able to ensure close proximity stimulation of corticofugal fibers throughout the ICC is reasonable.

FIG. 13. Experiment 2: curves showing how BF changes as a function of recording site location along the tonotopic axis of A1 (A and B) and the ICC (C and D) in the same animals used to obtain the A1 antidromic spread data. Only BFs ≥5 kHz were included in B and D to calculate constant slope values from the fitted lines. n = 5 for A and B and n = 8 for C and D.

FIG. 14. Experiment 2: spread of antidromic activation (mean ± SD) along the tonotopic axis of the ICC as a function of absolute ICC current levels (thin line). These values were calculated from the data in Fig. 12B using Eq. 1 and only include data for ICC sites with BFs ≥5 kHz. The number of sites used to calculate the mean and SD for each level is 1, 4, 11, 19, 23, 30, 30, 32, 35, 35, 35, and 35. The dark line is a fitted curve of Eq. 2 to the maximum spread value for each current level ($R^2 = 0.9, P < 0.001$) and describes how far from the stimulated site (D) fibers can be activated for a given current level (I). For more details on these calculations, see Data analysis: antidromic activation spread.
within the ICC and recording of single output neurons within A1. Furthermore, we were not able to simultaneously record from different layers, BF regions, and isofrequency locations within A1. So we had to perform two separate experiments in which we still could not record from the lowest antidromic threshold neurons with all our A1 sites. There are now available multi-site silicon probes with as many as 64 sites (NeuroNexus Technologies, Ann Arbor, MI) and more dense probes currently being developed that can allow us to simultaneously record from different layers to identify the optimal depth site and regions to assess BF and isofrequency location effects. Using more closely spaced electrode configurations, such as tetrodes (Gray et al. 1995), we can better isolate single units and assess how individual A1 neurons project to different subcortical regions. This would also enable us to use the collision test to more accurately identify antidromic spikes and isolate slower conducting neurons (Fuller and Schlag 1976; Swadlow 1998). In terms of ensuring close proximity stimulation of corticofugal fibers, particularly the axonal terminations within the ICC, more accurate methods will need to be developed. At least based on biophysical models, stimulation of axon terminals can achieve substantially lower thresholds compared with that of passing fibers under certain stimulus conditions (Rubinstein 1993). If such models are developed for the corticofugal fibers within the ICC and we use denser probes to stimulate more sites throughout a target region, then it may be possible to identify which site is in close proximity to an axon terminal based on antidromic thresholds.

Another limitation we encountered was the inability to directly compare our ICC site locations along a lamina across different sized animals and BF regions. This is important for assessing differences in projection patterns across BF regions and identifying if any systematic spatial distribution exists along the isofrequency dimension. Similar limitations will exist for identifying recording site locations within A1. One advantage of using higher-density probes that span a larger area is that we will be able to more efficiently identify the borders along the frequency and isofrequency dimensions. In this sense, we can normalize each region with respect to these borders. It may also be possible to identify other electrophysiological properties that can further separate out different functional zones, especially for regions that do not have a systematic tonotopic organization.

Projections from A1 to ICC

Some anatomical studies have shown that a substantial number of direct projections from A1 to the ICC exist that appear to be tonotopically organized (Andersen et al. 1980b; Bajo and Moore 2005; Bajo et al. 2006; Saldana et al. 1996) and glutamatergic (Feliciano and Potashner 1995). Using antidromic stimulation, we provided further evidence confirming that fibers projecting from A1 are amply scattered throughout the ICC and are spatially organized such that A1 neurons only project to ICC neurons with a similar BF. Also consistent with anatomical studies (Bajo and Moore 2005; Bajo et al. 2006; Coomes et al. 2005; Winer and Prieto 2001), we showed that these corticofugal projections originate primarily from layer V of A1. These findings across species (i.e., cat, ferret, gerbil, rat, guinea pig) and methods provide convincing evidence that the ICC is directly and highly modulated by A1 neurons that affect some spectral features of sound (see next section). Considering that an even denser projection of corticofugal fibers target the extralemniscal IC regions bilaterally (Bajo and Moore 2005; Bajo et al. 2006; Faye-Lund 1985; Herbert et al. 1991; Huffman and Henson 1990; Winer et al. 1998) that exhibit a tonotopic organization in register with that of the ICC (Andersen et al. 1980a; Saldana et al. 1996) and project onto ICC neurons (Jen et al. 2001), it is expected that this spectral modulation (and possibly other features) is also indirectly influenced by other cortical and subcortical regions.

