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Features of contralaterally evoked inhibition in the inferior colliculus

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Abstract

Cells in the central nucleus of the inferior colliculus (ICc) receive a large number of convergent inputs that are not only excitatory but inhibitory as well. While the excitatory responses of ICc cells have been studied extensively, less attention has been paid to the effects that inhibitory inputs have on auditory processing in the ICc. The purpose of this study was to examine the role of contralaterally evoked inhibition in single ICc cells in awake Mexican free-tailed bats. To study the contralaterally evoked inhibition, we created background activity by the iontophoretic application of the excitatory neurotransmitters glutamate and aspartate and visualized the inhibition as a gap in the carpet of background activity. We found that 85% of ICc cells exhibit a contralaterally evoked excitation followed by a period of inhibition. The inhibition acts primarily through $GABA_A$ receptors since the application of bicuculline eliminated or greatly reduced the inhibition in all cells. The inhibition has two parts: an early part which is coincident with the tone stimulus and a later persistent component which outlasts the tone stimulus by tens of milliseconds. The persistent inhibition typically is level-dependent, increasing in duration with increasing sound level. The persistent inhibition is also sensitive to the duration of the stimulus, with short (5 ms) tones being less effective than longer (>20 ms) tones in generating persistent inhibition. While the early inhibition has clear roles in the shaping of excitatory response properties to a stimulus, the later persistent component of the inhibition is more enigmatic. The fact that the persistent inhibition lasts well beyond the duration of excitatory inputs to the ICc cell implies that the persistent inhibition may be important for the temporal segregation of the responses to multiple sound sources. © 2000 Elsevier Science B.V. All rights reserved.

Key words: Inferior colliculus; Inhibition; Iontophoresis; Bicuculline; Strychnine; Glutamate

1. Introduction

The inferior colliculus is a nexus in the ascending auditory pathway in that the projections from the vast majority of lower auditory nuclei, whether monaural or binaural, converge at a common destination in its central nucleus (ICc) (reviewed by Aitkin, 1985; Oliver and Huerta, 1992). The projections not only provide excitatory innervation, but inhibitory innervation as well. The inhibitory innervation is both glycinergic and GABAergic and is at least as prominent as the excitatory innervation of the ICc (Saint Marie et al., 1989; Shneiderman and Oliver, 1989; Shneiderman et al., 1988; Winer et al., 1995).

The ICc has been the subject of numerous neurophysiological studies in which the discharge properties of single neurons were monitored with extracellular electrodes (e.g., Aitkin, 1985; Casseday et al., 1997; Irvine, 1986; Pollak and Casseday, 1986; Pollak et al., 1977a,b,c; Pollak and Schuller, 1981; Rose et al., 1963; Semple and Kitzes, 1987; Spitzer and Semple, 1993; Yin, 1994). Those studies directed far greater attention to the excitatory responses than to the inhibition of ICc neurons because it is the discharges evoked by excitation that are seen with extracellular electrodes. While it is recognized that inhibition also occurs at the ICc, with extracellular recordings there is always uncertainty about whether any suppression of discharges is due to inhibition at the ICc cell itself or whether the inhibition is occurring in a lower nucleus and is simply imposed on the ICc cell. Direct demonstrations that inhibition shapes ICc response properties came from studies in which inhibition was blocked by antagonists (Casseday et al., 1994; Faingold et al., 1989b, 1991;

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Fuzessery and Hall, 1996; Klug et al., 1995; Le Beau et al., 1996; Park and Pollak, 1993; Pollak and Park, 1993; Vater et al., 1992) or where post-synaptic potentials (PSPs) and currents were monitored with sharp and patch-clamp electrodes (Covey et al., 1996; Kuwada et al., 1997; Nelson and Erulkar, 1963; Pedemonte et al., 1997; Torterolo et al., 1995).

While the pharmacological and intracellular studies have revealed much about inhibition, each of those techniques has its strengths and limitations. Iontophoresis of drugs that antagonize inhibitory receptors, for example, clearly reveals the presence of inhibition. However, the ability to evaluate inhibition is somewhat limited because the only inhibition revealed is that inhibition that occurs simultaneously with the excitation. Intracellular and patch-clamp recordings offer direct insights into the integration of excitatory and inhibitory PSPs, but there is a low yield of stable recordings with these techniques, where investigators typically record only one or two cells per animal. Moreover, a problem particular to in vivo patch-clamping is that electrodes clog easily and successful recordings apparently can only be made from cells within a few hundred micrometers of the surface of the ICc (Covey et al., 1996).

To obtain more detailed information about the inhibition evoked by monaural stimulation of the contralateral and ipsilateral ears, as well as by binaural stimulation, we conducted a series of studies on the ICc. We employed a technique that affords some advantages of both intracellular and extracellular recordings by iontophoresing the excitatory transmitters glutamate and aspartate (Glu/Asp) thereby creating a background of firing in ICc cells. We did this because ICc cells normally have little or no spontaneous activity. This technique allowed us to visualize periods of inhibition during and after a stimulus, features that can be seen in intracellular and patch-clamp experiments. The inhibition appears as a gap in the carpet of Glu/Asp evoked background activity. This technique also offers the advantage of extracellular recordings in that it provides a much higher yield of cells and is not limited to the depth in the ICc at which the recordings can be made. In addition, since the background activity is created in the ICc cell which is being recorded, the suppression of background activity must also be occurring within that ICc cell, thus localizing the inhibition to the ICc and not to a lower nucleus. Finally, with multibarrel micropipettes, pharmacological agents such as blockers of inhibitory neurotransmission can be applied to the cell allowing us to determine which inhibitory transmitter contributed to the different components of the evoked inhibition (Faingold et al., 1989b, 1991; Klug et al., 1995; Park and Pollak, 1993; Pollak and Park, 1993; Vater et al., 1992).

The experimental subjects in these studies were Mexican free-tailed bats. We chose this bat as a subject because it uses a variety of acoustic signals for both echolocation and social communication and has a relatively hypertrophied auditory system making it well suited for studies of the brain's processing of acoustic cues. Moreover, several laboratories have used Mexican free-tailed bats in behavioral studies (Balcombe and McCracken, 1992; Gelfand and McCracken, 1986; McCracken, 1984; Schmidt and Thaller, 1994; Simmons et al., 1978, 1979) as well as anatomical (Grothe et al., 1994) and neurophysiological studies of their brainstem auditory nuclei (Bodenhamer and Pollak, 1981; Grothe and Park, 1998; Grothe et al., 1997; Park et al., 1996, 1998; Pollak et al., 1977a,b,c, 1978). Here we report on both the excitation and inhibition evoked in ICc cells by stimulation of the contralateral ear, with particular attention directed at evaluating inhibitory responses and how inhibition is affected by changes in stimulus parameters. Inhibition evoked by ipsilateral and binaural stimulation is the subject of another report (Klug et al., 1999).

2. Methods

2.1. Surgical procedures

Fifteen Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, were used in this study. Prior to surgery, each animal was anesthetized with methoxyflurane inhalation (Metofane, Pitman-Moore, Inc.). The hair on the head was removed with a depilatory, and the head was secured in a head holder with a bite bar. The muscles and skin overlying the skull were reflected and lidocaine (Elkins-Sinn, Inc.) was applied topically to all open wounds. The surface of the skull was cleared of tissue, and a foundation layer of cyanoacrylate and small glass beads was placed on the surface. A small hole was then drilled around the center portion of the inferior colliculus using the landmarks visible through the skull for orientation.

The bat was transferred to a heated recording chamber, where it was placed in a restraining cushion constructed of foam molded to the animal's body. The restraining cushion was attached to a platform mounted on a custom made stereotaxic instrument (Schuller et al., 1986). A small metal rod was cemented to the foundation layer on the skull and then attached to a bar mounted on the stereotaxic instrument to ensure a uniform positioning of the head. A ground electrode was placed between the reflected muscle and the skin. Recordings were begun after the bats recovered from the anesthetic. The bats typically lie quietly in the restraining cushion and show no signs of pain or discomfort. Supplementary doses of the neuroleptic Vetamine (Mal-

linckrodt Veterinary) were given if the bat struggled or otherwise appeared in discomfort.

Using visual landmarks viewed with an operating microscope, the electrode was then positioned on the surface of the brain on top of the inferior colliculus. Subsequently, the electrode was advanced from outside of the experimental chamber with a piezoelectric microdrive (Burleigh 712IW). Neurons were sampled at a depth between 400 and 1600 μm.

