Inferior Colliculus Responses to Multichannel Microstimulation of the Ventral Cochlear Nucleus: Implications for Auditory Brain Stem Implants

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Submitted 7 June 2007; accepted in final form 6 October 2007


The ventral cochlear nucleus (VCN) is the first processing site of sound information in the brain and receives direct excitatory synaptic inputs from the cochlea via the auditory nerve (AN) (Loren de de Nô 1933a,b). The neurons of the VCN are arranged in a tonotopic order, with low-frequency AN fibers innervating the most ventral and lateral regions of the VCN and high-frequency fibers innervating more dorsal and medial regions (Rose et al. 1959). On the basis of cytoarchitecture, there are several neuron types found in the VCN, each one different in anatomy (Hackney et al. 1990; Osen 1969) and function (Evans and Nelson 1973; Shofner and Young 1985). The posteroverentral cochlear nucleus (PVCN) in several animal species predominantly contains two types of cells that are known to project to higher auditory brain centers, such as the central nucleus of the inferior colliculus (CIC) of the midbrain, through both direct and polysynaptic pathways (Adams 1979a; Beyerl 1978; Cant 1992; Oliver 1987; Osen 1972; Schofield and Cant 1996). One such type, the T-stellate neurons (also known as type I multipolar neurons), are thought to be the prime encoders of monaural frequency (Brawer 1974; Cant 1981; Osen 1969; Paolini et al. 2004, 2005) and project directly to the CIC (Adams 1979a; Beyerl 1978; Cant 1992; Oliver 1987; Osen 1972; Schofield and Cant 1996). Another cell type, the octopus cells, are located in the most posterior regions of the PVCN and are a major source of inhibition to the CIC through the ventral nucleus of the lateral lemniscus (Adams 1997; Friaut and Ostwald 1988; Nayagam et al. 2005, 2006; Saint Marie and Baker 1990; Schofield and Cant 1997; Thompson 1998). The anteroventral cochlear nucleus (AVCN) is known to contain a high proportion of bushy cells (Harrison and Warr 1962; Osen 1969; Tolbert and Moster 1982a,b), which do not directly project to the CIC (Adams 1979b, 1983; Ryugo et al. 1981). However, these cells project to the superior olivary complex (Cant and Casseday 1986; Warr 1966), which in turn sends projections to the CIC (Browner and Webster 1975; Roth et al. 1978).

In several animal species, the CIC is tonotopically arranged from low to high frequencies in a dorsomedial–ventrolateral direction (Aitkin et al. 1972; FitzPatrick 1975; Huang and Fex 1986; Semple and Aitkin 1979). Although there are some differences in the anatomical characteristics of the VCN across species (Cant and Benson 2003), given that both the VCN and the CIC are tonotopically organized, it is likely that the projections between the structures are frequency-specific, which is supported by previous anatomical studies (Oliver 1987; Osen 1972). What remains to be investigated is whether these tonotopic projections are functionally valid for VCN stimulation. The knowledge gained by assessing this could provide significant further information on how to electrically stimulate the VCN with the auditory brain stem implant (ABI).

The ABI differs from the cochlear implant (CI) in that it stimulates the surface of the cochlear nucleus (CN) rather than along the tonotopic gradient of the cochlea (Otto et al. 1998). Commercially available implants consist of either 12 (MED-EL C40+, MED-EL, Innsbruck, Austria) or 21 (Nucleus 24 ABI, Cochlear, Sydney, NSW, Australia) active stimulating electrodes fixed on a Dacron fabric mesh that fits on the surface of the CN in the lateral recess of the fourth ventricle. The ABI has mainly been used to restore hearing in patients with profound sensorineural hearing loss caused by a nonsympathetic injury.
with Neurofibromatosis Type II (NF2), a genetic condition usually marked by bilateral tumor growths on the VIIIth cranial nerve (Otto et al. 2002). Damage to this nerve after removal of these tumors may result in profound sensorineural hearing loss. Although patients with this condition are unable to benefit from the CI designed to stimulate the spiral ganglion neurons of the cochlea, they may benefit from stimulation of the VCN when implanted with the ABI (Edgerton et al. 1982). In other conditions where a CI will not be effective, such as a temporal bone fracture with cochlear nerve avulsion, cochlear ossification, and possibly cochlear nerve aplasia, patients may also benefit from an ABI (Colletti and Shannon 2005). When compared with CI recipients several clinical studies have shown that NF2 ABI users obtain only limited sound perception (Otto et al. 1998, 2002; Schwartz et al. 2003). The typical ABI user will benefit from environmental sound perception, although in most cases is unable to understand speech without lipreading (Otto et al. 1998, 2002; Schwartz et al. 2003). A recent study (Colletti and Shannon 2005) reported that some non-NF2 patients can achieve significantly higher speech perception than NF2 patients. Although they suggested a separate pathway for speech and modulation coding, which might be damaged in NF2 patients, it is unclear why only some non-NF2 patients receive benefit.

