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Iontophoresis *In Vivo* Demonstrates a Key Role for $GABA_A$ and Glycinergic Inhibition in Shaping Frequency Response Areas in the Inferior Colliculus of Guinea Pig

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The processing of biologically important sounds depends on the analysis of their frequency content by the cochlea and the CNS. GABAergic inhibition in the inferior colliculus shapes frequency response areas in echolocating bats, but a similar role in nonspecialized mammals has been questioned. We used the powerful combination of iontophoresis with detailed analysis of frequency response areas to test the hypothesis that GABAergic and glycinergic inhibition operating in the inferior colliculus of a nonspecialized mammal (guinea pig) shape the frequency responses of neurons in this nucleus. Our analysis reveals two groups of response areas in the inferior colliculus: V-shaped and non-V-shaped. The response as a function of level in neurons with V-shaped response areas can be either monotonic or nonmonotonic. Application of bicuculline or strychnine in these neurons, to block inhibition mediated by $GABA_A$ or glycinergic receptors, respectively, increases firing rate primarily within the boundaries of the control response area. In contrast, neurons in the non-V-shaped group have response areas that include narrow, closed, tilted, and double-peaked types. In this group, blockade of $GABA_A$ and glycine receptors increases firing rate but also changes response area shape, with most becoming more V-shaped. We conclude that (1) non-V-shaped response areas can be generated by GABA and glycinergic synapses within the inferior colliculus and do not simply reflect inhibition acting more peripherally in the pathway and (2) frequency-dependent inhibition is an important general feature of the mammalian inferior colliculus and not a specialization unique to echolocating bats.

Key words: inhibitory neurotransmitters; inhibition; auditory system; microiontophoresis; inferior colliculus; frequency response area; GABA; glycine; guinea pig

Frequency analysis is fundamental to the processing of biologically significant sounds by the auditory system. This process begins in the cochlea (Helmholtz, 1863; von Békésy, 1963), and neurons in the inferior colliculus (IC), like those at other levels of the auditory pathway, are frequency selective (Rose et al., 1963; Merzenich and Reid, 1974; Aitkin et al., 1975; Semple and Aitkin, 1979; Ramachandran et al., 1999). The frequency response areas of many neurons in the IC are V-shaped, like auditory nerve fibers, but others are more complex, suggesting that they are shaped by inhibitory as well as excitatory inputs (Ehret and Merzenich, 1988). Neurons in the IC are recipients of both GABAergic and glycinergic inhibitory synapses. Whereas glycinergic inhibition in the IC originates extrinsically, GABAergic inhibition originates both extrinsically and intrinsically (for review, see Oliver and Shneiderman, 1991), with ~20% of neurons in the IC of cat considered to be GABAergic (Oliver et al., 1994). Single-cell recording alone cannot show whether inhibition operating within the IC shapes the frequency response areas of IC neurons or whether the response patterns reflect inhibitory processing at more peripheral levels in the pathway. A powerful means of resolving this issue is to combine neuronal recording in the IC with the iontophoretic application of inhibitory neurotransmitters or their antagonists. Studies using this method in the IC of the mustache bat found that application of the $GABA_A$ antagonist bicuculline produced broadening and other shape changes in the frequency tuning curves of ~40% of units (Yang et al., 1992). The majority of neurons tested were sharply tuned with a characteristic frequency of 60 kHz, the dominant frequency in the animal's calls. Similar findings were also reported in the IC of the horseshoe bat (Vater et al., 1992). In contrast, in chinchilla, bicuculline was reported to increase discharge rate primarily within the excitatory region existing before drug application (Palombi and Caspary, 1996), but because frequency responses were measured at a single intensity, changes in the shape of response areas could not be assessed. In addition, it has been argued that frequency response properties of neurons in the IC of cat reflect processing in more peripheral nuclei projecting to the IC (Ramachandran et al., 1999). It is important to discover, therefore, whether the modification of response areas by inhibition seen in the bat represents a unique specialization or is common to other mammals. Furthermore, the role of glycine in frequency processing in the IC has not been addressed.

The aim of this study was to determine whether $GABA_A$ receptor-mediated and glycinergic inhibition operating in the IC

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of a nonspecialized animal (guinea pig) influences the frequency responses of neurons in the IC. Using iontophoresis combined with a technique that provides detailed information about the frequency response area of a cell, we describe two broad classes of response areas for neurons in the inferior colliculus. In the first (V-shaped), both GABAergic and glycinergic inhibition modulate firing rate without changing response area shape. In the second group (non-V-shaped), inhibition within the IC is a major determinant of response area shape, as well as firing rate.

Parts of this work have been published previously in abstract form (Le Beau et al., 1994a,b).

MATERIALS AND METHODS

Surgical preparation, maintenance of the animal, procedures for single-unit recording, iontophoresis of drugs, and auditory stimulation were as described previously (Le Beau et al., 1996; Rees et al., 1997). In this account, only essential details of methods are given.

Anesthesia. Experiments were performed on adult pigmented guinea pigs (*Cavia porcellus*) of either sex weighing 300–800 gm. Two different anesthetic protocols were used. The first group of animals were anesthetized with urethane (1.5 gm/kg, i.p., in a 20% solution; Sigma, Poole, UK). Supplementary doses of urethane (0.5 gm/kg, i.p.) and phenoperidine (1 mg/kg, i.m.; Janssen Biochimica, Beerse, Belgium) were given as indicated. In the second group of animals, surgical anesthesia was obtained with a cocktail comprising 1 part Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; dose of 1.5 ml/kg, s.c.; Janssen Biochimica) and midazalem (Hypnoval; 1.5 ml/kg, s.c.; Roche Products, Hertforshire, UK) (Flecknell, 1996). Anesthesia was then maintained with α-chloralose (75 mg/kg, i.p.; Sigma) given every 2–3 hr as indicated. Atropine sulfate (0.05 mg/kg, s.c.) was given to all animals to minimize bronchial secretions.

Surgical preparation. The trachea was cannulated, and the animal was ventilated artificially with a small animal ventilator (Harvard Apparatus, Edenbridge, UK) when necessary. The animal's core temperature was monitored with a rectal probe and maintained at 37°C with a thermostatically controlled blanket (Harvard Apparatus). The animal was placed in a stereotaxic frame in which the ear bars were replaced by hollow speculi that seated securely in the external auditory meatuses. A midsagittal scalp incision was made, and the skull was exposed. A craniotomy was performed, and the dura was reflected to expose the cortical surface over the inferior colliculus. After electrode insertion, the exposed cortex was covered with a 2% agar solution to prevent desiccation.

Recording and iontophoresis. The recording electrode was advanced into the IC through the overlying cortex. Recording electrodes were glass-coated tungsten or, when iontophoresis was performed, glass electrodes attached to a multibarrel assembly (Stone, 1985; Le Beau et al., 1996). The recording pipette was filled with 2 M NaCl (resistance of 13–30 MΩ). One barrel of the seven barreled pipette, filled with 0.5 M NaCl, pH 3.5, was used for current balancing and to test for current and pH artifacts. The other barrels were filled with either 5 mM bicuculline methiodide, pH 3.0–3.5, or 10 mM strychnine hydrochloride, pH 3.0–3.5 (Sigma). Iontophoretic ejection and retaining currents were generated using a Neurophore BH-2 System (Medical Systems Corp., Greenvale, NY). Retaining currents of −15 to −12 nA were used for all drugs to prevent spontaneous drug diffusion from the tip. Ejection currents were usually in the range of 5–80 nA and never exceeded 200 nA. Drug barrel resistance could be tested during the experiment to identify blocked barrels.