2.2. Electrodes

In all experiments 'piggy back' multibarrel micropipettes were used for recordings and iontophoresis of drugs (Havey and Caspary, 1980). Single barrel micropipettes were pulled to a tip diameter less than 1 μm and blunted under microscope observation so that the tip diameter was 1–2 μm. A multibarrel electrode was pulled from a five barrel blank (H-configuration, Omega dot, Glass Co. of America) and the tip blunted so that the tip diameter of the multibarrel array was 15–20 μm. The single barrel pipette was attached to the five barrel pipette under microscopic observation and glued with cyanoacrylate so that the tip of the single barrel pipette protruded about 10–15 μm from the broken tip of the five barrel pipette. The single barrel micropipette was used for recording and filled with buffered 1 M NaCl and 2% Fast green (pH 7.4). Electrode impedances ranged from 5 to 15 MΩ. Fast green was used to enhance the visibility of the electrode for placement in the small hole made in the skull. One barrel of the five barrel pipette was the balancing (sum channel) barrel which was also filled with buffered 1 M NaCl and 2% Fast green (pH 7.4). The remaining four ejection barrels were filled with different drugs as follows: GABA (500 mM, pH 3.5–4.0, Sigma), bicuculline methiodide (10 mM, pH 3.0, Sigma), strychnine hydrochloride (10 mM, pH 3.0, Sigma), and a cocktail of glutamate and aspartate (each 500 mM, pH 8.0, Sigma).

The drug and balancing barrels were connected via silver-silver chloride wires to a six channel microiontophoresis constant current generator (Medical Systems Neurophore BH-2) that was used to generate and monitor ejection and retention currents. The sum channel that connected to the balancing barrel was employed to balance current in the drug barrels and reduce current effects. The recording barrel was connected by a silver-silver chloride wire to a Dagan AC amplifier (model 2400) for analysis of single unit activity.

2.3. Acoustic stimuli and data acquisition

Sine waves from a Wavetek function generator (model 136) were shaped into tone bursts with an analog switch (Restek Model 15). The tone bursts were 20 ms in duration and had 0.5 ms rise-fall times. Stimuli to both ears were delayed by 20 ms from the start of data acquisition, using a Binaural Pulse Delay (Restek Model 110). Stimuli were presented at a rate of 4/s which was controlled by a Restek Model 45 Real Time Clock which also timed spike events for the peri-stimulus time (PST) histograms. Tone burst frequency was monitored by a frequency counter. A 24 bit digital interface NuBus card (National Instruments DIO-24) and a digital distributor (Restek model 99) connected a Power Macintosh 8500/120 computer to the Restek equipment and a two channel digital attenuator (Wilsonics, model PATT). The output of each independently controlled channel of the attenuator was sent to two 1/4 inch Brüel and Kjaer (B&K) microphones biased with 200 V DC and driven as speakers. At the start of each experiment, speakers were inserted into the funnels formed by the bat's pinnae, and positioned adjacent to the external auditory meatus. The pinnae were folded onto the housing of the microphones and wrapped with Scotch tape. The acoustic isolation with this arrangement was at least 40 dB.

Only well isolated spikes with a high signal-to-noise ratio were studied. Action potentials were fed to a window discriminator (Frederick Haer and Co.) and then to the real time clock. The Macintosh 8500 computer read the spike data from the real time clock and generated PST histograms in real time. Upon encountering an ICc neuron, its best frequency (BF) and its threshold at the BF were determined by audio-visual inspection. The frequency of the tone burst was then set at the neuron's BF, and a rate-intensity function was acquired at intensities ranging from threshold to 50 dB above threshold. All stimuli were presented in a pseudo random order. PST histograms were generated from the spikes evoked by 20 presentations of each stimulus in the predrug condition.

When drugs were not being ejected, a retention current of 15–30 nA of appropriate polarity was applied to each drug barrel to prevent leakage of drugs. When drugs were ejected, the polarity of the retention current for that drug barrel was reversed. For each neuron, GABA was first applied where the ejection current was progressively increased until the neuron was completely inhibited. The purpose of applying GABA was to ensure that drug ejection could influence the discharges of that neuron. If GABA could not completely inhibit the neuron, or if current effects could be observed, recordings were stopped immediately and the electrode was replaced.

The roles of GABAergic and glycinergic inhibition were tested initially by applying the $GABA_A$ receptor antagonist, bicuculline methiodide, or the glycine receptor antagonist, strychnine hydrochloride, with low (10 nA, electrode positive) ejection currents while ob-

taining rate-intensity functions. During the application of each antagonist, rate-intensity functions were repeatedly taken until the shape of the function and maximum spike count stabilized. The ejection current was then increased and the procedure repeated until the maximum spike count no longer increased. The final currents ranged from 10 to 80 nA. At the end of the experimental protocol, all drugs were turned off and the cell was allowed to recover. During recovery, rate-intensity functions were taken every 2–3 min until spike counts and spike patterns were similar to those obtained before the antagonists were applied.

2.4. *Iontophoresis of drugs and measurement of inhibition*

Since ICc cells had little or no spontaneous activity, we evoked background activity by iontophoresing the excitatory transmitters Glu and Asp onto the cell from which we were recording. Both amino acids have been shown to be excitatory for ICc neurons (Faingold et al., 1989a), and we found that a cocktail containing both amino acids was more effective for evoking discharges than presenting either one by itself. Glutamate and aspartate were applied at ejection currents of 5–10 nA (electrode negative) at first, and ejection currents were adjusted until the desired background level of activity of between 4 and 40 spikes/s was achieved. Although high rates of 20–40 spikes/s could be evoked in a few cells, only low rates of 4–10 spikes/s could be evoked in the majority of cells regardless of the current used to eject the excitatory transmitters.

BF tone bursts always evoked excitation and in the majority of neurons that excitation was followed by an inhibition, seen as an uninterrupted gap of at least 5 ms in the background discharges (Fig. 1B). Since the PST histograms were monitored in real time, we observed the build-up of both tone evoked and background activity and could visualize whether or not an inhibitory gap was generated by repeated presentations of the tone burst. Tone bursts were repeatedly presented until a clear gap following the initial excitation was evident in the background activity or when it was apparent that there was no gap in the background activity following the excitatory response. Thus, a variable number of stimuli was presented during the application of Glu/Asp to generate each PST histogram, where the number of stimulus presentations was always larger than the 20 tone burst presentations that were used to generate the PST histograms in the control or predrug condition.

The period of inhibition, or gap, that we measured was the interval between the last discharge evoked by the tone burst and the next Glu/Asp evoked discharge which indicated the resumption of background discharges (Fig. 1B). To ensure that the gap we measured was indeed a stimulus evoked inhibition, and not a random gap in the background activity, tone bursts at four to six intensities were presented. We noted the lowest intensity at which the gap was evoked and defined this as the 'threshold for inhibition'. The gap then had to be evoked at higher intensities in the same temporal slot as it was at the threshold intensity (e.g., Fig. 2). During the application of antagonists in a few neurons, tone bursts evoked an excitation followed by a clear inhibitory gap in which there were one or even less frequently two scattered discharges. The gap with the extraneous discharges was always in the same temporal slot as the completely suppressed gaps that were evoked at different intensities. Although there were one or two discharges, the gap was clearly separated from the resumption of background discharges, which had a higher discharge rate, and we therefore used the gap as an estimate of the inhibitory period. Occasional discharges that occurred within the gap, however, were rare and the gaps that we measured in the vast majority of neurons were periods of complete suppression of any Glu/Asp evoked discharges.

We measured both the period of inhibition that occurred during the tone burst and the period of inhibition that persisted after the tone burst had ended. We did this by first measuring the duration of the total evoked response, which represents the excitation and any inhibitory gap that followed the excitation (Fig. 1C, total response was 36 ms). In most units, however, it was difficult to determine at what point in time the excitation began because the Glu/Asp discharges often blurred the beginning of the discharge train. We therefore measured the latency of excitation from predrug or control PST histograms, which was 8 ms in Fig. 1A, and corrected for that latency in determining the beginning of the tone evoked excitation in the Glu/Asp histograms. In Fig. 1C, and all other figures presented in the text, the beginning of the tone burst marker below each PST histogram was corrected for latency and indicates the beginning of the tone evoked excitation. From the duration of the total evoked response, the portion corresponding to the duration of the tone burst (20 ms) was subtracted. The period of the inhibition remaining after this subtraction was defined as the persistent inhibition, and was 16 ms in Fig. 1C, while the remaining, non-persistent inhibition was the portion that occurred during the tone burst (16 ms in Fig. 1C).

