Evidence of a Functionally Segregated Pathway From Dorsal Cochlear Nucleus to Inferior Colliculus

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Dorsal cochlear nucleus (DCN) evidence of a functionally segregated pathway from dorsal cochlear nucleus to inferior colliculus. J Neurophysiol 87: 1824–1835, 2002; 10.1152/jn.00769.2001. Type O units in the central nucleus of the inferior colliculus (ICC) of decerebrate cats are excited by best frequency (BF) tones near threshold, but are inhibited by high-level tones at all frequencies. Dorsal cochlear nucleus (DCN) principal cells display similar response map features and project directly to the ICC, and are thus supposed to be the dominant source of excitatory input for type O units. To test this hypothesis, the responses of type O units were compared before and after two pharmacological manipulations. When DCN to ICC axons were blocked by pressure injections of lidocaine, most type O units (~80%) were silenced or showed substantially reduced activity, but some units showed increased activity. All of the former units had low maximal rates to BF tones, whereas the latter units had high rates. When local circuit inhibitory mechanisms in the ICC were blocked by iontophoretic application of bicuculline or strychnine, type O unit responses also fell into two classes: low-rate units that showed increased spontaneous and driven activities and high-rate units that showed, in addition, altered response map features. Taken together, these results demonstrate that low-rate type O units are part of a functionally segregated pathway initiated by the DCN, whereas high-rate type O units are created at the level of the ICC.

INTRODUCTION

Single units in the central nucleus of the inferior colliculus (ICC) of unanesthetized decerebrate cats can be grouped into three distinct types (not including onset units) based on the patterns of excitation and inhibition evoked by contralateral tones of differing frequency and intensity (i.e., response maps) (Ramachandran et al. 1999). Type V maps exhibit a wide V-shaped excitatory area and no signs of inhibition; type O maps display an O-shaped island of excitation at low stimulus levels that is bounded by inhibition at higher levels. Based on resemblances to response maps in different lower-order nuclei, it has been speculated that type V units are shaped by excitatory inputs from the medial superior olive (MSO) (Goldberg and Brown 1969; Guinan et al. 1972), that type I units receive input primarily from the lateral superior olive (LSO) (Caird and Klinke 1983), and that type O units are derived from projections originating in the dorsal cochlear nucleus (DCN) (Spirou and Young 1991; Young and Brownell 1976). Consistent with this connectionist model, each ICC unit type shares the binaural response properties of its putative input (Davis et al. 1999; Ramachandran and May 1999a,b).

Several lines of evidence suggest, however, that the response map properties of ICC neurons depend on inhibitory as well as excitatory interactions. First, in vivo whole cell patch-clamp and intracellular recordings show that most ICC neurons receive synaptic inputs from both excitatory and inhibitory sources (Covey et al. 1996; Kuwada et al. 1997). Second, local GABAergic and glycnergic inputs are known to shape the tuning characteristics of ICC neurons (Fuzessery and Hall 1996; Le Beau et al. 1995; Palombi and Caspary 1996; Vater et al. 1992; Yang et al. 1992). In particular, blocking these inputs with their respective antagonists, bicuculline and strychnine, can result in ICC response maps showing broader excitatory tuning, a loss of sideband inhibition, or a significant upward expansion of closed excitatory areas. Finally, reversible inactivation of inhibitory nuclei that project to the ICC can lead to the expansion, or contraction, of excitatory areas in ICC unit response maps (Thornton and Rees 2001), suggesting that ICC response map features may reflect a complex interplay of convergent excitatory and inhibitory inputs.

The goal of the present study was to test directly the hypothesis that type O units receive a dominant excitatory input from DCN principal cells. The fidelity of this putative pathway was evaluated by comparing the responses of type O units before and after two different types of pharmacological manipulations: reversible inactivation of the output of the DCN by injection of lidocaine into the dorsal acoustic striae (DAS); and reversible blockade of local circuit inhibitory mechanisms in the ICC with bicuculline or strychnine. The results suggest that type O units can be divided into two distinct classes based on their maximum driven rates to best frequency (BF) tones. Type O units with low rates (~80% of the population) are often silenced when the DAS is blocked, and such units also retain their response map features under the influence of inhibitory antagonists. In contrast, high-rate type O units show increased activity after the DAS is inactivated and also show a loss of on-BF inhibition after the application of bicuculline or strychnine. These results thus support the idea that most, but not all,
type O units are part of a functionally segregated pathway initiated by the DCN.