Previous studies involving CIs (Friesen et al. 2001) and ABIs (Kuchta et al. 2004) have shown that the ability to perceive speech is largely correlated with the number of independent channels of frequency information. One way this may be achieved is by stimulation of localized groups of neurons within iso-frequency laminae of the VCN. However, the present surface implant technology may have limited access to the tonotopic map located within the VCN. A penetrating electrode array may overcome this limitation, as suggested by previous studies showing low thresholds, high dynamic ranges, and topographic specificity in higher brain centers to VCN stimulation (El-Kashlan 1999; El-Kashlan et al. 1991; McCreery et al. 1998, 2007; Takahashi et al. 2005).

In this investigation we examined the frequency specificity of activation of CIC neurons by microstimulation of the VCN across multiple sites. Our approach was to directly compare CIC multunit cluster responses obtained by electrical stimulation to those obtained acoustically. Unlike previous studies (El-Kashlan 1999; El-Kashlan et al. 1991; McCreery et al. 1998, 2007; Takahashi et al. 2005), we stimulated specific frequency regions in the VCN that were predetermined using acoustic stimulation. Multichannel electrodes were inserted in both structures along their tonotopic axes and, after determining the characteristic frequencies (CFs) of the multunit clusters at each electrode site, we stimulated each VCN site with single biphasic charge-balanced pulses. We recorded multunit spike activity in the CIC and analyzed multunit spike rate in response to stimulation, which allowed accurate measures of electrical thresholds, dynamic ranges, and frequency specificity of activation. The hypothesis of our study was that CIC clusters respond with lowest current threshold to electrical stimulation of a frequency-matched site in the VCN. We also hypothesized that this frequency specificity should depend on location of stimulation within the VCN. Our results have significant implications for ABIs.

**METHODS**

**Surgery**

Male Hooded Wistar rats (*n* = 8) weighing between 350 and 450 g were anesthetized with urethane in water (20% w/v; Sigma-Aldrich, Castle Hill, NSW, Australia) via the intraperitoneal cavity. The animals were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and fitted with hollow ear bars. Animal temperature was regulated at 37°C and monitored continuously using a DC homoeothermic blanket. Contralateral craniotomies were performed to access both the VCN and the inferior colliculus (IC). The cerebellum was aspirated after removing the outer duramer to expose the brain stem and VCN. Under visual control, using the lateral recess of the fourth ventricle as a guide, 32-channel electrodes (Neuronex Technologies, Ann Arbor, MI) were inserted both into the ipsilateral (left) VCN and the contralateral (right) CIC along their tonotopic axes. In all experimental animals, the VCN electrodes were inserted in a caudorostral direction to access the central parts of the PVCN, which have a high concentration of T-stellate cells (see RESULTS). The CIC electrodes for all experiments were inserted at a 10° rostrocaudal angle. A low-impedance silver reference electrode was placed under the skin and served as a common reference for spike recording. All surgical procedures and protocols were approved by the La Trobe University Animal Ethics Committee (Protocol # 03/13) and the St. Vincent’s Hospital Animal Ethics Committee (Protocol # 35/06).

**Multichannel electrodes**

Each multichannel electrode consisted of four silicon substrate shanks (200 µm apart), each 5 mm long, attached to a fiberglass circuit board with eight iridium electrode sites (413 µm² surface area, 200 µm apart) on each shank (referred to later as shanks a–d with sites 1–8 on each shank; see Figs. 1 and 2). Before each experiment, to increase their charge storage capacity, all 32 sites of the VCN electrode were electrochemically activated using cyclic voltammetry as described by Anderson et al. (1989) (660B Potentiostat and CHI200 Picoamp Booster, CH Instruments, Austin, TX). On activation, the impedance of each site was changed from 1 MΩ to <100 kΩ at 1 kHz, whereas each CIC electrode site had an impedance (unactivated) of 1–3 MΩ at 1 kHz.

**Acoustic protocols**

All stimuli generation and data acquisition were performed using Tucker Davis Technologies System III hardware (TDT, Alachua, FL), with a custom-designed software package using TDT’s OpenEx client-server applications. Acoustically driven units were first located in both structures by presenting a broadband (1–44 kHz) Gaussian-distributed noise stimulus (50-ms bursts, 500-ms intertrial interval) while advancing the electrode arrays into the VCN and CIC using a motorized microdrive (Sutter Instrument, Novato, CA). Once the arrays were in position, responses to various pure tones (1–4 kHz, 1-kHz steps, 500-ms bursts, 300-ms intertrial interval) of different amplitudes [10- to 70-dB sound pressure level (SPL), 10-dB steps] were obtained to generate a response area for each recording site (10 repetitions for each frequency–intensity combination). Ear bars were calibrated before each experiment using a 1/8-in. Brüel & Kjær (Nærum, Denmark) microphone and measuring amplifier unit.