Two features of inhibition as measured from gap durations should be kept in mind. The first is that in some cases the gap durations may indicate a slightly longer inhibitory duration than was actually evoked. This could have occurred because the low rates of background activity and the moment-to-moment variability of background activity occasionally resulted in short, 2–4 ms gaps that occurred randomly in the Glu/Asp

evoked background activity (e.g., Fig. 1C,D). Thus, there is the possibility that in some records a random gap may have occurred immediately following the inhibition. In these cases, the gap would be 2–4 ms longer than the actual duration of the inhibition. The second feature is that our analysis did not identify periods during which the strength of the inhibition may have been sufficient to reduce the background rate but was not strong enough to completely suppress discharges. In these cases the inhibition may have been substantially longer than that indicated by the duration of the gap. Unfortunately, there is no satisfactory way of evaluating changes in background spike rate quantitatively because background rates in most cells were not only low but also tended to be highly variable throughout each histogram. Thus although inhibitory durations may have persisted at a reduced strength beyond the period of complete spike suppression, we confined our measurements of inhibitory periods to the gap durations because that measurement provides a conservative estimate of the inhibitory duration.

The care and use of the animals reported in this study were approved by the National Institutes of Health (NIH Grant DC 00268).

3. Results

Here we report on the patterns of excitation and inhibition evoked by 20 ms tone bursts presented to the contralateral ear in 116 single units recorded from the ICc of the Mexican free-tailed bat. For each cell, we initially determined its BF and the temporal discharge pattern evoked by that frequency. The BFs ranged from 19 to 70 kHz, with most between 23 kHz and 31 kHz. The reported range of BFs in this species of bat is 12–80 kHz where frequencies from 22 to 27 kHz are overrepresented (Vater and Siefer, 1995). Forty-seven percent (55/116) of the units discharged throughout the duration of a 20 ms tone burst and thus had sustained discharge patterns. The remaining 53% (61/116) discharged only at the beginning of the tone with one or a few spikes and had phasic discharge patterns.

After evaluating the excitatory response, we next ex-

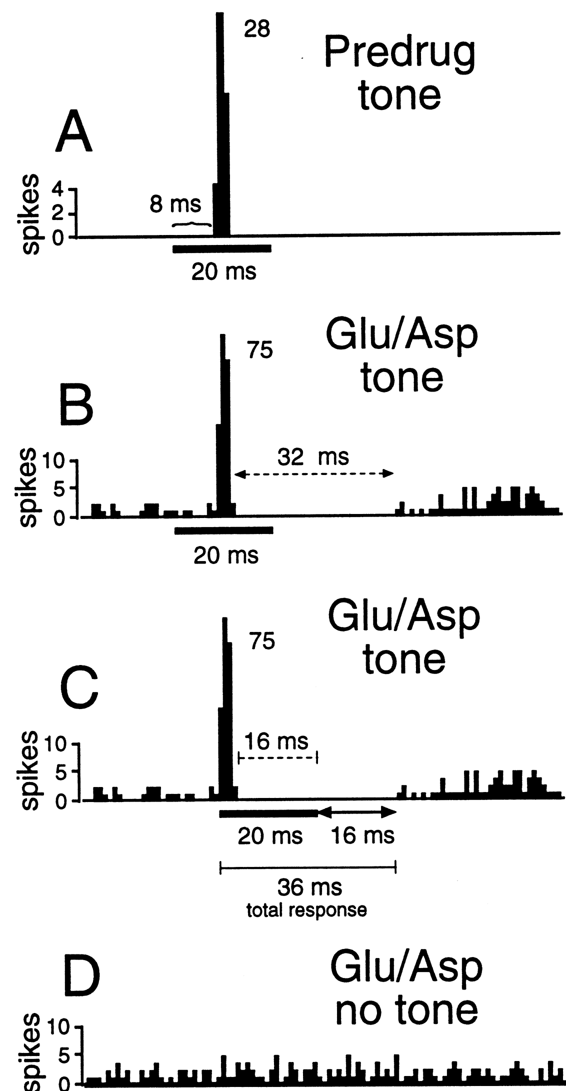

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Fig. 1. Visualization of tone evoked inhibitory periods through the application of the excitatory agonists glutamate (Glu) and aspartate (Asp). A: PST histogram recorded in response to 20 presentations of a 20 ms tone burst. The frequency of the tone burst was set at the cell's BF (23.9 kHz) and was 30 dB SPL. Black bar represents tone burst which was delayed 20 ms from the start of histogram. The response latency from the start of the tone burst was 8 ms. B: PST histogram generated by the same tone burst as in A, but while Glu/Asp background activity was evoked. Notice that the excitatory response to the tone was similar to the response evoked in the predrug condition, but there was also a 32 ms tone evoked inhibitory period (dashed line with double headed arrow) that can be seen as a gap in the background activity following the excitatory response. Histogram was generated by 50 tone burst repetitions. C: Same record as in B except that the representation of the tone burst has been shifted to the right by 8 ms to compensate for the cell's response latency. The total response was 36 ms and was measured from the beginning of the tone evoked discharge to the end of the inhibitory gap. The beginning of the tone evoked discharge is measured from the beginning of the tone burst after correcting for latency. The inhibition is divided into two periods. The first is the non-persistent inhibitory period, indicated by the dashed line, that was 16 ms and occurred during the duration of the 20 ms tone burst. The second period is the inhibition that persisted beyond the tone burst duration. The persistent inhibition in this case is 16 ms and is indicated by the solid line with the double headed arrow. D: Glu/Asp evoked background activity in the absence of a tone presentation. In all figures, the spike count of only the tone evoked excitatory response is indicated by the number to the right of the response peak.