**METHODS**

**Surgical preparation**

Adult cats (3–4 kg) with clean external ears and clear tympanic membranes were used under institutional animal care guidelines. Cats were premedicated with atropine (0.1 mg im) and anesthetized with xylazine (2 mg im) and ketamine (initial dose 40 mg/kg im; supplemental doses 15 mg/kg iv). Thereafter, core body temperature was maintained at 39°C with a feedback-controlled heating blanket. The cephalic vein was cannulated to allow administration of fluids, and a tracheotomy was performed to facilitate quiet breathing. The skin and temporalis muscles overlying the skull were reflected and the skull opened over the parietal cortex. Cats were decerebrated under visual control by aspirating through the thalamus; anesthesia was then discontinued. Both ear canals were transected to accept hollow ear bars, and the cat’s head was secured in a stereotaxic frame. The bullae were vented to prevent a buildup of static pressure in the middle ear. The left inferior colliculus was exposed by making a fenestration over the occipital cortex and aspirating the underlying cortical tissue. In some cases, partial removal of tentorium was required to increase accessibility. The right DAS was exposed by removing the skull below the nuchal ridge from the midline toward the right sigmoid sinus and aspirating the portion of the cerebellum above the floor of the fourth ventricle. At the end of experiments, cats were killed with an overdose of pentobarbital sodium (26 mg/kg iv). Some cats were perfused to allow histological confirmation of the completeness of decerebration and of the placement of pharmacological electrodes.

**Recording protocol**

Electrophysiological recordings were made in a double-walled, sound-attenuating chamber. Acoustic stimuli were delivered via electrostatic speakers that were coupled to the hollow ear bars. These closed-field acoustic systems were calibrated in situ with a probe tube microphone before each experiment and produced signals with a minimal variation in stimulus level (∆± 5 dB) from 40 Hz to 40 kHz. The test stimuli consisted of tone or broadband noise bursts, 200 ms in duration, with rise/fall times of 10 ms, and a presentation rate of 1 burst/s.

The activity of single units in the ICC was recorded with platinum-iridium metal electrodes. The signal from the electrode was amplified (10,000 to 30,000 times), band-pass filtered (100 Hz to 6 kHz), and displayed on an oscilloscope. A variable threshold Schmitt trigger was used to discriminate action potentials from background activity. Digitized spike trains were stored for off-line analysis by recording spike times relative to stimulus onset. Recordings were made before and after pressure injection of lidocaine into the DAS or iontophoresis of agents into the ICC. Only one type of manipulation was performed in a single experiment.

Hypodermic needles (30 gauge) were used to record from, and to deliver solutions of lidocaine hydrochloride (2%; Tech America) into, the DAS. These needles were tightly coupled to a 1-ml syringe to allow reliable injections of microliter amounts of lidocaine solution. Needles were initially placed in the DAS where its fibers are gathered in a discrete bundle to pass over the restiform body, and then moved medial toward the midline of the fourth ventricle to achieve maximum isolation from the intermingled fibers of the intermediate acoustic striae (Fernandez and Karapas 1967). Placement of the needle was guided by searching for noise-evoked background activity where the DAS was expected to be found. The needle was placed in a position judged to the center of the DAS (i.e., where there was the maximum background activity), and then left there for the entire experiment.

Piggyback multitarred electrodes (Havey and Caspary 1980) were used to deliver pharmacological agents in the ICC. These electrodes were made by gluing a three-barrel glass micropipette, with a tip diameter of 10–15 µm, approximately 10–15 µm behind the tip of the platinum-iridium recording electrode. Two barrels of the pipette were filled with strychnine hydrochloride and bicuculline methiodide solutions (each 10 mM, pH 3.5–4.0, Sigma). The third, balancing (or sum), barrel was filled with a pH-balanced buffer (pH 4.0, potassium hydrogen phthalate, CMS). The drug and balancing barrels were connected via silver-silver chloride wires to two microiontophoresis constant current generators (WPI, model 260) that were used to generate and monitor retention currents (20 nA, electrode negative) and ejection currents (50 nA, electrode positive).

**Data collection and analysis**

Recording electrodes were advanced dorsoventrally through the inferior colliculus, while 50-ms search tones or noise bursts were presented at the BF of the background activity. Entry into the ICC was indicated by a reversal from a decreasing to an increasing progression of BFs (Aitkin et al. 1975; Merzenich and Reid 1974) and by the prevalence of ICC response types that were identified in previous experiments (Davis et al. 1999; Ramachandran et al. 1999). When an ICC unit was isolated, its BF and threshold to contralateral tones were determined audiovisually, and then the following characterization protocol was initiated. Rate-level functions were obtained by sweeping the level of BF tones or noise bursts over a 100-dB range in 1-dB steps. Frequency response maps were created by sweeping the frequency of a sequence of tone bursts over a 4-octave range centered on unit BF. At a minimum, these sweeps were presented at levels of 10 and 40 dB re threshold, which is sufficient to classify unambiguously the receptive field properties of ICC units (Ramachandran et al. 1999). Each frequency-intensity combination was presented once.