Extracellularly recorded action potentials were amplified (10,000×) and filtered (0.3–3 kHz) by a preamplifier (Dam-80; World Precision Instruments, Aston, UK). The spikes were discriminated, converted to logic pulses, and time stamped to an accuracy of 10 μsec by a CED-1401 Laboratory Interface (Cambridge Electronic Design, Cambridge, UK). On isolating a single unit, the characteristic frequency and minimum threshold to contralateral stimulation were determined audiovisually.

Generation of frequency response area maps. The animal was situated inside a sound-attenuating booth, and stimuli were delivered through a calibrated, sealed acoustic system (Rees, 1990). Pure tones were shaped by trapezoidal waveforms with 5 msec rise–fall times and could be independently attenuated at the output to the transducers by a pair of digital attenuators. Frequency response areas for single neurons were obtained to either monaural or binaural stimuli. Binaural stimuli were presented at the same level to both ears and with zero interaural time delay. The method used here for the generation of response areas was similar to that described by Evans (1979). An audiovisual determination of the best frequency (BF) of a neuron was used to set the appropriate frequency range to be tested. The response area was constructed by counting the number of spikes elicited in response to 969 50-msec-tone bursts (repetition rate of five per second, 5 msec rise–fall time), which varied in 51 logarithmically spaced frequency steps and over an intensity range of 90 dB (in 5 dB steps). Tones were produced under computer control and presented in pseudorandom sequence. Order effects were minimized by adjusting the presentation sequence so that no tone was followed by another that was >40 dB lower in intensity. The number of spikes produced by each tone was counted and displayed on-line as a bar at the appropriate position in a plot of tone frequency versus attenuation level. The length of the bar was proportional to the number of spikes counted for each stimulus presentation. Response areas generated with a single presentation of each stimulus required ~4 min recording time. Some control recordings were, however, performed using multiple presentations of each stimulus to ensure that the maps obtained using one presentation provided an accurate representation of the response area of a neuron. In addition, because studies using iontophoresis require comparison of response areas up to 1 hr apart, we also made control recordings in some units at 25 min intervals to confirm that the response area pattern was stable over time in the absence of drug application.

To assess the expansion of the response area during the application of inhibitory antagonist, we measured the total area of the response areas in the control and drug conditions and calculated the percentage of change. The response markers on the response area plots are separated by equal fractions of an octave. To calculate the total area, we counted the number of driven response values in each intensity row of the response area and summed the values of all of the intensity rows to obtain the total area. In most cases, the edge of the response area was clearly discernible, but high spontaneous activity in some cases made it difficult to determine the edge. In these cases, we calculated the mean spontaneous rate by averaging the spike counts elicited by the stimuli at 90 dB attenuation, and the edges of the response area in each row of the plot were deemed to be the points at which the firing rate exceeded twice the spontaneous rate. In the iontophoretic experiments, control (predrug) recordings were followed by measurements during the application of the drug. Only data obtained after changes in activity had stabilized were used to determine any changes in response properties. Statistical analyses were performed using SigmaStat, and significance ($p < 0.05$) values were determined by a Student's t test, Wilcoxon signed ranks test, or Pearson's product correlation as specified in Results.

Histological verification of recording sites. At the end of each experiment, the animal was given a lethal dose of sodium pentobarbitone (Nembutal) and then perfused with a wash solution followed by fixative (Le Beau et al., 1996). Saggital or transverse sections were cut at 50 μm on a freezing microtome, stained with 0.1% cresyl violet, dehydrated, and covered. In experiments in which tungsten electrodes were used, the positions of recorded neurons and electrode tracks were marked with electrolytic lesions. In iontophoretic experiments, only two electrode penetrations were generally made in each colliculus to enable electrode tracks unequivocally to be identified. The position of individual recordings in a track was determined from depth and BF. Units were assigned to one of the three major subdivisions of the IC, the central nucleus of the IC (CNIC), the dorsal cortex of the IC (DCIC), or the external nucleus of the IC, as defined for guinea pig by Malmierca et al. (1995). In this study, we pooled neurons recorded from the CNIC and DCIC and, for convenience, refer to these locations collectively as the IC.

RESULTS

Classification of frequency response areas

Neurons were classified into one of seven types based on the shape and pattern of the frequency response areas. Monaural and binaural frequency response areas were recorded from a total of 177 neurons in the CNIC or DCIC. Two broad classes of response area were identified: V-shaped ($n = 136$) and non-V-shaped ($n = 41$), with the latter including narrow, closed, and tilted response areas. The characteristics of each group are described below, and examples of the different types are shown in Figures 1 and 2. For the quantification of the data obtained in this

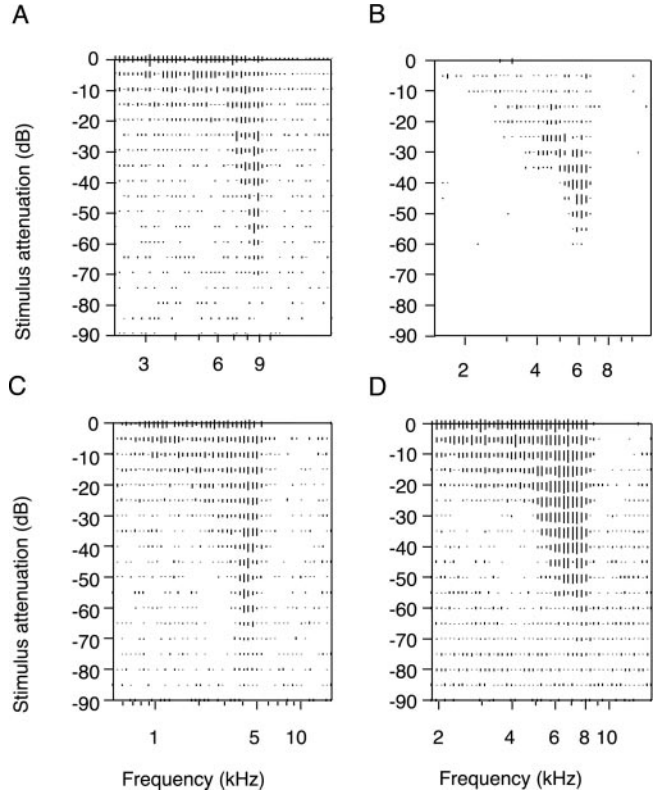

Figure 1. Frequency response maps for neurons with V-shaped response areas. Frequency response areas for a neuron with a monotonic V-shaped response area (*A*; BF of 9.0 kHz) and a neuron with a nonmonotonic V-shaped response area (*B*; BF of 6.0 kHz). *C*, V-Shaped response areas for two neurons with BFs of 4.1 (*C*) and 7.5 (*D*) kHz reveal the presence of inhibitory side bands.

study, we considered only responses obtained with contralateral monaural stimulation.