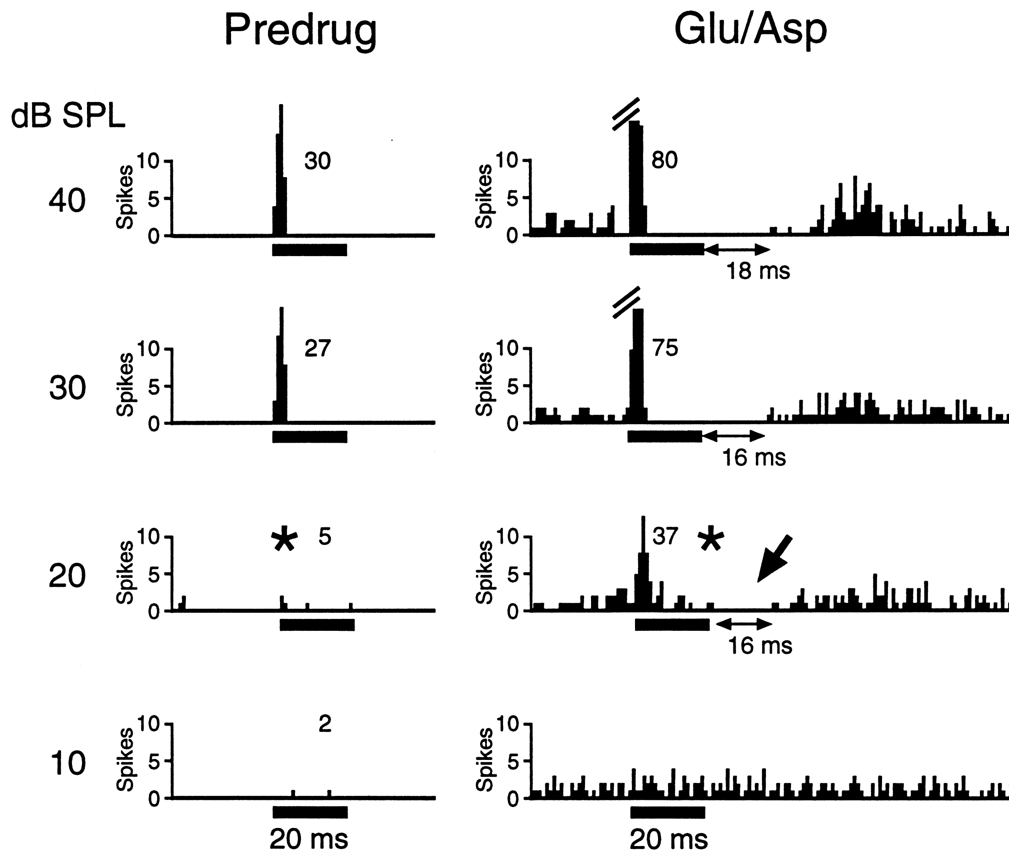

Fig. 2. Excitatory and inhibitory responses evoked by increasing intensities in an ICc neuron. Left panel shows phasic discharge pattern evoked by 20 ms tone bursts when background activity was not induced by Glu/Asp. Spike counts evoked during each tone burst are shown to the right of each response. The excitatory threshold was 20 dB SPL (asterisk), and was similar to the excitatory threshold estimated from the records obtained while background activity was evoked by Glu/Asp (records in right panel). Histograms with background activity in right panel also show that an inhibitory gap was first evoked at 20 dB SPL (arrow), the same intensity as the excitatory threshold. Thus we estimated that the thresholds for excitation and inhibition were the same in this neuron. In addition, the inhibitory gap was evoked at higher intensities in the same temporal slot as the gap evoked at 20 dB SPL. The inhibition at 20 dB SPL only had a persistent component (indicated by the double headed arrow) since spikes were evoked throughout the duration of the tone burst. At 30–40 dB SPL, the inhibitory duration was enhanced by the recruitment of a non-persistent component that suppressed the Glu/Asp discharges during the tone burst. However, the duration of the persistent component increased only marginally, from 16 ms to 18 ms, with intensity. Each predrug histogram in the left panel was generated by 20 tone burst repetitions while each Glu/Asp histogram in the right panel was generated by 50 tone burst repetitions. In this and all subsequent figures, the representations of the tone bursts were shifted to the right to compensate for response latency. Excitatory responses in 30–40 dB SPL histograms were truncated for illustrative purposes only (diagonal slashes). All tone bursts were 23.9 kHz and were 20 ms in duration.

amined whether BF tone bursts presented to the contralateral ear also elicited an inhibition that appeared as a complete suppression of background discharges immediately after the excitation. Inhibition evoked by contralateral stimulation was observed in 85% (94/110) of the cells in our sample. Examples are shown in Figs. 1 and 2. These figures also show that during the application of Glu/Asp, the excitatory response was similar to the predrug excitatory response.

3.1. Threshold of inhibition

Since contralaterally evoked inhibition was a common feature of ICc neurons, we next turned attention to an evaluation of two of its significant features: (1) the inhibitory threshold; and (2) the relative values of the inhibitory and excitatory thresholds. The inhibitory threshold was defined as the lowest sound intensity which elicited a gap of complete suppression in the background activity that followed the excitatory discharge. The excitatory threshold was defined as the lowest intensity that evoked stimulus locked discharges while Glu/Asp were applied (Figs. 2 and 4). Excitatory thresholds were also determined before application of Glu/Asp. With only a few exceptions, the same thresholds were obtained before and during the application of the excitatory transmitters (e.g., Fig. 2).

Inhibitory thresholds ranged from −20 to 50 dB SPL, although the thresholds tended to cluster between 0 and 20 dB SPL (Fig. 3A). The median inhibitory threshold for the 94 cells was 10 dB SPL. There was no apparent difference between phasic and sustained

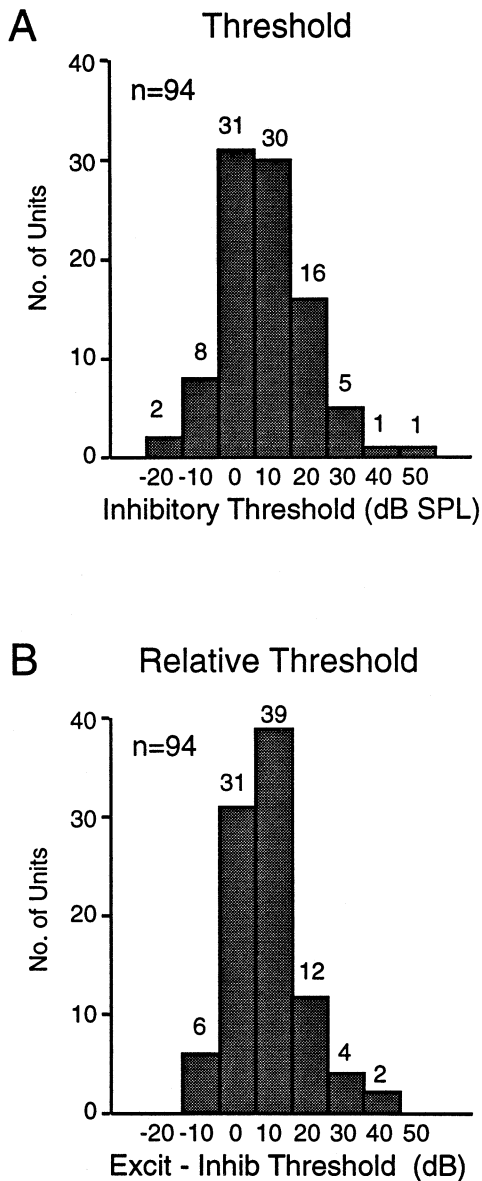

Fig. 3. A: Distribution of the inhibitory thresholds for 94 units. B: Distribution of inhibitory thresholds relative to excitatory thresholds for the same 94 neurons. Negative numbers indicate inhibitory thresholds that were lower than excitatory thresholds.

cells in terms of the distribution of inhibitory thresholds and in terms of their mean values.

Inhibitory thresholds were typically higher than excitatory thresholds. Sixty-one percent of cells (57/94) had inhibitory thresholds that were 10–40 dB higher than their excitatory thresholds (Fig. 3B), though for most of these cells (39/57) the excitatory and inhibitory thresholds did not differ by more than 10 dB. In 33% of cells (31/94), the inhibitory and excitatory thresholds were equal (Fig. 3B). Only 6% (6/94) of cells had inhibitory thresholds lower than their excitatory thresholds, and none of these cells had differences greater than 10 dB.

3.2. Persistent inhibition

A notable feature of the inhibition in 85% (93/110) of the cells was that it persisted for variable periods of time after the end of the tone burst, and thus was longer than the tone burst that evoked it. In Fig. 1C, for example, the inhibition persisted for 16 ms beyond the duration of the tone burst. The duration of the persistent inhibition is of interest because it differs from the excitation, in that discharge trains are either phasic, and thus much shorter than the duration of the tone burst, or are sustained, and have durations comparable to the tone burst duration. Only in rare cases was the discharge train longer than the tone burst duration and in those cases the difference in duration was only a few milliseconds (e.g., Fig. 4 at 50 dB SPL).

The duration of the persistent inhibition was dependent on sound intensity (e.g., Figs. 2 and 4). In more than half of the cells (54%, 50/93), the duration of the persistent inhibition increased monotonically with intensity (e.g., Fig. 4) or increased and then reached a maximum value at which it plateaued (Fig. 2). The remainder of the cells (46%, 43/93) showed non-monotonic effects of intensity, where the duration of the persistent inhibition first increased with intensity, attained a maximum value and then decreased as sound intensity was raised further. On average, non-monotonic cells reached their maximum persistent inhibition at 20 dB above their inhibitory thresholds. At higher intensities, the inhibition decreased to around half of the maximum duration, and in some extreme cases (9/43) it was completely eliminated.

The maximum duration of persistent inhibition varied among the sample and was often relatively long (Fig. 5). The mean maximum persistent inhibition was 26 ms and there was no difference in the mean persistent inhibition of phasic and sustained cells. Half of the cells had maximum persistent inhibitory periods between 15 ms and 33 ms, and the longest persistent inhibition, seen in one cell, was 91 ms.

3.3. The effect of tone duration on persistent inhibition

All of the data presented so far were obtained with 20 ms tone bursts. Interestingly, we found that the ability to evoke a persistent inhibition in some cells was dependent on tone duration. Seven cells were tested with short tone bursts of 5 ms, as well as with 20 ms. In all seven cells, the shorter tones (5 ms) were less effective than longer tones. In three cells, persistent inhibition was evoked by the 5 ms tones but the duration was less than that evoked by the 20 ms tones (Fig. 6).