Responses to acoustic stimuli were recorded after lidocaine was pressure injected into the DAS or inhibitory antagonists were iontophoresed into the ICC. In the case of the DAS experiments, a small volume (100 µl) of 2% lidocaine solution was ejected from the needle. Rate-level or rate-frequency functions were repeated until the lidocaine effects were gone (or the unit was lost). Whenever units were monitored long enough, their response rates returned to baseline levels within 30–60 min. Control experiments in which lidocaine-free saline was injected were not conducted. Therefore it is possible that the effects of injections are not due to the anesthetic effects of lidocaine, but are due to pressure or some other perturbation. For the purposes of this study, however, the cause of the disruption of ascending DCN influences is not of critical importance, as long as the effect is localized. The photomicrograph in Fig. 1 shows the location of the lidocaine electrode in one experiment. Note that the tissue damage caused by the injected lidocaine (enclosed by the circle) is confined to the right DAS (lines).

In the case of pharmacological manipulations in the ICC, the retention current was turned off and the ejection current was turned on for either bicuculline or strychnine. A minimum of 5 min was allowed for the drug concentration to stabilize before data were taken; the agents were applied continuously during data acquisition. Antagonist application was then discontinued and the cell allowed to recover to baseline activity, which usually required 20–30 min. In a few cases, the other antagonist was then applied, or occasionally both antagonists were applied simultaneously, and the process repeated. To ensure that response changes were due to the antagonists and not to the applied current or the low pH of the solutions, the buffer was sometimes ejected onto units. No effects were observed in these cases.

The responses evoked by auditory stimuli are described in terms of average steady-state rates. The minimal adaptation effects, stimulus-evoked rates were computed over the last 150 ms of the stimulus-on interval; and spontaneous rates were computed over the last 400 ms of the stimulus-off interval of each 1-s stimulation period. Excitatory (inhibitory) responses were defined as those for which the stimulus-
evoked rate was at least one SD above (below) the spontaneous discharge rate. All data were smoothed with a triangularly weighted moving average filter to reduce noise.

RESULTS

The effects of DAS inactivation were studied on 32 ICC neurons, including 22 type O units, in 9 cats. The effects of pharmacological blockade of inhibitory mechanisms within the ICC were studied on 17 type O units in 7 additional experiments.

Effects of DAS inactivation on ICC type O units

The effects of DAS inactivation were studied on 22 type O units; no effects were observed for 2 of these units. The responses of the remaining 20 units can be separated into 2 classes: those that showed a reduction in spontaneous and driven activities and those that showed an increase in these response properties. Figure 2A shows a partial frequency response map for a type O unit before and after pressure injection of lidocaine into the DAS. Each plot shows the unit’s discharge rate to pure tones as a function of frequency at a fixed sound level, given as an attenuation value at the right of the plot. Regions where the driven activity is consistently above the level, given as an attenuation value at the right of the plot.

Regions where the driven activity is consistently above the spontaneous rate (SR; horizontal lines) are excitatory areas (black fill), whereas regions with activity below the SR are inhibitory areas (gray fill). The vertical line through the response map is at the unit’s BF. Under control conditions (solid lines), type O response maps display an island of excitation at frequencies near BF and levels near threshold, and predominantly inhibition at higher sound levels. When lidocaine was applied to the DAS, the SR of the unit decreased from 30 to 0 spikes/s in approximately 100 s (Fig. 2B), and the unit lost entirely its tone-evoked responses (heavy dashed lines in Fig. 2A). One hour after the lidocaine injection, the map assumed its original features (dotted lines; Fig. 2A).

The BF-tone and noise rate-level functions in Fig. 2, C and D, offer an alternative method for visualizing the effects of DAS inactivation on type O units. In control conditions, the BF rate-level functions of type O units are highly nonmonotonic (solid line; Fig. 2C); as the level of the tone grows, the unit first exhibits a rate increase, then shows a strong rate decrease. Five minutes after the lidocaine injection (heavy dashed line), the response was zero at all stimulus levels. Similarly, the unit’s robust response to broadband noise under control conditions was completely eliminated when the output of the DCN was blocked (Fig. 2D). One hour after the injection, both rate-level curves assumed their original shapes (dotted lines).