V-shaped response areas

(1) "Monotonic V-shaped" (Fig. 1*A*) response areas have a narrow tip at the BF of the neuron, and the range of frequencies to which they responded became progressively wider on both sides of BF as sound intensity was increased. In many cases, the response areas were not symmetrical on a log axis, and a response "tail" extended into the low-frequency region. The stimulus-evoked firing rate in these neurons did not show any marked reduction as stimulus intensity increased. Because of this monotonic increase in firing rate with sound level, we have termed these response areas monotonic V-shaped.

(2) "Nonmonotonic V-shaped" response areas (Fig. 1*B*) have a V-shaped response area similar to that described above. However, as stimulus level increased, there was a marked reduction in discharge rate (denoted by the shorter bar lengths) and, in some cases, a complete absence of a response to some frequencies that were effective at lower intensities. Because of this nonmonotonic change in firing rate with sound level, we have called these response areas nonmonotonic V-shaped.

In most neurons recorded here, spontaneous firing rates are low (less than three per second), but occasionally there was sufficient spontaneous activity to reveal the presence of inhibition around the excitatory response area (Fig. 1*C*,*D*). The region of inhibition occurred predominantly in side bands bordering the excitatory response.

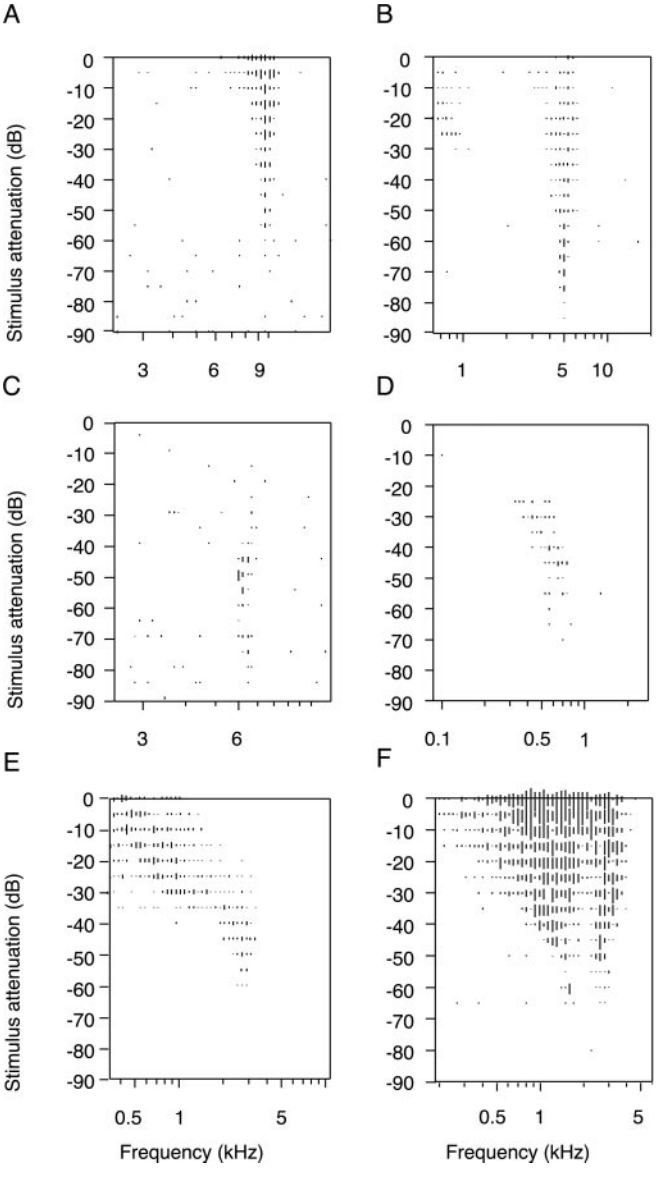

Figure 2. Frequency response maps for neurons with non-V-shaped response areas. Responses for two neurons with narrow response areas. The neuron in *A* has a single area of excitation at BF of 9.0 kHz, whereas the neuron in *B* has a narrow area of excitation at BF of 5.0 kHz but also a separate region of excitation at low frequency (<1.0 kHz). *C* and *D* show response areas for neurons with closed responses with BFs of 6.5 and 0.7 kHz. *E* shows a neuron with a low-tilt response (BF 2.7 kHz), and *F* shows a double-peaked responses with two BFs at 1.5 and 2.5 kHz.

Non-V-shaped frequency responses

(1) "Narrow" (Fig. 2*A*,*B*) response areas were defined by a limited expansion over frequency with increasing sound level. These response areas also lacked the low-frequency tail that was present in the response areas of most V-shaped neurons. They are similar to the "type I" of Ramachandran et al. (1999). However, in some neurons, an island of activity elicited at frequencies below BF was separated from the remainder of the excitatory response by a region in which the cell was silent at the intensities tested (Fig. 2*B*).

(2) "Closed" (Fig. 2*C*,*D*) response areas have a clearly circumscribed excitatory response area with no excitatory response above a particular stimulus level. These neurons have also been

Table 1. Proportions of frequency response area types recorded with urethane or chloralose anesthesia in the inferior colliculus of the guinea pig

	Urethane		Chlorolose	
Frequency response type	*n*	Percentage	*n*	Percentage
V-Shaped response areas				
Monotonic	76	72.4	35	48.6
Nonmonotonic	12	11.4	13	18.1
Subtotal (V-shaped)	88	83.8	48	66.7
Non-V-shaped response areas				
Narrow	6	5.7	15	20.8
Closed	2	1.9	5	6.9
High tilt	3	2.9	1	1.4
Low tilt	4	3.8	2	2.8
Double-peaked	2	1.9	1	1.4
Subtotal (non-V-shaped)	17	16.2	24	33.3
Total	105	100	72	100

termed "upper threshold" (Grinnell, 1963; Vater et al., 1979), because they possess both lower and upper intensity thresholds, below and above which the neuron fails to elicit an excitatory response. In the decerebrate cat preparation, Ramachandran et al. (1999) called similar response areas "type O." Some neurons with closed response areas responded only over an extremely restricted range of frequency and intensity, to the extent that only a small island of excitation occurred at BF (Fig. 2*D*).

(3) "Low- or high-tilt" (Fig. 2*E*) response areas also exhibit a nonmonotonicity of response with level, but unlike the nonmonotonic V-shaped responses described above, it is markedly asymmetric, resulting in a greater reduction in firing on either the high- or low-frequency side of the response area. The response areas, therefore, tilt toward the low frequencies (low-tilt) or high frequencies (high-tilt) at higher intensity levels. The tilt may be sufficiently marked so that there is no excitatory response at the BF of the unit at the highest stimulus intensities.

(4) "Double-peaked" (Fig. 2*F*) response areas have two tips of maximum sensitivity, separated by an area of reduced or no excitatory activity.

Neurons were classified into one of the above categories on the basis of their responses to monaural contralateral stimulation. Neurons with V-shaped response areas and all of the different non-V-shaped response areas were encountered in animals anesthetized with urethane or chloralose, although the proportions of the different types did vary between the two anesthetics (Table 1). The larger proportion of V-shaped response areas occurring under urethane may reflect some weakening of inhibition by this anesthetic compared with chloralose. Alternatively, other factors such as nonuniform sampling of units might contribute to this difference. In this study, therefore, we focus on the effects of inhibitory blockade in individual units rather than changes in the numbers of units in different groups.