**Electrical protocols**

Each acoustically driven electrode site in the VCN was electrically stimulated with charge-balanced biphasic current pulses using the site above it on the same shank as a reference (120 µs per phase, 80-µs interphase gap, bipolar configuration). For the topmost site on each shank, the site immediately below it was used as a reference. Microstimulators (32-channel RX7 microstimulators; TDT) were used to...
deliver currents \( \leq 100 \mu A \) (typically maximum current delivered was 54 \( \mu A \)). The pulse rate on every channel was fixed to one pulse every 500 ms (50 repetitions for each current amplitude). Because the electrode sites had a surface area of 413 \( \mu m^2 \) and our stimulus pulses were 120 \( \mu s \) per phase, even at 100 \( \mu A \), the maximum charge density was 2.9 \( mC/cm^2 \) and the charge per phase was 12 nC. This ensured that we were not above the electrochemical charge injection limit for iridium oxide (\(-3 mC/cm^2\)) (Beebe and Rose 1988) and that we were not above the electrochemical charge injection limit for the VCN neuron.

\[\text{Data analysis}\]

\[\text{INITIAL PROCEDURE. Multiunit activity from each of the electrode sites was analyzed off-line, using programs we developed in Matlab (The MathWorks, Natick, MA). A typical approach to analyze multiunit recordings is spike sorting (Lewicki 1998). One of the major challenges for any sorting technique has been to distinguish spike waveforms that occur as a result of two or more neurons firing simultaneously, resulting in fully or partially overlapping spikes (Bar-Gad et al. 2001). In structures like the VCN and the CIC, it is expected that when an acoustic stimulus is presented, not only one but several neurons close to a recording site will respond simultaneously. Therefore it was not possible to determine whether any spike waveform acquired by our system corresponded to a single neuron’s spike; thus we analyzed all our data using the multiunit activity from each site. Also, the method of on-line spike detection meant that while the spike detector was recording a waveform, it could not be triggered by another spike until it had recorded 31 samples of the signal. This could result in the recording system not accurately keeping track of all the spikes, especially those that occurred very near in time to each other. Therefore a rethresholding procedure was used off-line to accurately determine the occurrence of spikes. This procedure involved finding the mean and SD of each 31-sample spike recorded by the system, and then checking how many times any particular sample out of the 31 exceeded 1.5SDs above the mean. Each threshold crossing was picked up by a Schmitt trigger function, which kept track of the occurrence of spikes. This provided a more accurate estimate for spike rate calculations for analysis of the data.}\]

\[\text{ACOUSTIC RESPONSE AREAS. Spike rate calculations were made for each frequency–amplitude combination of sound presented using a}\]
Electrodes were inserted along the dorsoventral axis of the CIC at an approximately 10° angle to the vertical.

Histological analysis

At the end of each experiment, the animal was deeply anesthetized and perfused transcardially using 0.1 M phosphate-buffered saline and 10% neutral buffered formalin (Sigma–Aldrich). After tissue fixation, the brain was removed and snap-frozen. Serial coronal sections of 60 µm were collected using a freezing sledge microtome (Thomas Scientific, Swedesboro, NJ). Sections were placed on gelatin-coated slides, stained with thionine, and coverslipped. Positions of the electrodes through the VCN and CIC were confirmed using bright-field microscopy and VCN shank locations were reconstructed by manual drawings using a combination of histological data and known visual placements of the electrodes. Outlines of the CN were determined in the horizontal and parasagittal planes with reference to the rat brain atlas (Paxinos and Watson 2005). On viewing the coronal histological sections, the placement of the VCN probe was estimated and dots representing the shanks were added to the outlines, giving a relative indication of where the VCN electrodes were placed within the boundaries of the model outlines (see Fig. 1). Histological data were obtained in six of the eight experimental animals. Due to multiple VCN penetrations, histological data could not be verified for the remaining two animals.