The range of effects seen on the rate-level curves of type O units is illustrated in Fig. 3. A and B show two examples of units that, like the one in Fig. 2, show a strong decrease in driven activity, defined as a complete cessation in tone-evoked activity. The example in C shows a weak decrease in driven activity, in which the tone-evoked rate is reduced but not entirely eliminated. This arbitrary distinction is made to provide qualitative information about the magnitudes of responses in various unit types. The example in D shows an increase in driven activity after the lidocaine injection. Table 1 summarizes the DAS inactivation results by unit type, with the use of the response classes defined in Fig. 3. It is clear from the top row of Table 1 that a strong decrease is the most common response observed in type O units (10/22, 45%), and that a majority of units showed a decrease in activity (17/22, 77%) after the DAS was blocked. Units showing an increase in activity were less prevalent (3/22, 14%).

Type O units that showed a decrease in activity after DAS inactivation exhibited weak responses to BF tones, whereas type O units that showed an increase in activity were strongly excited by tones (e.g., compare Fig. 3, B with D). To demonstrate the association between initial response rates and response class, Fig. 4A shows a plot of each type O unit’s maximum driven rate for noise versus its maximum BF-tone driven rate under control conditions. The filled circles indicate the units that showed a decrease (strong or weak, see legend) in activity after the lidocaine injection, whereas the open circles indicate units that showed an increase in activity. The X’s mark units that failed to show a response to DAS inactivation. Units showing a decrease in activity gave relatively weak responses to tones (<55 spikes/s). In contrast, units showing an increase in response gave stronger responses to tones. A range of response rates to noise relative to tones was observed among units showing a decrease; all three units showing an increase had weaker responses to noise than to tones.

Figure 4B shows the magnitude of the effects of DAS inactivation on the response properties of type O units. These results are presented in terms of scale factors: the ratio of the maximum stimulus-driven rate during lidocaine injection versus control conditions. Each type O unit’s scale factor for noise is plotted against its scale factor for tones; the diagonal line indicates where the responses were equally affected by the manipulation. Fewer symbols are shown in this figure than are shown in Fig. 4A because the symbols overlie one another (e.g., 10 symbols at the origin), or the unit was lost before both rate-level curves were obtained (2 cases). Two trends are apparent in the data. First, all of the units that showed a strong decrease in their response to tones also showed a strong decrease in their response to noise (10 of 10 units; black filled
circle at the origin). Second, all of the units that showed a weak decrease in their response to tones showed a smaller decrease in their response to noise (gray filled circles above the diagonal). Tone and noise data were both obtained for only two units showing an increase in tone-driven activity, but those two also showed increases in their noise responses.

Effects of DAS inactivation on other ICC unit types

The responses to DAS inactivation were studied for two type V, four type I, and four onset units; qualitative results are summarized in Table 1. Representative data for each unit type are shown in Fig. 5. Type V units (Fig. 5A) showed weak decreases in both tone and noise-driven activities that were most pronounced at levels within 30 dB of threshold. Type I units showed similar weak effects near threshold (Fig. 5B), but tone and noise responses could be oppositely affected. In addition, some type I units, like the example shown, also exhibited decreases in SR after the lidocaine was applied. Onset units, on the other hand, tended to show the greatest effects in their noise responses. These units give one or two spikes at the onset of a tone burst, but sustained responses to noise (Ramachandran et al. 2000). As shown in Fig. 5C, the tone response is little affected by the application of lidocaine to the DAS, but the noise response decreased to almost one-half of its original value. A summary of the magnitudes of the effects of DAS inactivation on the tone and noise responses of these three unit types is given in Fig. 5D. In general, these unit types were less affected by DAS inactivation than type O units (compare with Fig. 4B) with none showing a complete loss of activity.

Effects of bicuculline and strychnine on ICC type O units

The effects of bicuculline (n = 12) and/or strychnine (n = 6) were studied on 17 type O units. All of these units exhibited changes (primarily increases) in their levels of spontaneous and stimulus-evoked driven activities under the influence of the inhibitory antagonists. In some cases, these changes were accompanied by alterations in the frequency response character-
istics of the unit. Thus type O responses to local inhibitory blockade can also be separated into two classes: those that show increased activity without affecting the basic response type ("rate change") and those that show, in addition to increases in discharge rate, a change in their response pattern ("pattern change").

Figure 6 shows data from two representative type O units that exhibited rate changes in their activity after iontophoresis of bicuculline (left column) or strychnine (right column). Notice that before the application of bicuculline, the response map in Fig. 6A displayed the characteristic type O low-level island of excitation that was bounded by inhibition at higher levels. Bicuculline increased the SR and low-level tone-evoked discharge activity of this unit but did not abolish the inhibition at high levels. In fact, the inhibition appears even stronger during GABAergic blockade because of the higher SR. Thus bicuculline had virtually no effect on the pattern of excitation and inhibition in the response map or on the shape of the rate-level function (Fig. 6B) but caused the maximum driven rates to increase almost twofold. On the other hand, bicuculline not only increased the maximum noise driven rate of this unit (Fig. 6B) but also eliminated its inhibitory response to high-level noise. Strychnine often produced similar changes in type O units (Fig. 6, C and D), although the magnitudes of the effects were usually smaller.