V-shaped and non-V-shaped response areas occurred in neurons with best frequencies covering the range of frequencies studied (0.18–20.5 kHz). However, in view of the small number of neurons with some of the non-V-shaped response areas, we have not attempted a detailed analysis of response type as a function of best frequency.

The effect of iontophoretically applied inhibitory antagonists on frequency response areas

To assess the contribution of $GABA_A$ and glycine receptor-mediated inhibition to the generation of these different frequency response types, we assessed the effect of iontophoretic application of the $GABA_A$ receptor antagonist bicuculline and the glycine receptor antagonist strychnine in animals anesthetized with urethane. The effects of bicuculline were tested on 33 neurons (25 V-shaped and eight non-V-shaped), and strychnine was tested on 14 neurons (10 V-shaped and four non-V-shaped).

Effect of bicuculline and strychnine on neurons with V-shaped response areas

An example of the effect of bicuculline on the response of a neuron with a V-shaped response area is shown in Figure 3. Compared with the control condition (Fig. 3*A*), application of bicuculline (Fig. 3*B*) results in a marked increase in the stimulus-evoked firing rate across the whole of the response area. The firing rate fully recovers to its original level once ejection of bicuculline is discontinued (Fig. 3*C*). In this example (Fig. 3*A*), the spontaneous firing rate of the neuron was unaffected by the application of bicuculline, despite the changes in stimulus evoked activity. We used two different analyses to illustrate any changes in firing rate and response area after iontophoresis of bicuculline: "subtraction" and "drug-only" response plots (Fig. 4). This figure shows the control response (Fig. 4*A*) and the response in the presence of bicuculline (Fig. 4*B*) for another neuron with a V-shaped frequency response area. The result of subtracting the control response from the bicuculline response (Fig. 4*C*) represents the increased firing rate that occurred with the blockade of $GABA_A$ receptor-mediated inhibition. This subtraction plot shows that an increase in firing rate was evident for all combinations of frequency and intensity that elicited a stimulus-evoked response in the control condition. In the drug-only response plot (Fig. 4*D*), only frequency–intensity combinations that elicited an excitatory response with bicuculline, but not in the control condition, are plotted. In other words, this plot highlights any expansion of the stimulus-driven response area. In this example, there was no expansion of the response area with bicuculline, and, therefore, only residual spontaneous activity is apparent on the plot.

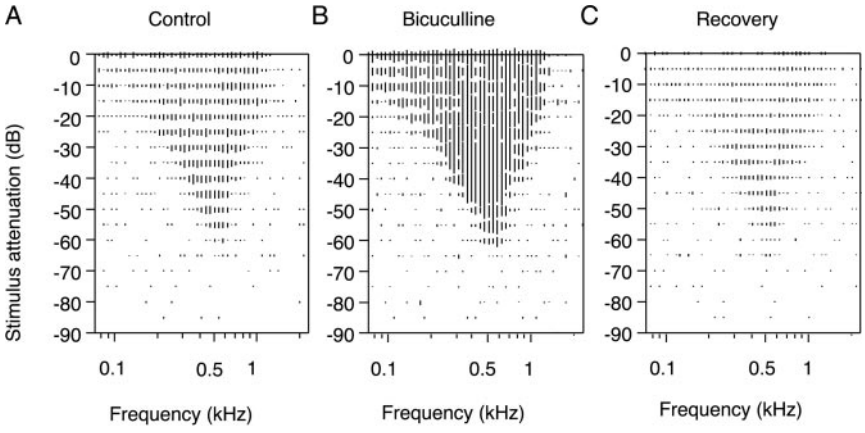

Figure 3. Effects of bicuculline on neurons with V-shaped response areas. Control frequency response area (*A*) reveals a V-shaped response area (BF of 0.6 kHz). In the presence of the iontophoretically applied $GABA_A$ receptor antagonist bicuculline, the stimulus-evoked firing rate increased (as evidence by the increased *line length* on the plot), but there was no change in the shape of the response area. These effects were reversed (*C*) once ejection of bicuculline ceased.

Occasionally, units had sufficient spontaneous activity to reveal the presence of inhibitory side bands on either side of the excitatory response area (Fig. 5*A*). In these cases, there was an increase in firing rate within the existing excitatory response region when bicuculline was applied but only limited expansion into the inhibitory side-band region (Fig. 5*B*). Spontaneous firing did, however, increase in the side-band regions with bicuculline.

In total, 16 of 25 neurons with V-shaped response areas showed only small, below criterion (<20%) increases in the size of their response area (see Materials and Methods). In the remainder of the neurons with V-shaped response areas ($n = 9$), changes of 20–126% were observed in the size of the response area. However, all neurons ($n = 25$) showed some increase in discharge rate with increases ranging from 18 to 786%, with a median of 112%. Interestingly, the effects on response area and discharge rate appeared to occur independently because there was no correlation ($p > 0.05$) between the magnitude of the increases in response area with changes in mean discharge rate. In addition to changes in response area size and discharge rate, bicuculline application also caused a reversible 5–15 dB reduction in threshold in 5 of 25 neurons with V-shaped response areas. Such a change in threshold is demonstrated in the response shown in Figure 6, in which there is a 15 dB reduction in threshold at BF and some expansion in the response area, particularly in the region of the low-frequency tail. This neuron and the other four that changed threshold contribute to the group of 9 of 25 neurons with V-shaped response areas that showed modest changes in response area after application of bicuculline. Despite the change in area, the response area remains V-shaped. In contrast to the response areas shown in Figure 3, this neuron also displayed a noticeable increase in spontaneous as well as stimulus-evoked firing in the presence of bicuculline.

The application of strychnine to block glycinergic inhibition

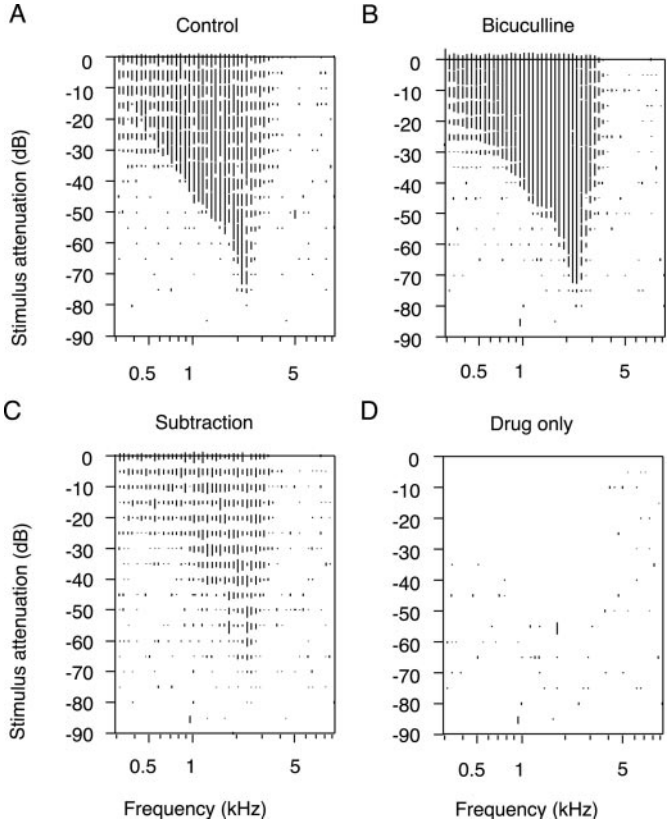

Figure 4. Neurons with V-shaped response areas show little expansion with bicuculline. A V-shaped response area (BF of 2.5 kHz). Control (*A*) and with bicuculline (*B*). Bicuculline causes an increase in discharge rate within the response area but little expansion of the area. The subtraction plot (*C*) shows the difference between control and bicuculline responses and represents the increase in firing rate. The drug-only plot (*D*) shows that bicuculline did not produce any change in the size of the response area.