It is interesting to note that there are some anatomical differences in projection patterns that have been observed across species. In rat (Saldana et al. 1996), cat (Andersen et al. 1980a), and ferret (Bajo et al. 2006), the primary auditory field has shown to project bilaterally to the ICC, whereas in the gerbil (Bajo and Moore 2005), such projections appear to terminate only in the ipsilateral ICC. Based on auditory cortex ablation (Feliciano and Potashner 1995) and tracer (Coomes et al. 2005) studies in guinea pig, it appears that A1 predominantly projects to the ipsilateral ICC as well. We did not investigate antidromically activated spikes in the contralateral A1 to confirm these guinea pig findings. In addition to differences in bilaterality, there also appears to be differences in the density of projections to the ICC. In rat, gerbil, and guinea pig (based on our findings), it appears that corticofugal projections from A1 are amply scattered throughout the ICC. However, in cat and ferret, the projections appear to be localized to the dorsomedial portion of the ICC with fewer terminal fibers observed in the cat (Winer et al. 1998).

One possible explanation for differences across species is that the methods used did not effectively and completely stain the thin corticofugal fibers within certain ICC subregions and/or species. This could occur if fibers in certain regions were thinner or less prone to transporting the tracers. This would further suggest that subpopulations of cortical neurons exist that project to different regions within the ICC and exhibit different anatomical and biophysical properties. Antidromic stimulation could then be used to activate those corticofugal fibers to determine their distribution within the ICC and A1 bilaterally and characterize some of their electrophysiological properties. On the other hand, these differences may represent features of auditory coding that is inherently different across species. Bilateral cortical modulation of the ICC in cat, ferret, and rat may relate to their more predatory behavior and keen awareness of the environment compared with the guinea pig and gerbil. The more dense projection of corticofugal fibers throughout the ICC, compared with just the dorsomedial region observed in cat and ferret, may be a feature inherent in rodents. The question remains as to what these differences in projection patterns mean in terms of function and, more importantly, how to interpret these differences to better understand cortical modulation of ascending auditory information in humans.

Functional implications

The strict tonotopic organization of corticofugal fibers from A1 to the ICC suggests that their role is to provide modulation (possibly excitatory) of frequency-specific information. Similar conclusions have been made based on electrophysiological studies in bats (Gao and Suga 2000; Yan and Suga 1998) and mice (Yan and Ehret 2002; Yan et al. 2005). These studies
have demonstrated that focal stimulation of corticofugal neurons can shift an ICC neuron’s BF (and minimum threshold) to that of the A1 neurons. Although these studies did not provide direct evidence that the BF shifts were caused by monosynaptic projections from A1 to the ICC, they did demonstrate that A1 neurons generally modulate ICC neurons in a frequency-specific manner. Thus it appears that sounds containing behaviorally meaningful spectral features strongly activate cortical neurons (and cognitive centers) that in turn activate ICC neurons, making them more sensitive to those features. Considering that we observed differences in descending projection patterns depending on BF region (Fig. 7), these spectral features may be processed and modulated in different ways. Overall, this auditory cortical feedback appears to enable the brain to modify its intrinsic circuitry to accommodate changes in the environment and to allow for selective hearing of sounds that may be of importance to an individual.