Examples of type O units that exhibited pattern changes under the influence of bicuculline (left column) or strychnine (right column) are shown in Fig. 7. In contrast to the units described above, the closed excitatory areas of these units were substantially expanded by the administration of bicuculline (Fig. 7A) or strychnine (Fig. 7C). The effect was most dramatic at higher stimulus levels near BF where inhibitory responses under control conditions were transformed into excitationary responses. With the elimination of most, if not all, of its inhibitory responses to tones, the bicuculline-altered map in Fig. 7A resembles that of a type V unit. Given its prominent inhibitory responses to off-BF tones, the strychnine-altered map in Fig. 7C resembles that of a type I unit. Consistent with these pattern changes, the units show strictly excitatory responses when BF

### Table 1. Effects of DAS inactivation on the BF-tone responses of ICC unit types

<table>
<thead>
<tr>
<th></th>
<th>Strong Decrease</th>
<th>Weak Decrease</th>
<th>No Change</th>
<th>Increase</th>
<th>Totals</th>
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</thead>
<tbody>
<tr>
<td>Type O</td>
<td>10</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>Type V</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Type I</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Onset</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4</td>
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</table>

DAS, dorsal acoustic striae; BF, best frequency; ICC, central nucleus of the inferior colliculus.
against its maximum tone-driven rate. The maximum driven rate for noise is plotted in Figs. 6 and 7. It is clear from the top row (Fig. 7, B) that tones or broadband noise are presented at high stimulus levels via bicuculline (10/12; 83%) or strychnine (4/6, 67%). In Fig. 8A, rate change is the most common response observed for either pharmacological agent, with the use of the response classes de changes in response magnitude to both stimuli.

Table 2 summarizes the inhibitory blockade results by pharmacological manipulation, whereas all three of the high rate units exhibited pattern changes.

Figure 8B shows the magnitude of the effects of inhibitory blockade on the maximum BF-tone and noise responses of type O units; summary data are in Table 2. Each type O unit’s scale factor for noise vs. its maximum driven rate for noise is plotted against its scale factor for tones; the diagonal line represents equal magnitude of effect. Several trends are apparent in the data. First, almost all of the scale factors are greater than one suggesting that the inhibitory blockers primarily increase the discharge activity of type O units. The second trend is that the bicuculline data points are broadly distributed along the line of equality, whereas the strychnine points appear to cluster along a horizontal line at a noise factor of about one. This latter result suggests that strychnine has little effect on the noise responses of units. Third, the effects of bicuculline on tone responses are greater than those of strychnine. Finally, units that show a pattern change exhibit among the largest releases from inhibition observed for units under the influence of a given antagonist.

DISCUSSION

The results described above suggest that most, but not all, type O units receive a dominant excitatory input from the DCN. In particular, type O units with low maximum driven rates to BF tones (~80% of the population) are often silenced when the output of the DCN is blocked with lidocaine, and such units also retain their DCN-like properties under local inhibitory blockade with bicuculline or strychnine. These data thus demonstrate that low-rate type O units are part of a functionally segregated pathway initiated by the DCN.

Origins of type O units

The general hypothesis underlying these studies is that ICC unit response map types (V, I, and O) differ because they reflect dominant inputs from different sources of excitatory inputs, not because they represent points along a continuum dependent on the balance of their excitatory and inhibitory inputs. In support of this connectionist model, anatomical evidence suggests that, in addition to its well-known tonotopic organization (Aitkin et al. 1975; Merzenich and Reid 1974), the ICC contains a regional or nucleotopic organization (Oliver and Huerta 1992). That is, evidence suggests that the diverse ascending projections that converge in the ICC terminate in separate, but overlapping, zones creating functional synaptic domains (Aitkin and Schuck 1985; Bruno-Bechtold et al. 1981; Maffi and Aitkin 1987; Oliver et al. 1997; Roth et al. 1978; Ryugo et al. 1981; Schneidman and Henkel 1987; Zook and Casseday 1987). Consistent with an organization of preferential inputs, type V, I, and O units share monaural and binaural response properties with principal cells in different lower-order nuclei, the MSO, LSO, and DCN, respectively (Davis et al. 1999; Ramachandran and May 1999a,b; Ramachandran et al. 1999). In the present study, in which pressure injections of lidocaine were used to inactivate in isolation the output tract of the DCN, it was thus anticipated that type O units would show the greatest reductions in their spontaneous and stimulus-driven activities.