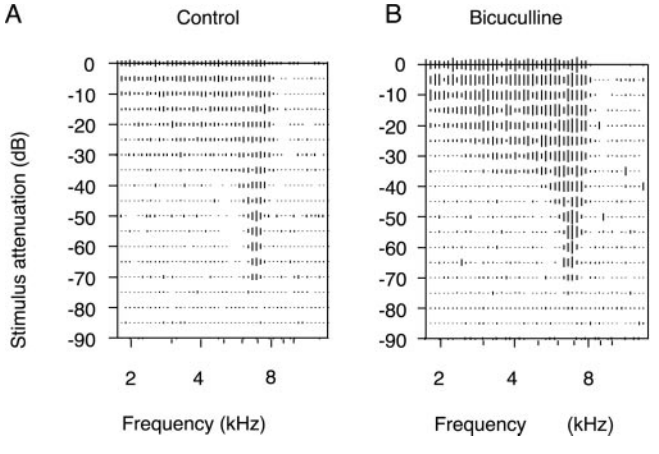

Figure 5. Bicuculline does not block inhibitory side bands. *A*, A V-shaped response area from a neuron (BF of 7 kHz) with sufficient spontaneous activity to reveal the presence of inhibitory side bands on both the low- and high-frequency side of the response area. *B*, Application of bicuculline caused an increase in firing rate within the excitatory response area but did not abolish the inhibitory side bands.

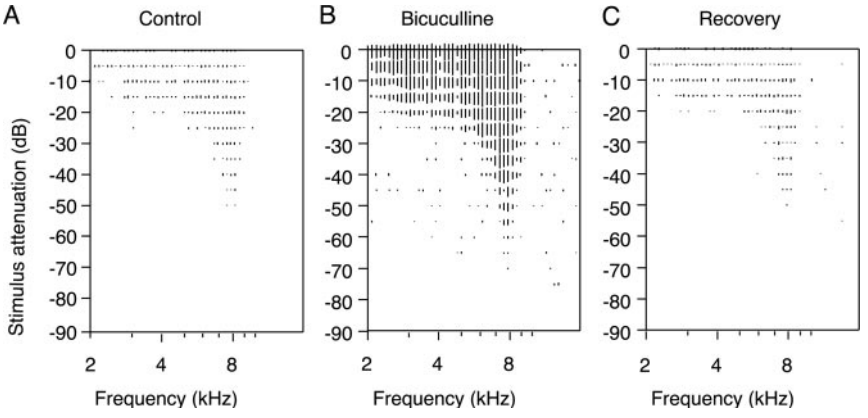

Figure 6. Bicuculline causes a reduction in threshold. A neuron (BF of 8.0 kHz) with a V-shaped response area in control (*A*) shows a marked increase in discharge rate with bicuculline (*B*) and also a 15 dB decrease in threshold. Both of these changes are fully reversed on recovery (*C*).

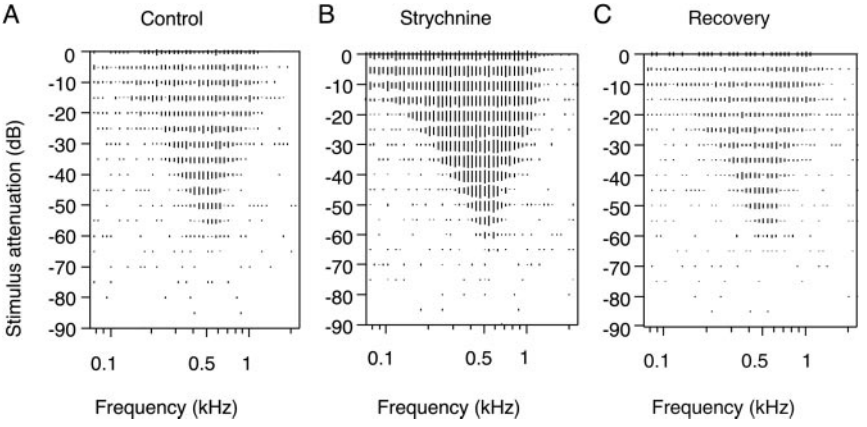

Figure 7. Effects of strychnine on neurons with V-shaped response areas. *A* shows a V-shaped control response for the same neuron as illustrated in Figure 3. Strychnine application (*B*) caused an increase in discharge rate but no change in response area. The effects of strychnine were fully reversed (*C*) once application ceased.

produced qualitatively similar effects to the blockade of $GABA_A$ receptor-mediated inhibition. The response areas in Figure 7 are from the same neuron as that shown in Figure 3. As with the bicuculline, strychnine caused an increase in stimulus-evoked activity across the whole response area, but there was no expansion of the response area and no change in the spontaneous rate. In total, 9 of 10 neurons with V-shaped response area showed small increases (<20%) in the size of the response area after application of strychnine, with only one neuron showing a 30% increase. Strychnine also caused a 5–10 dB reduction in threshold in 4 of 10 neurons.

Our results show that both $GABA_A$ and glycine receptor-mediated inhibition operates on V-shaped neurons, as evidenced by the changes in stimulus-evoked activity produced with bicuculline and strychnine, respectively. Importantly, this inhibition does not alter the shape of the response area and, in the majority of neurons, does not sharpen frequency tuning.

The effects of bicuculline and strychnine on non-V-shaped frequency response areas

In contrast to the changes in neurons with V-shaped frequency response areas, more dramatic changes in the size and shape of the response area were found when inhibition was blocked in neurons with non-V-shaped response areas.

A neuron with a narrow control response area (Fig. 8*A*) was sharply tuned around BF (5 kHz) with very little increase in width at higher intensities, but there was also a second, isolated region of excitation at low frequencies (<1 kHz). Application of bicuculline produced a marked increase in stimulus-evoked firing in both of these areas of excitation and also revealed that there was an excitatory input at all frequencies in between (Fig. 8*B*). Thus, blockade of $GABA_A$ receptor-mediated inhibition converted the narrow response into a V-shaped response area. There was also an increase in the spontaneous rate of the neuron in the presence of bicuculline. The subtraction plots for this neuron (Fig. 8*C*,*D*) again show that stimulus-evoked firing increased over the whole of the control response area, but new areas of excitatory response are also revealed in the drug-only response plot. The most dramatic changes in response area shape occurred for closed units. The highly restricted control response area (Fig. 9*A*) was substantially expanded with bicuculline to give a V-shaped response area (Fig. 9*B*). In a second neuron (Figs. 9*D*,*E*), the control response (determined audiovisually) was almost undetectable and therefore not collected, but a low-tilt response appears in the presence of bicuculline (Fig. 9*D*). The highly restricted excitatory response becomes evident on recovery (Fig. 9*D*). When strychnine was applied to the same neuron after recovery from bicuculline, a similar low-tilt response was again revealed (Fig. 9*F*).