In four other cells, no persistent inhibition was evoked by 5 ms tone bursts, although prominent persis-

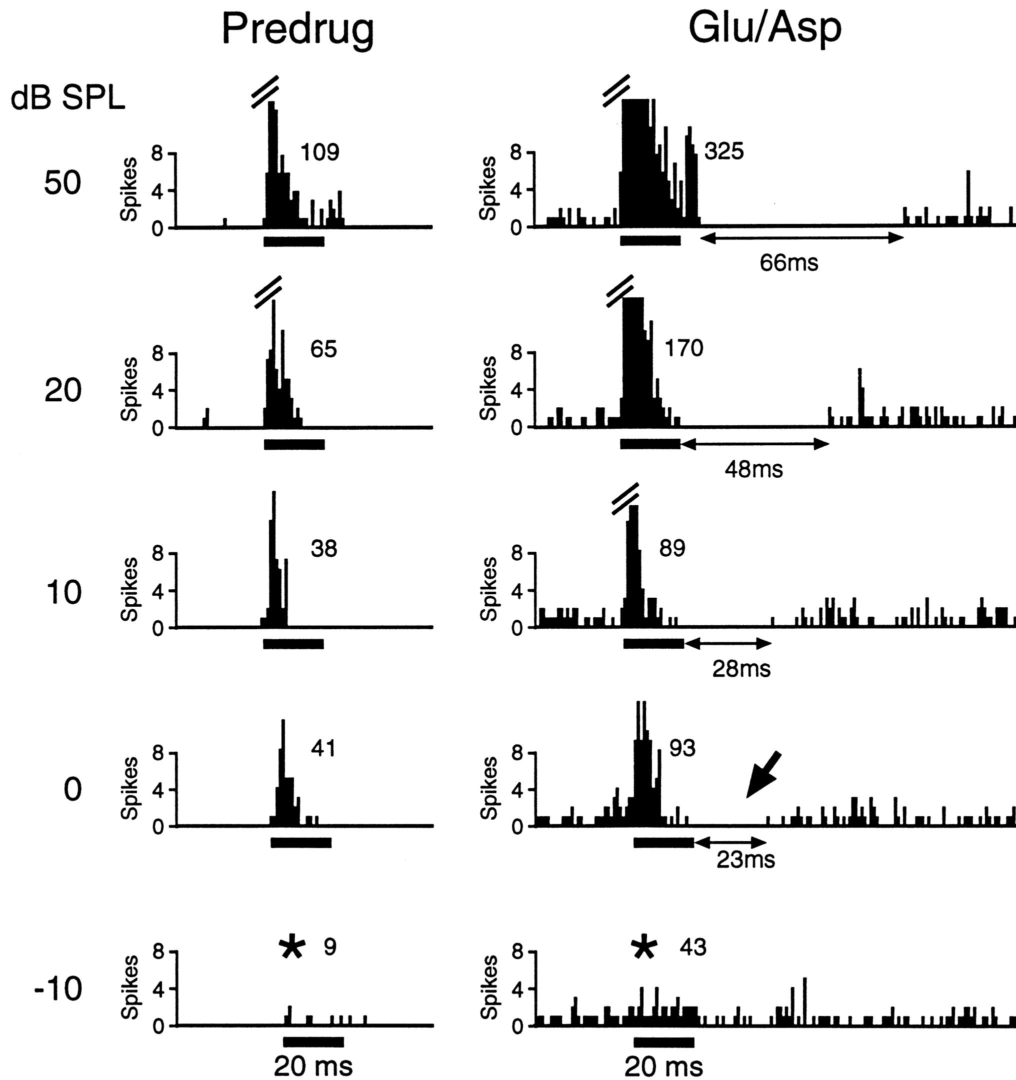

Fig. 4. Neuron in which the duration of persistent inhibition increased with intensity and did not plateau. Left panel shows phasic discharge pattern evoked by 20 ms tone bursts at low intensities and a sustained pattern evoked at high intensities when background activity was not evoked by Glu/Asp. Spike counts evoked during each tone burst are shown on the right of each response. The excitatory threshold was −10 dB SPL (asterisk), and was similar to the excitatory threshold estimated from the records obtained while background activity was evoked by Glu/Asp (records in right panel). Histograms with background activity in right panel show that an inhibitory gap was first evoked at 0 dB SPL (arrow). Thus we estimated that the threshold for excitation was about 10 dB lower than the threshold for inhibition in this neuron. The inhibition at all intensities only had a persistent component (indicated by the double headed arrow) since discharges were evoked during the tone bursts. The duration of the persistent component, however increased substantially, from 23 ms to 66 ms, with intensity. Each predrug histogram in the left panel was generated by 20 tone burst repetitions while each Glu/Asp histogram in the right panel was generated by 38 tone burst repetitions. Histograms generated by 30–40 dB SPL are not shown for purposes of presentation clarity, although duration of persistent inhibition increased progressively at those intensities. Excitatory responses in some histograms were truncated (diagonal slashes). All tone bursts were 26 kHz and were 20 ms in duration.

tent inhibition was evoked in these cells with tones of 20 ms or longer.

We also evaluated the effects on the persistent inhibition of longer stimulus durations, ranging from 20 to 80 ms, in 15 cells. Even though it was a relatively small sample, we were able to see several distinct types of responses to the longer durations. In almost half of the cells (7/15) the persistent inhibition remained constant or increased monotonically as tone duration increased (Fig. 7A). In a smaller number (4/15), the inhibition at first increased but then decreased as tone duration increased (Fig. 7B). The remainder of the cells (4/15) had irregular responses to different tone durations (Fig. 7C). In these cells, the persistent inhibition increased and decreased in an unpredictable manner as tone duration increased.

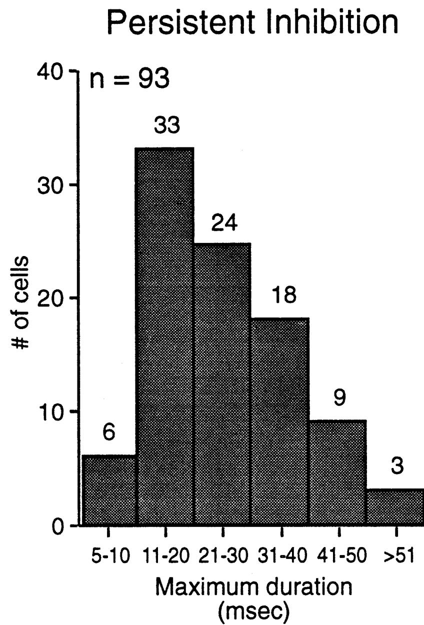

Fig. 5. Graph showing the distribution of the maximum duration of persistent inhibition for 93 ICc cells.

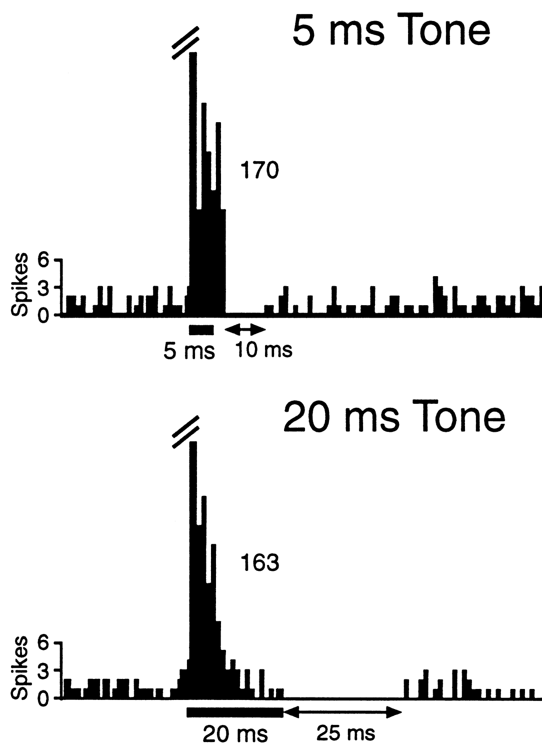

Fig. 6. Neuron in which short tones generated a shorter persistent inhibition than did longer tones. The persistent inhibition is indicated by the solid lines with double headed arrows. The 5 ms tone burst generated a 10 ms persistent inhibition while a 20 ms tone burst generated a longer (25 ms) persistent inhibition. Both tone bursts were 22.7 kHz at 40 dB SPL. 100 tone burst repetitions generated each histogram. Excitatory responses in histograms were truncated (diagonal slashes) and the tone evoked spike counts are to the right of the response peaks.