When lidocaine injections were made into the DAS, type O units often exhibited a complete loss of spontaneous and driven J Neurophysiol • VOL. 87 • APRIL 2002 • www.jn.org
activities (10/22 cases; Figs. 2 and 3, A and B; Table 1). These results suggest that DCN principal cells, so-called type IV units (Spirou and Young 1991; Young and Brownell 1976), provide the dominant excitatory input to these units because the considerable amount of tissue between the injection site and recording electrode would not allow spread of the lidocaine directly to the ICC cell under study. Of course, it is likely that the injection pipette was in contact with fibers descending to the CN from more central auditory nuclei (Adams and Warr 1976; Elverland 1977; Kane and Finn 1977), but blocking the activity of efferents to the DCN would not alter the conclusion that the DCN was the source of the ascending input.

Inactivating the DAS may also block descending inputs into the posteroventral cochlear nucleus (PVCN) (Adams and Warr 1976). It is also possible that, despite the apparently restricted region of damage caused by the lidocaine injections (Fig. 1), lidocaine came into contact with its output fibers in the nearby intermediate acoustic striae (Fernandez and Karapas 1967), but blocking the activity of efferents to the DCN would not alter the conclusion that the DAS was the source of the ascending input.

Inactivating the DAS may also block descending inputs into the posteroventral cochlear nucleus (PVCN) (Adams and Warr 1976). It is also possible that, despite the apparently restricted region of damage caused by the lidocaine injections (Fig. 1), lidocaine came into contact with its output fibers in the nearby intermediate acoustic striae (Fernandez and Karapas 1967). Most of the principal cells in the PVCN have monotonic BF-tone rate-level functions and simple V-shaped response maps (Shofner and Young 1985), thus inputs from the PVCN would need to converge with inhibitory inputs at, or below, the ICC to endow an ICC neuron with type O unit properties. The results of the current study suggest that most type O units are not created by local inhibitory inputs (Table 2), while previous studies have shown that most lower-order targets of PVCN inputs, neurons in the intermediate and ventral nuclei of the lateral lemniscus, also have monotonic rate-level functions (Aitkin et al. 1970; Covey and Casseday 1991). Therefore PVCN inputs are not likely to provide the primary inputs to type O units.

The second most common effect of blocking the DCN’s input on type O units was to reduce, but not to zero, the tone-driven rates of some units (Fig. 3C; Table 1). These results could suggest that some type O units receive additional ascending excitatory inputs from a non-DCN source. For example, principal cells in the anteroventral CN occasionally show type IV unit properties (Shofner and Young 1985) and project directly to the ICC (Adams 1979; Osen 1972), and thus could contribute to the response of these units. On the other hand, the fact that the response properties of type O units that showed a weak rate reduction do not differ as a group from those of type O units that showed a strong rate reduction (Fig. 4A) argues against the necessity for a second input. Instead, the variability in rate effects may have resulted from nonoptimal positioning of the lidocaine electrode over the DAS, despite the small size of the structure. An ill-situated electrode could also explain the observation that two type O units showed no effects at all when lidocaine was injected into the DAS.

Interestingly, the tone and noise responses for incompletely
silenced type O units were not reduced in the same proportion after the DAS was inactivated; rather, the noise responses decreased comparatively less than the tone responses (Fig. 4B). These results suggest that type O units receive, in addition to DCN inputs, a source of wideband excitation. The source of this second input is unknown; however, it must respond weakly to tones so as to not affect type O unit response map properties. One possibility is onset units in the ICC because they respond with single onset spikes to pure tones, but exhibit sustained robust responses to noise bursts (Fig. 5C) (Ramachandran et al. 2000). The fact that onset units are driven, in part, by convergent inputs from the DCN (Fig. 5D), may also explain why type O units that show no tone-driven activity after the DAS is inactivated also show no noise-driven activity. However, there are other units that give weak tone responses and strong noise responses, including onset units in the cochlear nucleus, and their contribution via currently unknown pathways cannot be ruled out.

Some type O units showed enhanced activity after the DCN’s output was blocked (Fig. 3D). In contrast to the units that showed rate decreases, all of these units had high rates of driven activity to tones (Fig. 4A). These results suggest that type O units are not a homogeneous population, but can be divided into two groups: low-rate units that receive a dominant excitatory input from the DCN and high-rate units that receive net inhibition (via interneurons) from the DCN. A similar rate-based separation of type O units is suggested by their different responses to blockade of local inhibitory mechanisms (Figs. 6 and 7). Because maximum rate decreases with increasing BF for type O units (Ramachandran et al. 1999), this rate-based dichotomy thus suggests that low-BF type O units (≤3 kHz) are more likely than high-BF units to have a non-DCN origin. The fact that high-rate type O units show an increase in rate after the DAS is inactivated does not rule out the possibility that the DCN contributes excitatory inputs to such units, but it does suggest that indirect inhibitory inputs are stronger. This inhibition may be mediated by inputs from the nuclei of the lateral lemniscus (Saint Marie et al. 1997), which in turn are excited by collaterals from DCN axons (Fernandez and Karapas 1967; Osen 1972).