For the results described here so far, the changes in response area had stabilized in the presence of the antagonists. However, Figure 10 illustrates the progressive expansion of a closed response area after application of bicuculline. Before application of bicuculline, this neuron responded over a very limited range of frequencies and intensities centered on BF, as is evident in the recovery response area (Fig. 10*D*). Interestingly, it can be seen that an excitatory response is first revealed at the edges with a progressive infilling of the response. This suggests that inhibition is strongest at the best frequency of the neuron, although we cannot exclude the possibility that diffusion of bicuculline from the electrode tip may result in the blockade of some inhibitory inputs sooner than others. The nonmonotonicity that persists,

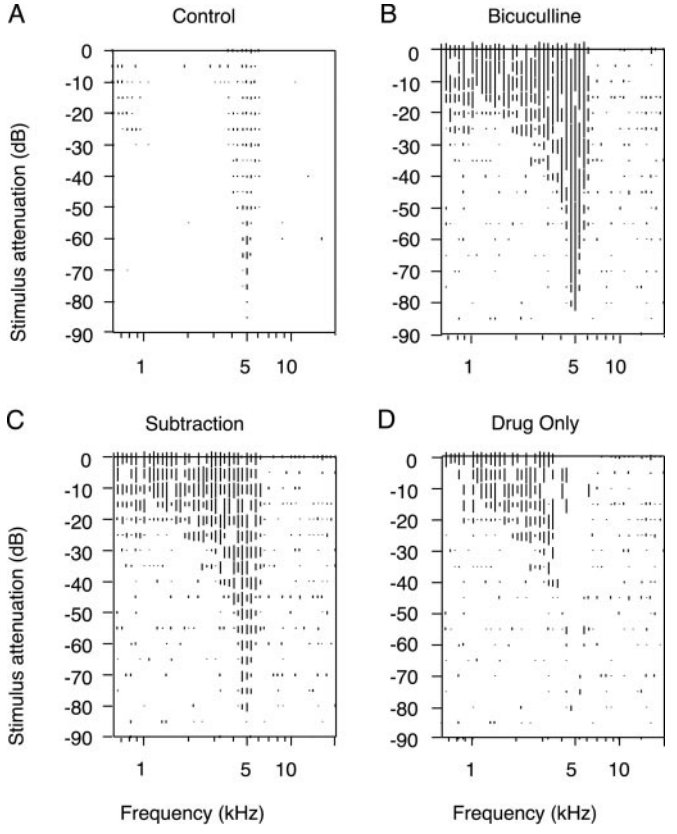

Figure 8. Effects of bicuculline on a neuron with a narrow response area. *A* shows the control response area for one neuron with a narrow response area at BF separated from a smaller area of excitation at low frequency. Application of bicuculline (*B*) caused an increase in firing rate within the existing excitatory areas but also an expansion of the response area to create a V-shaped response area. The subtraction plot (*C*) shows the difference between control and bicuculline response maps, and the drug-only plot (*D*) shows the new areas of excitation revealed by blockade of $GABA_A$ receptor-mediated inhibition. The neuron was lost shortly before completion of the response area collected under bicuculline, and some data points were not collected.

even in the presence of bicuculline, may reflect either incomplete blockade of GABAergic inhibition or a process mediated by glycinergic inhibition. Alternatively, the nonmontonicity may be present in the excitatory input.

The effect of bicuculline and strychnine on frequency tuning and response area size

Conventionally, the sharpness of frequency tuning of auditory neurons is described by measurement of Q_{10} values (Kiang et al., 1965; Evans, 1972), defined as the characteristic frequency divided by the bandwidth at 10 dB above threshold. This measure originally introduced for auditory nerve fibers is limited in that it does not provide information about changes in bandwidth at higher intensities. For neurons in more central auditory nuclei, therefore, Q values at other intensities, e.g., $Q_{30,}$ are often taken (Suga et al., 1997). To assess the effect of bicuculline and strychnine on the sharpness of tuning, we measured Q_{10} and Q_{30} for neurons with V-shaped response areas and, when possible, those with non-V-shaped response areas. Comparison of the Q_{10} values for neurons with V-shaped control response areas ($n = 25$) before and during application of bicuculline suggests that, for most neurons, there was little difference in the Q_{10} between the two conditions (Fig. 11*A*). Statistical testing using the Wilcoxon

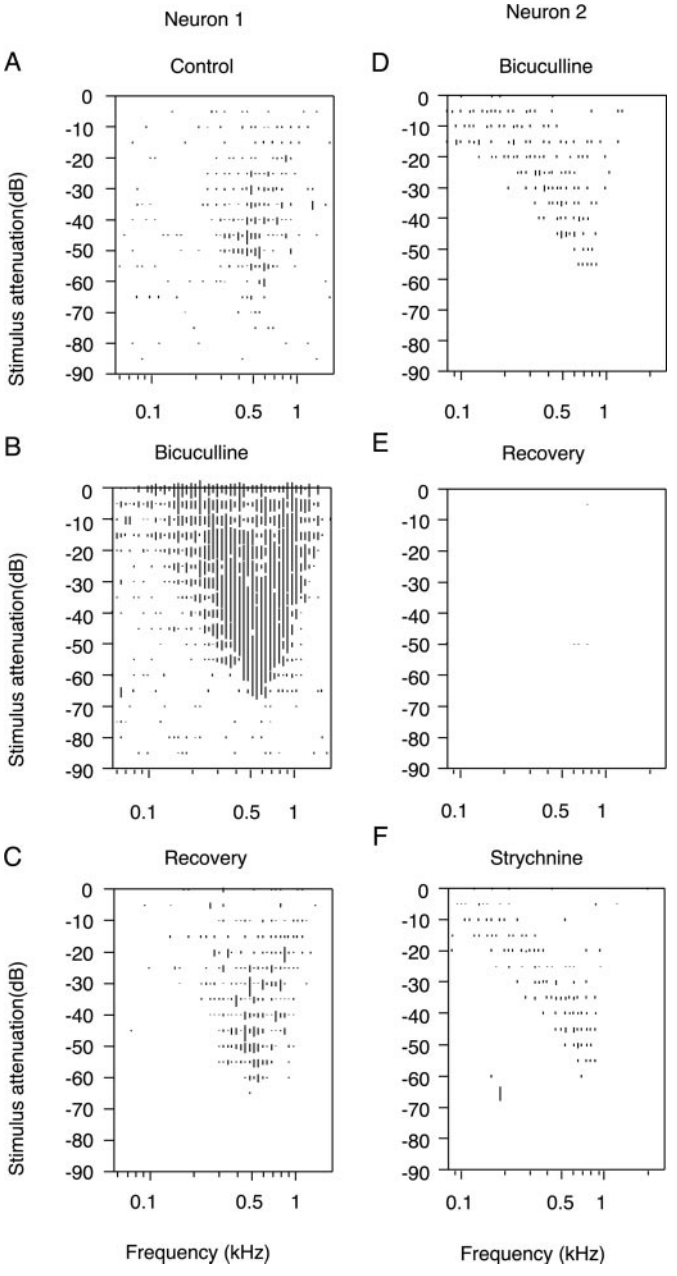

Figure 9. Effects of bicuculline and strychnine on neurons with non-V-shaped response areas. *A*, *Control*, shows a closed response area that, in the presence of bicuculline (*B*), expanded to become V-shaped. The effects are fully reversible once bicuculline application is discontinued (*C*). In a second neuron, application of bicuculline (*D*) reveals a low-tilt response, but, on recovery from drug application, only a small area of weak response remains with just three frequency–intensity combinations at ~0.7 kHz evoking any excitatory response (*E*). Subsequent application of strychnine (*F*) to the same neuron reveals a similar low-tilt response.