3.4. Neurotransmitters responsible for the contralaterally evoked inhibition

We also attempted to evaluate whether GABAergic or glycinergic inhibitory inputs were involved in the creation of inhibition at the IC by iontophoretically applying bicuculline and/or strychnine. We evaluated inhibition in 26 ICc cells before and during application of bicuculline. In 30% of the cells (8/26), the application of bicuculline reduced or eliminated both the inhibition occurring during the tone as well as the persistent inhibition, indicating that the source of the inhibition was GABAergic innervation in these cells (Fig. 8A). In the remaining 70% of the cells (18/26), the application of bicuculline shortened the duration of the inhibition but did not eliminate it. Partial elimination of GABAergic inhibition is illustrated by the neurons in Fig. 8B,C. The application of bicuculline to the neuron in Fig.

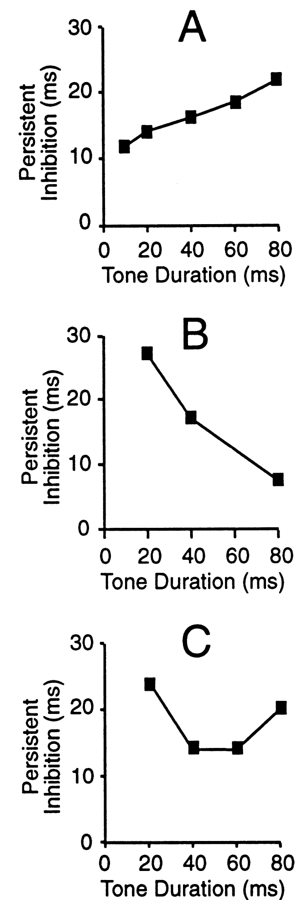

Fig. 7. Graphs from three neurons show the effects of increasing tone burst duration on persistent inhibition. A: A cell in which persistent inhibition increased in duration monotonically as tone duration increased. B: Persistent inhibition changed non-monotonically with tone burst duration. C: Persistent inhibition in this cell was not affected by increases in tone duration in a predictable fashion. Tone burst in A was 22 kHz at 10 dB SPL, in B it was 32.2 kHz at 0 dB SPL and in C it was 27.3 kHz at 30 dB SPL.

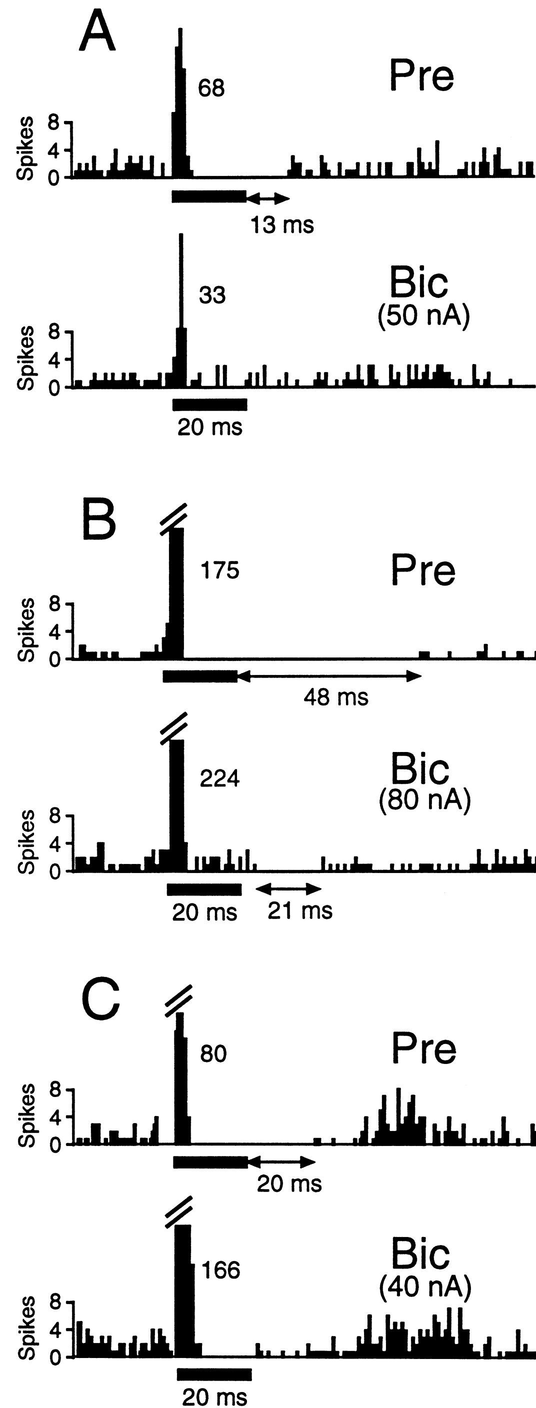

Fig. 8. Effects of blocking GABAergic inhibition with bicuculline on contralaterally evoked inhibition in 3 ICc neurons. A: A neuron in which the application of bicuculline reduced or eliminated both the non-persistent inhibition occurring during the tone as well as the persistent inhibition. Excitatory response during bicuculline was smaller than pre-drug response because 29 presentations of the tone burst were presented to generate the bicuculline histogram while 56 repetitions were presented to generate predrug histogram. Tone bursts were 35 kHz at 40 dB SPL. B: Another neuron in which bicuculline shortened the duration of the inhibition but did not eliminate it. Bicuculline eliminated the non-persistent inhibition during the tone burst and also reduced the duration of persistent inhibition from 48 ms to 21 ms. Tones were 27.2 kHz at 20 dB SPL. C: Neuron in which bicuculline reduced or abolished the persistent inhibition but had little or no effect on the non-persistent period of inhibition. Tones were 24 kHz at 50 dB SPL. Excitatory responses in histograms in B and C were truncated (diagonal slashes) and the tone evoked spike counts are to the right of each response peak.
→

8B reduced the persistent inhibition from 48 ms to 21 ms, but did not completely eliminate it. Bicuculline also eliminated the non-persistent inhibition during the tone burst. For the neuron in Fig. 8C bicuculline only eliminated the persistent inhibition but had no apparent effect on the inhibition evoked during the tone burst.

We also applied strychnine to 10 cells in which an inhibitory gap still remained after the application of bicuculline to evaluate whether or not the remaining inhibition was caused by glycinergic innervation. In one cell, the gap was abolished when strychnine was applied in conjunction with bicuculline, indicating that the inhibition in this cell was the result of both GABAergic and glycinergic innervation. However, in nine other cells, the addition of strychnine did not substantially reduce the persistent inhibition over the reduction caused by bicuculline. Rather, the more prominent effect of strychnine was to partially remove inhibition that occurred during the tone burst. This, as well as some of the uncertainties of measuring inhibition from gap durations, is illustrated by the neuron in Fig. 9. The predrug excitation evoked by BF tone bursts was followed by a clear period of inhibition that had both a non-persistent and a 16 ms persistent component. The discharge rate following the gap of persistent inhibition was at first low and then increased, a feature that was also seen during the application of bicuculline and strychnine. The period of lower discharges indicates a continuing inhibition whose strength was not sufficiently strong to completely suppress the Glu/Asp discharges. Notice, from the middle record in Fig. 9, that bicuculline had little effect on the non-persistent inhibition but seemed to weaken the persistent inhibition, as suggested by the presence of two discharges during this period. This was one of the few units in which we measured a period of persistent inhibition that had one or two discharges. When strychnine was added to the bicuculline, the gap of persistent inhibition was still present in the same time slot and its duration was only slightly reduced to 11 ms. However, the duration of the tone evoked discharge train increased substantially over the predrug discharge train, and the non-persistent inhibition was eliminated as indicated by the discharges that occurred during the remainder of the tone burst and for a few ms beyond. Thus, the primary influence of glycinergic inhibition

appeared to be on the non-persistent inhibition rather than on the persistent inhibition.

3.5. Contralaterally evoked inhibition shapes phasic responses in some neurons

In some neurons, e.g., Figs. 8A,B and 9, blocking inhibition eliminated the non-persistent inhibition that occurred during the tone burst. After blocking inhibition, the discharge rates following the vigorous excitatory responses appear to be similar to the overall background rate. The low discharge rates during the tone bursts suggest that the excitatory input to the ICc cell was itself phasic and was followed by an inhibition. In these cells, blocking inhibition simply allowed Glu/Asp discharges to be evoked for the duration of the tone burst. In other cells, however, blocking either GABAergic inhibition or GABAergic and glycinergic inhibition resulted in a far more vigorous discharge rate for the duration of the tone burst. As illustrated by two neurons in Fig. 10, the non-persistent component of the inhibition in those neurons suppressed the later part of a sustained excitatory input with the consequent creation of a phasic excitatory output from a sustained input.