RESULTS

Tonotopic maps and histological results

Figure 1A summarizes the placement of electrode shanks at their entry point into the CN in the horizontal plane. In four of the six animals, electrodes were inserted either toward the medial edge or the lateral edge of the VCN, whereas in two animals, placements were made in the central regions of the VCN. In one experiment (07_007), although the electrode was inserted into the dorsal CN (DCN) at its entry point, it was inserted with a greater caudorostral angle compared with the other experiments. As a result, few of the dorsally located electrode sites from this experiment were either in the DCN or close to the granule cell lamina between the DCN and the VCN.
FIG. 3. Acoustic and electrical responses from a characteristic frequency (CF) matched VCN–CIC pair (Exp 002; VCN Site c2; CIC Site a5). A: peristimulus time histograms (PSTHs) of CIC site a5 in response to a 10-kHz tone at various SPLs (10 repetitions each SPL, bin width = 1 ms). B: PSTHs of CIC site a5 in response to stimulation of VCN site c2 at various current levels (20 repetitions each current level, bin width = 1 ms). Arrow in PSTH at 30 μA indicates time-locked activity corresponding to presumed antidromic activity. C and D: response areas of a CF-matched pair of multiunit clusters in the VCN and the CIC, respectively. Both sites had a CF of about 10 kHz with colors representing normalized spike rate from threshold (blue) to saturation (red). E: rate-level functions recorded at CIC site a5 in response to tones at different frequencies (each frequency is shown by a different color). F: electrical thresholds of responses at CIC site a5 to stimulation of multiple VCN sites. Each VCN site is shown by a circle with the color representing the CF of that VCN site. Where 2 sites in the VCN had the same CF, the thresholds are shown by colored squares. Two VCN sites with CFs of 10 and 21 kHz when stimulated gave no response at CIC site a5 respectively. Both sites had a CF of about 10 kHz with colors representing normalized spike rate from threshold (blue) to saturation (red). Solid line corresponds to the range of CF regions stimulated in the VCN.

Characteristics of VCN electrical stimulation

In 334 of 512 possible sites (32 sites in each structure, 8 animals), multiunit cluster responses to frequencies at multiple intensity levels were obtained (response areas: 141, VCN; 193, CIC). Because our experiments involved sophisticated methods of surgery and recording techniques, due to experimental constraints it was on occasion not possible to stimulate all VCN sites in a given experiment. Of the VCN sites, 126 (89.4%) were electrically stimulated, of which 100 initiated a response in the CIC. The remaining 26 VCN sites did not show any response in the CIC even when stimulated at the highest current amplitude (100 µA). For the CIC, all 193 sites showed a response to electrical stimulation of at least one site in the VCN. To further analyze CIC responses to VCN stimulation, electrode sites in both structures were paired according to their CF. Those CIC sites that had a CF equal to or 1 kHz away from a single VCN site. These were classified into the “Lowest Threshold Aligned” group, consistent with Lim and Anderson (2006). VCN sites that did not have a CF-matched site in the CIC (n = 32) were not paired with any CIC sites and not included in the analysis. For both the groups, analyses were performed on the data obtained from stimulation of 68 VCN sites.

A total of 364 VCN–CIC pairs belonging to the CF Aligned group were analyzed for electrical stimulation (Fig. 3). In response to increasing levels of both acoustic and electrical stimulation, CIC sites exhibited an increase in discharge (Fig. 3, A and B) with multiunit spike activity generally observed
between 4 and 60 ms from stimulus onset. In contrast to acoustic stimulation, a well-timed onset peak was seen with higher stimulus currents in PSTHs from electrical stimulation, 4–5 ms from stimulus onset (Fig. 3B), possibly a result of antidromic activation in the VCN.

In a given experiment, it was possible for multiple VCN sites to have a similar CF; thus a given CIC site may have been paired to more than one site in the VCN. The CIC sites belonging to these pairs did not necessarily respond with the same threshold to stimulation of their VCN counterparts (Fig. 3F). Of the 364 CF-matched pairs analyzed, 289 (79.4%) responded to VCN stimulation. A total of 142 CIC sites were part of these responding pairs. The remaining 75 VCN–CIC pairs (20.6%) gave no response, the majority of which (45.3%) had electrode sites located in more anterolateral regions of the VCN (Exp 06_028 and Exp 07_005), as shown in Fig. 4. A total of 51 CIC sites made up these nonresponding pairs. However, 29 (57%) of these 51 CIC sites responded to stimulation of another CF-matched VCN site and thus were part of the 289 responding pairs. From the CIC sites that responded to stimulation, we were able to plot spike discharge as a function of stimulus current and designated CFs of stimulated sites within the VCN to construct electrical response areas (Figs. 3H and 5), the tuning characteristics of which could be compared directly to those of the acoustic response (see Electrical thresholds versus acoustic thresholds).