signed ranks test confirmed that this difference was not significant ($p > 0.05$). In some V-shaped neurons ($n = 17$), a Q_{30} could also be measured, but again there was no statistical difference between the control and bicuculline values ($p > 0.05$). $GABA_A$ receptor-mediated inhibition does not, therefore, cause any increase in sharpness of tuning, as determined by the Q_{10} and Q_{30} values, for neurons with V-shaped response areas. For neurons with non-V-shaped response areas (Fig. 11*A*), there was also no statistical difference between the control measurements of Q_{10} and those

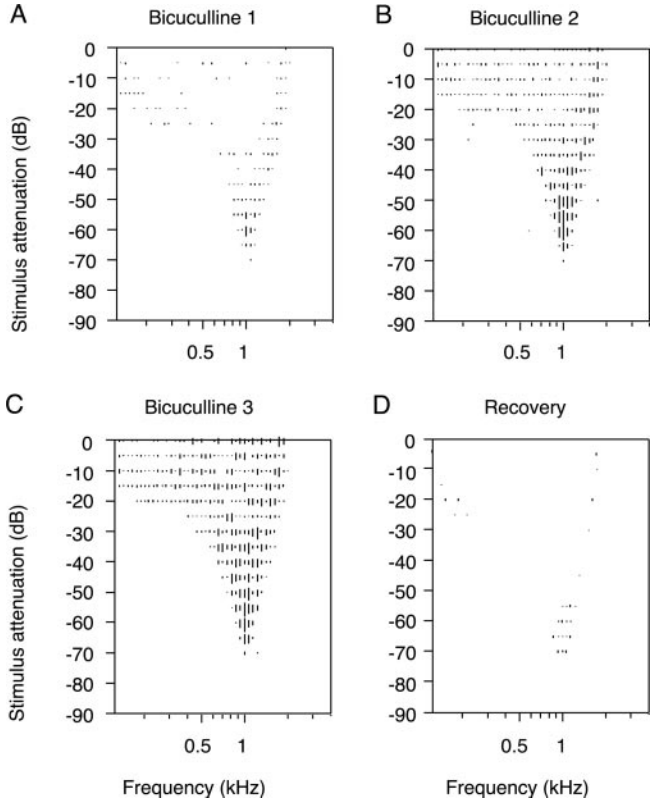

Figure 10. Bicuculline reveals excitation at the edge of response area first. For a neuron with a closed response area, response areas measured at increasing times after the onset of bicuculline application (*Bicuculline 1–3*; *A–C*) progressively reveal a V-shaped excitatory response area, with activity at the edge of response area emerging first. When bicuculline application is ceased (*D*), only a very small island of excitation at the BF and a few responses at the edge of the area yield an excitatory response.

measured during the application of bicuculline ($p > 0.05$). Q_{30} could not be measured in enough neurons with non-V-shaped response areas (for example, under control conditions, closed neurons with no excitatory response 30 dB above threshold yield an infinite Q_{30}) to permit statistical analysis. However, it is clear that substantial changes in the size of the response area do occur with bicuculline (Figs. 8–10) and strychnine (Fig. 9) in neurons with non-V-shaped response areas that are not reflected by these values of Q_{10} and Q_{30}. To quantify these changes, we took a measure of the total area (see Materials and Methods) of the frequency response area. For neurons with V-shaped response areas, bicuculline produced a median change of 13.8% ($n = 25$) change in area, whereas the median change for those with non-V-shaped responses was 114% ($n = 8$) (Fig. 11*B*). This difference was significant ($p < 0.05$). The number of neurons tested with strychnine was too small for meaningful statistical analysis, but similar changes were obtained (Fig. 11*C*). In the presence of strychnine, two of four of the non-V-shaped response areas showed changes of >100%, whereas the largest change for the V-shaped group ($n = 7$) was 30%.

DISCUSSION

By combining iontophoresis with detailed mapping of frequency response areas in the inferior colliculus of the guinea pig, we show that GABAergic inhibition in the IC controls the firing rate of all neurons and shapes non-V-shaped response areas in this nonspecialized mammal. This finding is important because it shows that mechanisms reported previously only in echolocating bats (Vater et al., 1992; Yang et al., 1992) are a general feature of the mammalian inferior colliculus. In addition, we show, for the first time, that glycinergic inhibition can contribute to the shaping of frequency response areas.

Neurons in the IC in guinea pig can be divided into two broad groups on the basis of their frequency response properties: neurons with V-shaped response areas and those with non-V-shaped response areas. The firing rate in V-shaped response areas either increases monotonically as a function of intensity or, less frequently, nonmonotonically. Non-V-shaped response areas include closed, narrow, and tilted types. The non-V-shaped response areas seen here are similar to those described in the IC in several other species (Ehret and Merzenich, 1988; Vater et al., 1992; Yang et al., 1992; Ramachandran et al., 1999) and at other levels in the auditory pathway (Sutter, 2000), suggesting that they are generic and fundamental to sound processing. Recent intracellular studies (Covey et al., 1996; Kuwada et al., 1997) demonstrate that frequency-dependent inhibition does influence the frequency responses of neurons in the IC and could be responsible for shaping these response area types recorded extracellularly. By their nature, however, such studies do not provide detailed information about the relationship between inhibition and the response area.