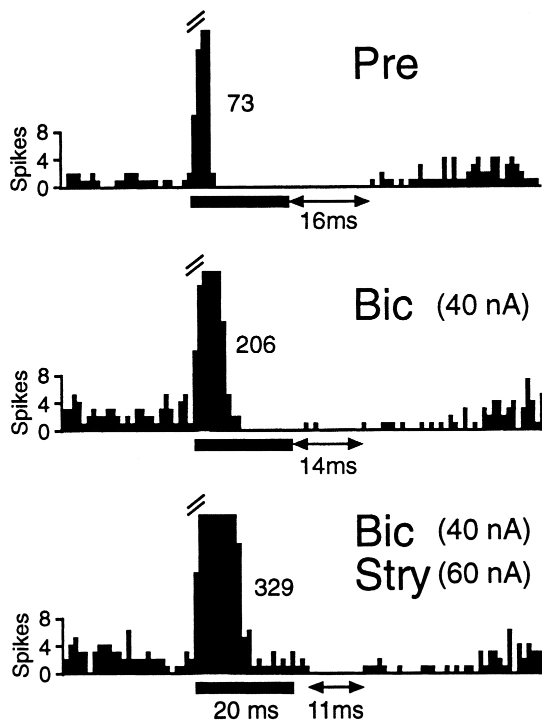

Fig. 9. Neuron in which bicuculline had only a small effect on inhibition while strychnine eliminated the non-persistent inhibition. See text for further explanation. Tone was 23.9 kHz at 30 dB SPL. Excitatory responses in histograms were truncated (diagonal slashes) and the tone evoked spike counts are to the right of each response peak.

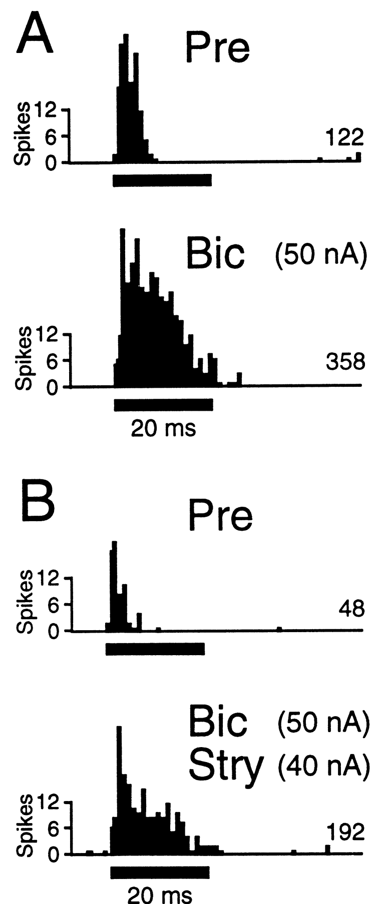

Fig. 10. Contralateral inhibition suppresses the later part of a sustained excitatory input with the consequent creation of a phasic excitatory response in two neurons. Glu/Asp was not presented in any of the histograms. A phasic discharge pattern was evoked by 20 ms tone bursts in both neurons before application of antagonists. Bicuculline alone transformed a phasic discharge pattern into a sustained pattern for the neuron shown in panel A (tone was 26 kHz at 20 dB SPL). Bicuculline together with strychnine transformed a phasic into a sustained pattern for the neuron in panel B (tone was 34.3 kHz at 10 dB SPL). All histograms were generated by 20 repetitions of the tone bursts.

3.6. Contralaterally evoked inhibition also shapes sustained responses

Many sustained cells also had a persistent inhibition. Moreover, in at least some sustained cells, blocking inhibition caused the duration of the discharge train to become longer than the duration of the tone burst. In these cells, the initial portion of the persistent inhibition interacted with the later part of a sustained excitation, thereby restricting the duration of the sustained excitatory response. We showed this effect of the persistent inhibition by applying bicuculline and strychnine to 11 cells with sustained discharge patterns. In five of the sustained cells, bicuculline and strychnine eliminated the persistent inhibition and lengthened the duration of the discharge train (Fig. 11). In one cell the discharge

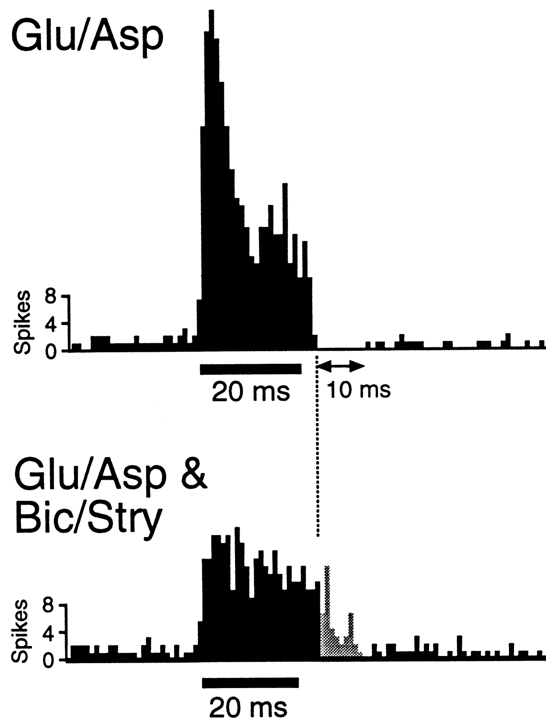

Fig. 11. The initial portion of the persistent inhibition interacted with the later part of a sustained excitation, thereby restricting the duration of the sustained excitatory response as well as decreasing the magnitude of the last half of the sustained response. Before drug application, the cell responded throughout the duration of the 20 ms tone. The response at the onset of the tone was much larger than the response that was evoked during the last half of the tone burst. In addition, the spike trains did not outlast the stimulus and the excitatory response was followed by a persistent inhibition. The PST histogram in the lower record shows that the application of bicuculline and strychnine removed the persistent inhibition. The removal of the persistent inhibition prolonged the discharge train which was now longer than the duration of the tone burst (indicated by the lighter portion of the histogram). The drugs also removed the part of the inhibition that occurred during the last half of the tone. Thus the drugs preferentially increased the magnitude of the responses during the last half of the tone as compared to that during the first half of the tone. Excitatory response evoked during the application of antagonists is smaller because 20 tone burst repetitions generated the BIC/STRY histogram in the lower panel while 69 tone burst repetitions generated the histogram in the top record. Tone bursts were 20.9 kHz at 20 dB SPL.

train was lengthened although the persistent inhibition was still present. In the five other sustained cells, the drugs were unable to remove the persistent inhibition and the duration of their discharge trains did not change.

There was also evidence that the earlier, non-persistent component of inhibition also influenced the temporal discharge pattern of sustained cells. The magnitude of the initial discharge was typically larger than the magnitude of the later portion of the discharge train. That inhibition partially suppressed the later portion of the discharge train was confirmed in seven of 11 cells when bicuculline and strychnine were applied. As shown in Fig. 11, blocking inhibition increased the magnitude of the last half of the response more dramatically than the first half.

4. Discussion

The main finding of this study is that monaural contralateral stimulation elicits both an excitation and an inhibition in nearly all ICc cells. The thresholds of the inhibition and excitation are typically similar and in most cells do not differ by more than 10 dB. Finally, the duration of the inhibition increases with intensity and in most cells the inhibition persists for tens of ms after the stimulus has ended.

These results are consistent with those reported in previous studies. The findings from studies of recovery of responsiveness in the ICc, which used single electrodes to record unit activity extracellularly, suggest that contralateral stimulation evokes a long lasting inhibition that persists well beyond the duration of the tone burst (Carney and Yin, 1989; Casseday and Covey, 1995; Friend et al., 1966; Suga, 1964). In those studies, conducted both on bats and on other mammals, two signals were presented that were separated by various time intervals. The initial signal always evoked discharges whereas the second signal had to be delayed by tens of ms to evoke a response comparable to that which the second signal would evoke if it were presented alone. The interpretation given to these results was that the initial signal evoked a long lasting inhibition and that the neurons recovered their responsiveness to the second signal only after the inhibition had ended. However, the methods used in those studies could not determine whether the inhibition that suppressed discharges to the second tone was evoked in the ICc cell or whether the suppression of responses to the second tone occurred in a lower nucleus.