The effects of lidocaine injections on type V and type I units were studied on a small sample of units. Most of these units...
showed reductions in their tone-driven activities when the DCN’s input to the ICC was blocked. These changes were typically small, centered on BF, and usually most noticeable at low stimulus levels (Fig. 5, A and B), suggesting that these unit types receive a minority of DCN inputs with BFs that are similar to that of their predominant inputs. Consistent with these findings, recent projection studies have suggested that DCN inputs are tonotopically matched/aligned with other inputs to the ICC (Oliver et al. 1997). In some cases, type I units also showed reductions in their SR after the DAS was inactivated, suggesting that DCN inputs were net inhibitory. The role of DCN inputs to these unit types thus requires further study.

The effects of manipulating in isolation the pathway from the DCN to the ICC have also been measured previously in anesthetized cats (Semple and Aitkin 1980). In this earlier study, electrical stimulation was used to activate the DAS and thereby to identify ICC units receiving this input. Similar to the widespread effects observed in the current study, electrical stimulation evoked responses in many units. Most of the single units judged to have received a direct input from the DCN had high BFs, were excited by contralateral tones, and showed highly nonmonotonic BF-tone rate-level functions. In response to binaural tones, these units often showed excitatory/inhibitory (EI) interactions, but none demonstrated any sensitivity to delay in interaural phase. This constellation of properties matches closely that of type O units in the decerebrate cat (Davis et al. 1999; Ramachandran and May 1999a,b; Ramachandran et al. 1999). The results of the current study are thus consistent with these prior findings and suggest a direct pathway from DCN type IV units to ICC type O units.

### Table 2. Effects of bicuculline and strychnine on type O unit response properties

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<tr>
<th></th>
<th>Bicuculline</th>
<th>Strychnine</th>
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</tr>
<tr>
<td>Relative spontaneous rate</td>
<td>1.88</td>
<td>1.51</td>
</tr>
<tr>
<td>Relative max rate for BF-tones</td>
<td>2.23</td>
<td>1.58</td>
</tr>
<tr>
<td>Relative max rate for noise</td>
<td>2.06</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Table entries are average values. Relative changes in a response parameter due to a pharmacological agent are computed as the ratio of the parameter under the influence of the agent versus control conditions. BF, best frequency.
Effects of blocking inhibition on type O units

Consistent with previous neuropharmacological studies, iontophoretic application of either the GABA<sub>A</sub> antagonist bicuculline or the glycine antagonist strychnine altered the response properties of type O units in the ICC, including their SR and stimulus-driven rates (Faingold et al. 1989, 1991; Le Beau et al. 1996; Pollak and Park 1993; Vater et al. 1992; Yang et al. 1992). Bicuculline application increased the SR and maximum driven rates in 93% of type O units and strychnine in 100% of units, although bicuculline effects tended to be larger (Fig. 8A; Table 2). This disparity in the relative strength of GABAergic and glycinergeric inputs, which has also been observed in other species (guinea pig, Le Beau et al. 1996; bat, Vater et al. 1992), could reflect a difference in the proximity of the injection pipette and those particular synapses, or a difference in the relative density of GABA and glycine puncta in the ICC.

Increases in SR after the removal of local inhibition suggest that type O units receive tonic inhibition. Faingold et al. (1993) reported a large increase in the SR of ICC units in rat when input from the contralateral dorsal nucleus of the lateral lemniscus (DNLL) was blocked by injection of a GABA agonist, and therefore concluded that the DNLL is one source of tonic GABAergic inhibition. DNLL units in decerebrate cat are known to have high SRs (Davis 2001) and could thus mediate a similar effect in cat. A possible source of spontaneous glycinergeric inhibition is the LSO (Brownell et al. 1979). Increases in tone-driven rates after the removal of inhibition are larger than those for SR (Table 2), suggesting that GABAergic and glycinergeric inputs also provide a level-dependent inhibition to type O units. In contrast to their similar effects on SR and tone-driven activity, bicuculline produced large increases in noise-driven discharge rates, whereas strychnine did not (Fig. 8B). This observation suggests that the glycinergeric sources of input to ICC type O units respond stronger to tones than to noise.