Differing rate-level functions were recorded from CIC multiunit clusters to stimulation of CF-aligned VCN sites (Fig. 3G). The shape of the rate-level function was used to classify multiunit cluster responses into four categories: monotonic, plateau, nonmonotonic, and complex (Aitkin 1991; Fig. 6). Monotonic responses (Fig. 6, Ai and Bi) were classified in cases where spike rates did not reach a saturation level and kept increasing with stimulus levels, whereas those that did saturate were classified as plateau-type responses (Fig. 6, Aii and Bii). Nonmonotonic responses (Fig. 6, Aiii and Biii) were classified in cases where spike rates first increased with stimulus levels and then decreased, indicating a possible loss of input or a decrease in excitatory drive.

FIG. 4. Horizontal representation of the CN with electrode shanks. Numbers show the percentage of the CF Aligned VCN–CIC pairs on each shank that did not give any response relative to the total CF Aligned pairs on that shank. Shanks on which >30% of the pairs gave no response are shown by open circles, whereas those shanks on which <30% gave no responses are shown by closed circles. Two of the shanks that were not stimulated are indicated by “NS.” One shank that had no CF matches in the CIC is indicated by “NCF.”
The distribution of all orthodromically activated thresholds, saturation points, and dynamic ranges obtained from the electrical rate-level functions of all VCN–CIC pairs is shown in Fig. 7. The method to obtain electrical thresholds and saturation points described earlier was applied to all responses regardless of the type of rate-level function. For monotonic responses, the saturation point was chosen as the highest current level in our range. For nonmonotonic and complex responses, the saturation point was chosen as the current level that elicited the overall maximum spike rate. The mean threshold ± SD for the pairs belonging to the CF Aligned group \((n = 289, \text{Fig. 7A}, i–iv)\) was found to be \(17.24 ± 11.34 \, \mu\text{A}\) and mean dynamic range was \(12.44 ± 9.64 \mu\text{A} (5.17 ± 2.95 \, \text{dB})\). The Lowest Threshold Aligned group consisted of VCN–CIC pairs \((n = 118)\) that were most sensitive to stimulation of a single VCN site. This group gave a lower mean threshold than that of the CF Aligned group \((12.63 ± 10.11 \, \mu\text{A})\) and a mean dynamic range of \(11.39 ± 10.22 \mu\text{A} (6.23 ± 3.68 \, \text{dB})\) (Fig. 7B, i–iv).

The threshold of the presumed antidromic activation (arrow in Fig. 3B) was noted as the current level that first elicited the time-locked onset peak that continued to be present at all current levels above threshold. Although the timely locked nature and the sudden appearance of activity above threshold are shown to be representatives of antidromic activity (Lim and Anderson 2007; Swadlow 1974; Swadlow et al. 1978) these properties are not always sufficient to discern antidromic spikes from orthodromic spikes; thus we labeled our responses as “presumed” antidromic activity. Of 1,313 total CIC responses to stimulation of the 100 VCN sites, 565 responses (43%) showed the presence of antidromic activity. In 541 (96%) of these responses, the threshold of antidromic activity to VCN stimulation was found to be equal to or higher than the threshold of the delayed orthodromic activity. Mean electrical threshold of all antidromic responses was found to be \(30.72 ± 13 \, \mu\text{A} (\text{mean} ± \text{SD})\). Although we have included these responses in our analysis, they did not influence our overall threshold measures.

**Electrical thresholds versus acoustic thresholds**

To directly compare the acoustic and electrical tuning at threshold levels for all CIC sites, both on-CF and off-CF, we plotted regression lines for the thresholds elicited by acoustic stimulation at given frequencies against the electrical thresholds elicited by stimulation of VCN sites with CFs equal to those frequencies (Fig. 8). Only CIC sites that responded to stimulation of at least three VCN sites were analyzed \((n = 135)\), of which 25.93% exhibited a high correlation (Pearson’s coefficient of correlation, \(r > 0.7\)), whereas 11% showed moderate correlation \((0.5 < r ≤ 0.7)\) between acoustic and electrical thresholds (Hinkle et al. 1998). Negatively correlated sites accounted for about 35% of the total number, whereas 20% of the total sites fell in the \(-0.2 < r ≤ -0.5\) range (Fig. 8f). As seen from the single experiment plots in Fig. 8, A–H, the majority of negatively correlated CIC sites came from two experiments with centrally located VCN electrode positions (Fig. 1, Exp 06_002 and Exp 06_003), whereas the remaining

![Graph of rate-level functions](image-url)
experiments had CIC sites that showed positive correlation between acoustic and electrical thresholds.