Our findings in guinea pig are at variance with the hypothesis of Ramachandran et al. (1999) based on response areas recorded in decerebrate cat. In their experiments, V-shaped response areas only occurred at low frequencies, and they hypothesize that their type O (closed in our study) and type I (narrow in our study) response types are created by inhibition in more peripheral nuclei in the pathway, which is simply reflected in responses recorded in the IC. Similarly, our findings contrast with a preliminary report of iontophoretic studies in the decerebrate cat (Davis, 1999), which found, except in the case of some type O neurons, that the

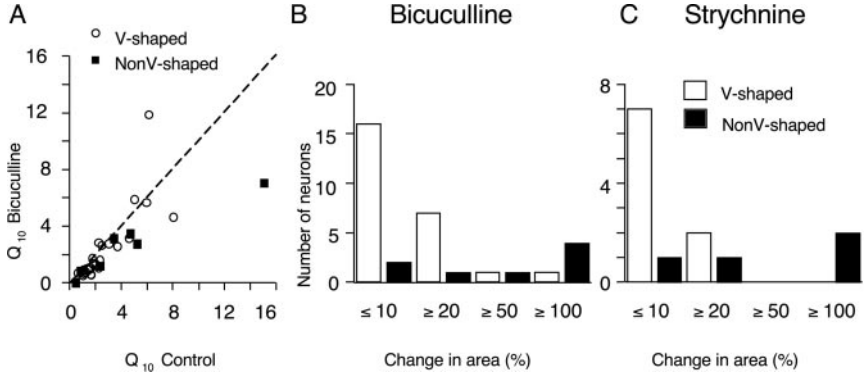

Figure 11. Bicuculline does not alter Q values but increases the area of response. Scatter plot for Q_{10} (*A*) in control and bicuculline for V-shaped (○) and non-V-shaped (■) show that bicuculline did not cause any significant sharpening of tuning. Measurements of the percentage of change in area with bicuculline (*B*) and strychnine (*C*) for V-shaped (*white bars*) and non-V-shaped (*black bars*) response areas shows that there is a significant broadening of non-V shaped response areas with bicuculline.

shapes of response areas in the IC did not change when inhibition was blocked. In our study, all neurons with non-V-shaped response areas became V-shaped or showed marked expansion in the presence of either bicuculline or strychnine, thus suggesting that non-V-shaped response types can be generated *de novo* within the IC by inhibition acting on neurons with V-shaped response areas. The reasons for the differences between these studies are unclear but may reflect the differences in species and preparation. In the medial geniculate nucleus of the mustache bat, an expansion of narrow response areas has also been reported with blockade of GABA receptors (Suga et al., 1997), suggesting that this response type is created at more than one site in the pathway.

Inhibition is not just important for shaping non-V-shaped response areas; it modulates the responses of all IC neurons. Neurons with V-shaped response areas receive GABA and glycinergic inhibition, as evidenced by their increased stimulus-evoked activity in the presence of bicuculline or strychnine. However, for these neurons, changes in firing rate are predominantly restricted to frequencies falling within the control response area. The increases in area seen in V-shaped response areas (9 of 25) can primarily be accounted for by a reduction in threshold at BF, abolition of any nonmonotonicity, or a small expansion at the edges of the response area as subthreshold excitatory inputs near the edges become suprathreshold when inhibition is removed (Fig. 6). Similar changes have been reported in the mustache and horseshoe bat (Vater et al., 1992; Yang et al., 1992).

One interpretation of these results in V-shaped response areas is that the frequency tuning of the inhibition is similar to the excitatory input of the neurons (Palombi and Caspary, 1996). In addition, some neurons with V-shaped response areas might receive a tonic, untuned inhibitory input that operates as a gain control similar to that described in the ventral cochlear nucleus (Evans and Zhao, 1993). Our data support the former because the inhibitory response area generated by subtracting the control response from the bicuculline response (Fig. 4) shows that inhibition is strongest in the center of the response area and falls off toward the edges. However, an element of tonic inhibition may also be present because application of bicuculline in some neurons produced changes in spontaneous activity as well as driven rate.

Interestingly, when V-shaped response areas were flanked by inhibitory side bands (Fig. 5), the application of bicuculline did not result in an abolition of the side bands by expansion of the excitatory region into the inhibitory area. We were unable to test these spontaneously active units with strychnine, but, as with bicuculline, the remaining neurons with V-shaped responses showed little expansion at the edges of excitatory response area with strychnine (Fig. 7). These findings suggest that the excitatory response in neurons with V-shaped response areas does not overlap the inhibitory side bands, confirming that the side bands were not, at least in these examples, suppressing an excitatory input at those frequencies. This conclusion is consistent with intracellular recordings in bat showing that inhibitory currents at frequencies on either side of the excitatory range occur in the absence of excitatory input (Covey et al., 1996).

In contrast to the V-shaped response areas, neurons with non-V-shaped responses generally showed larger changes in area, and in all cases, these were associated with a change in the shape of the response area. In the majority of cases, the response areas became more V-shaped with the application of antagonist, and, as with the V-shaped response areas, there was an increase in firing rate over the whole of the response area. Our findings in guinea pig are consistent with results obtained from neurons tuned to 60 kHz in mustache bat (Yang et al., 1992).

We also demonstrate that glycinergic inhibition influences frequency response areas in the IC, and an interesting feature is the similarity of the effects of strychnine to those of bicuculline. Such similarities have been observed previously for other aspects of sound processing. For example, in various bat species, bicuculline and strychnine had similar effects on the processing of binaural interactions (Klug et al., 1995), frequency modulation (Koch and Grothe, 1997), and duration tuning (Casseday et al., 2000) in the IC. However, different effects of bicuculline and strychnine on the responses of IC neurons have also been reported. In the horseshoe bat, changes occurred in excitatory tuning curves in the presence of bicuculline but not with glycine (Vater et al., 1992). GABAergic inhibition in the IC is of intrinsic and extrinsic origin, but glycinergic inhibition originates from the superior olivary complex (SOC) and ventral complex of the lateral lemniscus (VCLL) (for review, see Malmierca et al., 1998). The SOC is involved in binaural processing, and it is tempting to speculate that, in addition to its role in temporal processing (Covey and Casseday, 1991), VCLL is involved in determining frequency response areas in the IC.

We grouped non-V-shaped response areas together because the shapes of these response area types are all sculpted by inhibition. However, such a grouping may also be appropriate because the different patterns appear not to be discrete entities but rather represent points on a continuum of inhibitory effects. Thus, in the presence of bicuculline, a neuron with a closed response area becomes a low-tilt or a nonmonotonic V-shaped response, suggesting that different response types reflect different strengths of inhibitory input. Two types of inhibition appear to contribute to this shape change. In closed response areas, there is a strong intensity-dependent inhibition. In some cases, firing occurs over a wide range of intensities, but the response declines with intensity. In others, the degree of inhibition is so strong that the neuron only fires over an intensity range of a few decibels close to best frequency (Fig. 10). The progressive expansion of the excitatory response area of this closed response area begins at the edges, suggesting that inhibition is strongest toward the center of the response area. In contrast, inhibition in units with narrow response areas is most effective in frequency regions more remote from BF (Fig. 8). This pattern and that of the tilt response types raise the possibility that neurons with higher- or lower-frequency best frequencies contribute to their inhibition (cf. Yang et al., 1992). The precise shape of response area observed (e.g., narrow or tilt) might depend on the difference in BF between the recorded neuron and its inhibiting neighbors or the shapes of the response areas contributing the inhibition. Nevertheless, firing rate increases over the whole response area when inhibition is blocked, suggesting that either a second source of inhibition is present or there is overlap of the interacting response areas. Evidence supporting a role for across-frequency inhibition in the generation of narrow response areas comes from the expansion of their excitatory regions after exposure to traumatizing tones at frequencies above the BF of the neuron (Wang et al., 1996). This technique does not identify where in the pathway the effect of the traumatizing tone occurs, but our results with bicuculline demonstrate that inhibition in the IC shapes these response areas and suggests that inhibitory connections between different frequencies operate within the IC. Extensive local connections have been

reported within and between different frequency-band laminas in this nucleus (Oliver et al., 1991; Malmierca et al., 1995).

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