For most type O units, application of bicuculline or strychnine did not alter the shape of their frequency response maps (Fig. 6), despite the significant increases in firing rates at all frequencies and levels that excited the cell. In the remaining cases, the unit’s response map showed dramatic changes in the patterns of excitation and inhibition such that the map then resembled that of a type V or type I unit (Fig. 7). Similar to the rate-based dichotomy found for type O responses to DAS inactivation, type O units that retained their response map shape under the influence of either inhibitory antagonist had low discharge rates to BF tones under control conditions, whereas those that showed a pattern change initially had high rates (Fig. 8A). These results suggest that low-rate type O units primarily reflect the basic monaural response properties of principal cells in a lower-order nucleus, most likely the DCN as argued above, while inhibitory inputs to these units regulate, among other properties, the magnitude of neural responses. In contrast, high-rate type O units are created at the level of the ICC by convergence of excitatory and inhibitory inputs, where the response maps of the underlying dominant excitatory inputs resemble those of principal cells in many brain stem nuclei including the ventral CN, MSO, and LSO.

In agreement with these observations, previous pharmacological studies have also shown that local inhibitory inputs may, or may not, play a role in forming the tuning characteristics of units with upper threshold (closed) tuning curves (Fuzessery and Hall 1996; Le Beau et al. 1995; Palombi and Caspary 1996; Pollak and Park 1993; Vater et al. 1992; Yang et al. 1992). One clear difference between these studies and the current study, however, is the proportion of units showing pattern changes after application of an inhibitory antagonist, particularly bicuculline. In two species of bat and chinchilla, over 80% of units with upper threshold tuning curves showed substantial upward expansion of their excitatory response areas in the presence of bicuculline (Palombi and Caspary 1996; Vater et al. 1992; Yang et al. 1992). Similarly in the guinea pig, over 50% of group II units, including those with closed tuning curves, showed dramatic changes in the size and shape of their excitatory response areas (Le Beau et al. 1995). In contrast, only 16% of type O units in decerebrate cat showed a pattern change after application of bicuculline. Considering the large difference in the prevalence of such unit types in the ICC of these other species (≤30%) (Le Beau et al. 1995; Palombi and Caspary 1996; Pollak and Park 1993) and cat (55%·
achandran et al. 1999), this discrepancy could reflect a species difference.

Alternatively, this discrepancy could reflect a methodological difference. In the previous pharmacological studies, all of the tonal stimuli were short duration (≤50 ms), thus the rate estimates were dominated by the onset component of the response. In contrast, the rate responses in this study were measured over the last 150 ms of a 200-ms stimulus and thus represent a steady-state rate. Under the influence of bicuculine, most type O units gave sustained responses to low-level BF tones, but only bursts of 5–10 spikes at the onset of high-level tones (up from 0–2 spikes in the control condition) followed by a complete cessation of activity for the duration of the stimulus. If the analysis of rate in this study is restricted to the first 50 ms of the stimulus, then the proportion of units showing substantial upward expansion of their excitatory response areas rises to 92%, which is comparable to the percentages observed in other species. The increased responsiveness at the onset of high-level stimuli clearly shows that inhibitory influences modulate the information processing capabilities of ICC type O units. However, the subsequent cessation of activity strongly suggests a DCN origin because projection neurons in other lower-order brain stem nuclei show significantly different responses to long duration stimuli (CN, Shofner and Young 1985; MSO, Goldberg and Brown 1969; Guinan et al. 1972; LSO, Caird and Klinke 1983).

Functional significance

The results of this paper suggest that type O units can be grouped into two distinct populations: a majority low-rate unit type (~80% of type O units) (Ramachandran et al. 1999) that receives a dominant excitatory input from DCN type IV units; and a minority high-rate unit type that is created in the ICC by the convergence of non-DCN inputs and inhibitory inputs. By virtue of receiving dominant DCN inputs, one likely role for low-rate type O units is that they are involved in sound-source localization. In the cat, the filtering properties of the pinna add prominent notches to the spectra of broadband sounds (Mus cant et al. 1990; Rice et al. 1992) that must be present for accurate localization of wideband stimuli (Huang and May 1996). DCN principal cells are uniquely sensitive to such spectral features and are thus thought to initiate a pathway specialized for the processing of spectral cues for sound localization (Imig et al. 2000; Nelken and Young 1994; Young et al. 1992). Consistent with this interpretation, lesioning the DAS causes cats to exhibit poorly directed sound localization behaviors (May 2000; Sutherland et al. 1998). Preliminary observations suggest, however, that low-rate type O units do not simply reflect the processing of their DCN inputs, rather additional excitatory and inhibitory inputs transform the ascending representation of spectral notches into a more selective representation of sound-source location (Davis et al. 2001). By contrast, the roles of high-rate type O units in audition are unclear because, in part, their dominant excitatory inputs are unknown. The signal processing pathways in which these and other ICC unit types participate remain to be studied.

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REFERENCES


ORIGINS OF ICC TYPE O UNITS

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