**Frequency specificity of CIC activation**

The data were analyzed on a VCN shank by shank basis across all experiments to test whether location of VCN stimulation correlated with the degree of frequency-specific CIC activation. For each VCN site stimulated that had at least one CF-matched site in the CIC (n = 68), the tonotopic gradient of the CIC was examined for the lowest threshold response (Fig. 9A). For a perfect tonotopic point-to-point mapping between the VCN and the CIC, each frequency region in the VCN when stimulated should elicit the lowest threshold response in the same frequency region in the CIC (Fig. 9A, solid line). For each point in Fig. 9A, the frequency difference between the stimulated VCN site and the lowest threshold CIC site was calculated. The average frequency difference for each VCN shank was used as a measure of shank effectiveness in eliciting frequency-specific CIC activation. VCN shanks with ≥67% of the lowest threshold VCN–CIC pairs that had frequency differences ≤3 kHz were classified into the “Group 1 shanks” (closed circles, Fig. 9, A and B). Remaining VCN shanks were classified into a second group (“Group 2 shanks”; open circles, Fig. 9, A and B). The overall degree of frequency-specific CIC activation was found to be higher for the Group 1 shanks, which were placed more centrally, on the medial edge or more posterior in the VCN, whereas more lateral and anterior VCN placements (Group 2 shanks) showed a lower degree of frequency specificity (Fig. 9C).

Frequency specificity was also maintained at higher current levels. Figure 9D shows the CF of each CIC site plotted against the CF of the VCN site that elicited maximum spike discharge on that CIC site across all current levels, 2–25 ms from stimulus onset. Only CIC sites that had at least one CF-matched VCN site were analyzed (n = 96). Most CIC sites (69.8%) responded with maximum spike discharge to stimulation of VCN sites belonging to the Group 1 shanks. Mean frequency difference for these maximum discharge VCN–CIC pairs was 4.6 ± 5.2 kHz. Fewer CIC sites responded with maximum discharge to stimulation of VCN sites, which were either on the Group 2 shanks (11.5%; mean frequency difference = 5.5 ± 3.8 kHz) or on shanks with unknown placements (18.7%; indicated by crosses; mean frequency difference = 6.7 ± 4.7 kHz).

In addition to being frequency specific, the mean threshold elicited in the CIC for the Group 1 shanks (12.8 ± 6.4 µA) was found to be significantly lower (P < 0.001) than the mean threshold for the Group 2 shanks (21.2 ± 11.5 µA). The mean dynamic range was found to be larger (P < 0.001) for the Group 2 shanks (6.67 ± 3.2 dB) compared with the Group 1 shanks (4.93 ± 2.7 dB) (Fig. 9, E and F; mean ± SD).

**Effect of broadness of tuning on frequency specificity**

Figure 10 shows the relationship between changes in electrical thresholds for all CIC sites from the threshold of a CF-matched VCN–CIC pair, plotted against their corresponding frequency differences from the VCN site in that pair. In cases where there was more than one CF-matched CIC site for a given VCN site, the change in threshold was calculated from the one that responded with the lowest threshold. From the acoustic response area of each CIC site, the Quality factor at 10 dB above threshold (Q10) was calculated as the CF divided by the bandwidth in kHz, as a measure of sharpness of tuning (Kelly et al. 1991). Sites that gave a Q10 ≤1 (n = 72) were classified as broadly tuned, whereas those with a Q10 >1 (n = 121) were classified as narrowly tuned. For the CIC sites that responded to VCN sites in the Group 1 shanks, both broadly tuned (Fig. 10, A1 and A2) and narrowly tuned (Fig. 10, B1 and B2) CIC sites showed a trend for threshold differences to increase with a rise in frequency difference (narrowly tuned, r = 0.41, P < 0.01; broadly tuned, r = 0.27, P < 0.01). For the Group 2 shanks, only the broadly tuned CIC sites exhibited this trend of increasing threshold differences with increasing frequency differences (r = 0.22, P < 0.05).

**Discussion**

In this investigation, we examined the spatial distribution of excitation within the CIC, evoked by electrical stimulation of...
selective VCN sites. Given that VCN neurons project both monosynaptically and polysynaptically to contralateral CIC neurons (Cant and Benson 2003), and in a tonotopic manner based on anatomical studies (Oliver 1987; Osen 1972), we expected that CIC neurons would be most sensitive to stimulation of VCN neurons with the same CF and, conversely, less sensitive to stimulation of neurons with different CFs. In support of this, a high degree of frequency specificity was seen in VCN–CIC pairs between the CFs of regions activated in the CIC with the lowest threshold and CFs of regions stimulated in the VCN. This frequency specificity was not only present at threshold levels but was also maintained across all current levels in our stimulation range. A major factor contributing to this high degree of frequency specificity was the placement of the electrodes within the VCN. Best results were obtained when electrodes were placed toward the medial, central, or posterolateral regions rather than more anterolateral regions of this nucleus.