The mechanism behind corticofugal plastic BF changes is not well understood. One hypothesis is that a center-surround pattern of excitation and inhibition exists such that BF-aligned activation is excitatory, whereas BF-misaligned activation is inhibitory (Yan and Ehret 2002; Yan et al. 2005; Zhang et al. 1997). As described by Yan et al. (2005), when the BF of an ICC neuron is shifted due to corticofugal activation, the ICC neuron’s tuning curve generally broadens to include the target BF and its thresholds for different frequencies, including the original BF and partially excluding the target BF, increase. This pattern of increased thresholds ensures that the neuron has its lowest threshold at the new target BF. If the A1 neuron and its target ICC neuron have similar BFs, then no BF shift occurs. If the center-surround hypothesis is correct, then the question arises as to how these A1 neurons actually achieve inhibitory modulation if their projections are proposed to be excitatory (Feliciano and Potashner 1995; Saldana et al. 1996). We have also demonstrated that A1 neurons only project to ICC neurons with a similar BF. This raises another question as to how BF-misaligned ICC neurons are modulated by these corticofugal inputs. One possibility is that A1 neurons only excite ICC neurons with a similar BF. These activated ICC neurons then provide their own inhibitory modulation of neighboring ICC neurons with different BFs to reduce their sensitivity to those BFs. Simultaneously, these activated ICC neurons may also provide excitatory modulation of these neighboring ICC neurons to make them more sensitive to the target BF. The fact that these A1 to ICC projections are necessary to initially induce these plastic BF shifts yet not required to maintain these effects over time (Gao and Suga 1998) provides further evidence that these plastic shifts in BF coding must be induced by intrinsic mechanisms within the ICC. Thus it appears the role of these corticofugal projections may be to provide an initial excitatory signal to the ICC that then triggers a cascade of intrinsic activation patterns within the ICC that modifies the acoustic properties of the neurons. This is not to claim that polysynaptic inputs from A1 to the ICC via extralemniscal IC regions do not contribute to the inhibitory effect. There are a large number of monosynaptic projections from A1 to the external nucleus and dorsal cortex of the IC bilaterally that appear to coincide with the tonotopic organization of the intracollicular connections of the IC (Saldana et al. 1996). Because these monosynaptic projections appear to be excitatory and synapse onto GABAergic neurons that then project onto ICC cells (Jen et al. 2001),

it is possible that frequency-specific inhibition associated with corticofugal plastic BF changes may also be caused by this polysynaptic pathway. Whatever the mechanism, it does not appear to involve rapid feedback from the auditory cortex. We obtained antidromic latencies that were several milliseconds to as much as 10 ms. Based on our current-distance curve for activation of corticofugal fibers within the ICC (Fig. 14) and comparison with data presented in Fig. 1 of Ranck (1975) (see Experiment 2: ICC spread of activation), these fibers appear to have slow conduction velocities due to axon diameters that are less than a micrometer (Winer 2005b).

One question we were unable to answer from this study was whether or not a systematic organization of projections from A1 to the ICC exists along the isofrequency dimension. We presented data (Fig. 7, G vs. H) showing that A1 neurons from different animals but with a similar BF could exhibit different projection patterns. However, it was not clear if these differences were caused by the limited sampling of ICC locations or actually due to the A1 neurons projecting from different isofrequency locations within A1. To determine this, we could simultaneously record antidromic spikes within multiple regions along the isofrequency dimension of A1 in response to stimulation of different regions along an ICC lamina. This would provide evidence for whether or not different A1 neurons with a similar BF project to similar and/or different ICC regions. It has been proposed that topographic maps associated with other sound features (e.g., binaurality, latency, intensity, frequency tuning, periodicity) are mapped along the isofrequency dimension of the ICC (Aitkin et al. 1985; Ehret 1997b; Schreiner and Langner 1988; Stiebler 1986) and A1 (Ehret 1997a; Middlebrooks et al. 1980; Rutkowski et al. 2000; Schreiner et al. 2000; Schulze and Langner 1997; Velenovsky et al. 2003). In the same way A1 neurons may modulate ICC neurons with respect to spectral properties, it is possible that this modulation could occur for these other sound features. If this is true, then we could characterize these projections from A1 to the ICC with respect to these different features and determine how they are spatially organized along the isofrequency dimension.

Understanding the functional significance of the descending pathway from A1 to the ICC is important. However, it represents just one component of a more complex auditory corticofugal system that projects to various auditory and nonauditory cortical and subcortical regions (for review, see Winer 2005a). Descending fibers within this system originate from different cortical layers and target many regions, including the thalamus, midbrain, brain stem, striatum, amygdala, and other cortical regions. Thus it is believed that the auditory corticofugal system influences our perception of sound as well as our behavior and emotional state in response to the sounds. Although anatomical studies have provided us with an initial roadmap of various corticofugal pathways to make these inferences, little is known about the actual functional properties of the different descending components. By combining current anatomical methods with other electrophysiological techniques, such as those presented in this paper, we can begin to identify the functional features of the auditory corticofugal system and expand our traditional views on ascending auditory coding.
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