The posterolateral and central placements would stimulate regions within the PVCN where a high concentration of T-stellate cells are found (Smith and Rhode 1989). Moreover, a high proportion of CIC sites exhibited maximum spike discharge to stimulation of the central and posterolateral VCN regions, which is consistent with a large number of T-stellate cell projections arising from these regions that correspond to the main excitatory inputs to the contralateral CIC. Medial and a few of the extreme lateral placements close to the free surface of the VCN would most likely stimulate the granule cell domain (Mugnaini et al. 1980a,b; Weedman et al. 1996). These cells are known to project to the fusiform cells (also known as pyramidal cells) of the DCN (Mugnaini et al. 1980b; Ryugo et al. 1995), which in turn project to the CIC (Berrebi and Mugnaini 1991). The medial extent of the granule cell domain is more developed in rats (Mugnaini et al. 1980b), thus increasing the likelihood of granule cell stimulation with medial shank placements in our experiments.

In contrast, the anterolateral placements would predominantly excite the AVCN, which is known to contain a high proportion of bushy cells (Harrison and Warr 1962; Osen 1969; Tolbert and Morest 1982a,b). Because bushy cells do not project directly to the CIC (Adams 1979b, 1983; Ryugo et al. 1981), higher levels of current would be required to evoke a CIC response for these anterolateral placements as current spreads to neighboring T-stellate regions. This indiscriminate activation of VCN T-stellate neurons may explain the high proportion of unmatched VCN–CIC pairs with lowest threshold from anterolateral VCN placements. This was manifested predominantly in narrowly tuned CIC neurons, whereas broadly tuned neurons were generally unaffected. This alludes to the possibility that narrowly tuned CIC neurons may receive projections from the narrowly tuned T-stellate neurons of the PVCN, whereas broadly tuned CIC neurons may receive polysynaptic projections from a wide variety of cells types, including those from the AVCN. Furthermore, a large proportion of these anterolateral placements did not elicit a response in CF-aligned VCN–CIC pairs and very few CIC sites responded with maximum spike discharge to stimulation of these regions. This suggests that the current spread from these placements was confined to the AVCN. It is possible that in those cases, even the highest current amplitude (54 μA) was not enough for current to spread to the central PVCN T-stellate regions.
From a CIC perspective, over a third of the CIC sites analyzed showed a moderate to high correlation between their acoustic tuning and electrical tuning. However, for CIC sites in the experiments that had central VCN placements, a poor correlation was observed (Exp 06.002 and 06.003). In these experiments, we found that although lowest threshold was obtained by stimulating a CF-matched site in the VCN, when VCN sites away from CF were stimulated, the thresholds did not increase substantially. One possible explanation for this could be related to the nature of current spread in the VCN. Central regions of the VCN are known to have a high proportion of AN fibers entering via the AN trunk (Arnesen and Osen 1978; Arnesen et al. 1978; Lorente de Nó 1933a,b; Osen 1970). The high probability of stimulating these fibers of passage may affect electrical tuning in the CIC at higher current levels.

Electrical stimulation thresholds obtained in our study were consistent with the results obtained by McCreery et al. (2000) who obtained CIC activation through chronic VCN stimulation at current levels as low as 6 µA. We also found that two VCN sites within an experiment that had the same CF as a CIC site, elicited different electrical threshold values and some VCN sites when stimulated, did not elicit any response in CF-matched CIC sites. This lack of stimulus driven activity in higher-order auditory structures resulting from stimulation of some CN sites was also noted by Takahashi et al. (2005) who observed a lack of auditory cortical activation from a large proportion of CN sites. In this paradigm, we made use of bipolar stimulation to minimize current spread across frequency laminae. It is possible that neurons within the CIC receive convergent input from several points within a VCN iso-frequency lamina. It is unknown whether it is sufficient to stimulate only a part of an iso-frequency lamina to convey meaningful frequency information in higher-order centers. There may be benefit in stimulating a greater number of sites within an iso-frequency lamina to maximize frequency-specific activation in the CIC.

Back-propagation of fibers may also influence the responses obtained within the CIC to electrical stimulation of the VCN. Multunit clusters in the CIC often exhibited a well-timed onset response to VCN electrical stimulation, which occurred at latencies in the order of 4–5 ms from stimulus onset. This time-locked onset response may be a result of antidromic activation of descending projections known to exist from the IC to the CN (Coomes and Schofield 2004; Faye-Lund 1988; Okoyama et al. 2006; Schofield 2001; Schofield and Cant 1999).
Implications for an ABI

Our study examined the degree of frequency specificity of CIC activation achievable by electrical stimulation of the VCN. The results of this study are directly applicable to the development of ABIs. We have identified the following four major implications from our research.

VCN ELECTRODE PLACEMENT. The need for the electrodes to access the tonotopic organization of the VCN is an important consideration in achieving better performance. Based on our results, it is evident that the placement of the implant array into the VCN is a critical factor in conveying frequency-specific information to higher-order brain centers. We have shown using a penetrating electrode array that medial, central, and posterolateral regions of the VCN when stimulated produce greater frequency-specific CIC activation compared with the anterolateral regions. This is consistent with McCreery et al. (1998) who showed that central regions of the PVCN are suitable for penetrating ABI implantation and McCreery et al. (2007) who recently showed that the rostrolateral and rostromedial region of the VCN when stimulated chronically elicited significantly lower degrees of CIC frequency specificity compared with the caudolateral and caudomedial VCN regions. Based on our results, if a surface array is placed on the posterolateral surface of the VCN in a dorsoventral direction using the present commercial ABI, some frequency-specific stimulation may be achievable but a penetrating array in the PVCN would result in an increased likelihood of frequency-specific CIC activation. However, a correct placement of the electrode array is not sufficient on its own to guarantee best results. One must consider the effects of deafness periods on the structure and function of the VCN. Hearing loss can evoke significant morphological and physiological changes within the auditory brain stem, and these changes become greater with duration of deafness (Hardie and Shepherd 1999). In particular, hearing loss is associated with a reduction in overall CN volume and an increase in neural density (Hardie and Shepherd 1999), which are likely to influence the effectiveness of the ABI.

CURRENT LOCALIZATION. We have shown some similarity between electrical and acoustic response areas in the CIC but this was not found in all cases. In fact, regardless of whether CIC neurons were broadly or narrowly tuned acoustically, they responded with similar thresholds to stimulation of VCN regions within a 2-kHz range for our classified Group 1 shanks, which were placed medially, centrally, or posterolaterally in the VCN. For the Group 2 shanks that were placed more anterolaterally, similar threshold CIC activation was within a 6-kHz range. Although bipolar stimulation of the VCN was implemented using electrode sites with relatively small surface areas, we did not always achieve localized, frequency-specific CIC activation. Perhaps more sophisticated methods like tripolar or even quadrupolar stimulation (Jolly et al. 1996) or using different pulse widths and shapes may be an advantage over bipolar stimulation. However, one must consider to what extent stimulation of passing fibers might be a limiting factor in achieving frequency-specific activation.

We realize that our results were obtained by stimulating a rat VCN, where the frequency laminae would be less widespread as compared with a human and, in addition, the electrode dimensions that we used in this study were in the order of micrometers instead of millimeters. Humans and several other...
primate species have their best hearing frequencies (indicated by audiograms) centered around 4–8 kHz and the upper hearing limit of most humans is centered around 17 kHz (Heffner 2004). This is in contrast to the rat, which has an upper hearing limit of ≤80 kHz (Kelly and Masterton 1977). Thus we would expect the human VCN to have a tonotopic map with frequencies shifted toward lower-frequency scales compared with those of the rat.

ABI POWER REQUIREMENTS. Our results using penetrating electrodes show that charge deliveries as low as 0.36 nC (3 μA, 120 μs per phase) are sufficient to elicit a frequency-specific response in the CIC. Present surface electrodes have been reported to elicit thresholds in the order of 2 nC (Colletti and Shannon 2005). CIC multiunit clusters in our study exhibited average spike rate saturation at current levels close to 30 μA. These results imply that a penetrating ABI would have more efficient power requirements.

FACILITATION OF INFORMATION TRANSFER. A final implication of our results is that not all acoustically driven VCN stimulation sites when electrically stimulated produced a response in the CIC. This implies a need for an electrode design that will incorporate sufficient redundancy to optimize performance. In addition, individual stimulation of two or more VCN areas with similar CFs often elicited different thresholds and different rate-level functions in the CIC. We propose that a greater number of electrode sites will not only provide sufficient redundancy but also allow stimulation of multiple sites within VCN isofrequency laminae to maximize frequency-specific CIC activation.

ACKNOWLEDGMENTS

We thank Dr. Conor Hogan from the chemistry department at La Trobe University for assistance in electrochemical activation of the electrodes. We also thank Dr. Karina Needham, R. Argent, and C. Suhr for assistance in histological verification of the electrode sites and comments on the manuscript.

GRANTS

This work was supported by the Garnett Passe and Rodney Williams Memorial Foundation and the Bionic Ear Institute. Research was conducted at the Auditory Neuroscience Laboratory at the School of Psychological Science, La Trobe University, Australia and the Auditory Clinical Neuroscience Unit, The Bionic Ear Institute, Melbourne, Australia.

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