Contralaterally evoked inhibition in the ICc was shown more convincingly in recent studies in which extracellular discharges were monitored while pharmacological blockers of inhibitory neurotransmission were focally applied to ICc cells from multibarrel electrodes (Faingold et al., 1989b; Fuzessery and Hall, 1996; Lu et al., 1997; Park and Pollak, 1993; Pollak and Park, 1993; Vater et al., 1992; Yang et al., 1992). Those studies found that upon blocking inhibition, many onset cells were changed into sustained cells, showing that a contralaterally evoked inhibition in the ICc shaped a sustained excitation into an onset response. While this method showed that an inhibition coincides with an excitation in ICc neurons, it could not reveal whether periods of inhibition extended beyond the duration of the excitation generated by the tone burst.

Contralaterally evoked inhibition at the ICc has also been reported in in vivo intracellular or patch-clamp studies from anesthetized and awake animals. The first intracellular study in the ICc by Nelson and Erulkar (1963), as well as the recent intracellular study by Kuwada et al. (1997), showed that in some ICc cells, contralateral stimulation evoked discharges followed by a more or less long lasting hyperpolarization, though the length of the inhibition was not specifically mentioned in either study. Patch-clamp recordings in the voltage clamp mode performed in awake bats by Covey et al. (1996) visualized synaptic currents in IC cells. In one cell, an IPSC lasted 20 ms beyond the end of the stimulus and could be representative of the persistent inhibition observed in the extracellular studies of the ICc mentioned above.

4.1. Source(s) of the contralaterally evoked inhibition

One of the striking features of responses evoked by contralateral stimulation is the difference between the durations of the inhibition and the excitation that are evoked in the same cell by the same signal. Contralaterally evoked inhibition is long lasting and commonly persists for tens of milliseconds beyond the duration of the tone burst. In contrast, the durations of the contralaterally evoked discharge trains, even in the most extreme cases, are only slightly longer than the durations of the tone bursts that evoked them. How does one explain the long duration of the persistent inhibition when such persistence is not expressed in the excitation, and what functional significance might be attributed to persistent inhibition? Below we first discuss four possible explanations that could account for the inhibitory persistence. These are not necessarily mutually exclusive and may function cooperatively.

The first explanation for the source of persistent inhibition is that the ICc cell receives inhibitory innervation not only from ascending projections, but also from other ICc cells or from descending cortical projections that have relatively long latencies. In this scenario, the inhibition occurring during the tone burst is from the ascending afferents whereas the persistent inhibition is generated either by the innervation from a number of other ICc cells or from descending neurons, each with a progressively longer latency than the cell they innervate. Cortical cells have longer latencies than ICc cells and descending inputs have been shown to evoke inhibition in some ICc cells. Both features are appropriate for evoking a persistent inhibition in ICc cells. The possibility that innervation by other ICc cells could evoke persistent inhibition is consistent with studies which have shown that many ICc cells are GABAergic (Oliver et al., 1994; Winer et al., 1995), and that these cells innervate both the medial geniculate body and other ICc cells through collateral innervation (Gonzalez-Hernandez et al., 1996; Saint Marie et al., 1997; Saldana and Merchan, 1992).

The second explanation invokes a GABA receptor with slow kinetics. Recently, two types of $GABA_A$ receptors were isolated in rat hippocampal neurons (Banks et al., 1998). One type of $GABA_A$ receptor is typical of the traditional $GABA_A$-fast receptor, whereas the other $GABA_A$ receptor has much slower activation and inactivation kinetics and was termed the $GABA_A$-slow receptor. Both receptors are blocked by bicuculline and thus both receptors would be blocked in the experiments we conducted. If $GABA_A$-slow receptors occur in ICc cells, they could explain persistent inhibition since the postsynaptic effects of the GABA released during a stimulus would be extended for many milliseconds beyond the end of the tone.

The third possibility is that persistent inhibition is generated by $GABA_B$ receptors which are insensitive to bicuculline and strychnine (Sivilotti and Nistri, 1991). $GABA_B$ receptors act through G proteins and thus are slower and have more prolonged actions than the classical fast $GABA_A$ receptor (Andrade et al., 1986; Holz et al., 1986; Otis et al., 1993; Pfrieger et al., 1994; Sivilotti and Nistri, 1991). $GABA_B$ receptors are present in the ICc although they occur in low density (Chu et al., 1990; Fubara et al., 1996). Consistent with their low density, responses of ICc cells are only increased slightly when $GABA_B$ receptors are blocked (Burger and Pollak, 1998; Faingold et al., 1989a,b). Thus, the partial involvement of $GABA_B$ receptors in the generation of persistent inhibition cannot be ruled out.

The fourth explanation is that the persistent inhibition may be partially generated by an intrinsic property of ICc cells, such as a voltage sensitive current. Some possibilities include a slowly activating voltage sensitive potassium current or a calcium activated potassium current. Both of these currents could be activated by the depolarization and action potentials caused by excitatory inputs. We should point out that a confounding factor might be that these currents may have been partially activated by the iontophoresis of Glu/Asp. However, if such currents contribute to persistent inhibition, they presumably would have been activated more potently by the synchronous burst of sound evoked activity than they would have been by the relatively low rate of intermittent discharges evoked by the iontophoresis of Glu/Asp. Alternatively, channels opened by hyperpolarization, such as an I_h current that has a reversal potential lower than the threshold for evoking discharges, might also contribute to the persistent inhibition (Pape, 1996). Due to a paucity of ICc brain slice studies, the existence of these or other currents in ICc cells is unknown. Intracellular studies in

ICc brain slices or cultured neurons would go a long way to addressing these possibilities.

4.2. Bicuculline and strychnine did not eliminate persistent inhibition in all ICc cells

Here we briefly discuss the basis of the early inhibition, the inhibition that occurs during the duration of the stimulus, and then discuss persistent inhibition and why we believe that bicuculline sensitive receptors as well as other features presented in the preceding section may contribute to the generation of persistent inhibition. Our results suggest that the primary mechanism for both the early inhibition evoked during the duration of the tone as well as the persistent inhibition after the tone is synaptic inhibition, specifically glycinergic innervation and GABAergic innervation acting through $GABA_A$ receptor. Apparently glycinergic inhibition contributed primarily to the early inhibition and was only marginally responsible for the persistent inhibition. We point out that our results from blocking glycinergic innervation are more suggestive rather than conclusive due to the small number of units in our sample. Our results also suggest that GABAergic inhibition, while also contributing to the early inhibition, is the primary agent responsible for the generation of persistent inhibition, since in most neurons persistent inhibition was partially or completely eliminated when $GABA_A$ receptors were blocked by bicuculline. However, the failure to completely eliminate persistent inhibition in some cells when both bicuculline and strychnine were applied simultaneously suggests that other factors may at least be partially involved in generating persistent inhibition in some cells. One possibility is that the cells were refractory. However, we consider this unlikely, since the gaps remaining after application of bicuculline and strychnine were longer than 1–2 ms. For reasons outlined below, we suggest the possibilities that $GABA_B$ receptors, which are insensitive to both strychnine and bicuculline, or voltage sensitive currents that were discussed above, are more likely to contribute to the persistent inhibition.

We suggest the possible involvement of factors other than $GABA_A$ mediated inhibition with trepidation since there is always the possibility that the drugs failed to block all inhibitory receptors. If true, this could explain why the bicuculline and strychnine did not completely eliminate persistent inhibition in all cells and then would not require the involvement of other receptors or voltage sensitive currents. However, we think it unlikely that key receptors were not blocked because a number of measures were taken to minimize that possibility. For example, we titrated the currents used for drug ejection. The antagonists were initially ejected with a low current and while the drugs were being applied rate-intensity functions were repeatedly taken until their shape and magnitude stabilized. The ejection current was then increased and rate-intensity functions taken again until the functions stabilized. This process was repeated until the rate-level function did not increase beyond that obtained at the previous ejection current. In this way we attempted to block most, if not all of the synaptic inhibition to the cell. Thus, we are left with two possible explanations for persistent inhibition: (1) that we did not block all GABAergic receptors in all cells, which would suggest that GABAergic innervation can completely account for persistent inhibition, an explanation that we believe is unlikely but cannot entirely rule out; or (2) that in some cells bicuculline insensitive receptors or voltage activated currents contribute at least partially to its generation.

4.3. Functions of contralateral inhibition

We turn next to a consideration of the functions of both the early, or non-persistent inhibition and the persistent inhibition. With regard to the early inhibition, we and others have shown that the early inhibition can modify and shape response properties evoked by contralateral stimulation (Casseday et al., 1994; Covey et al., 1996; Faingold et al., 1989a,b; Le Beau et al., 1996; Palombi and Caspary, 1996; Park and Pollak, 1994; Pollak and Park, 1993; Vater et al., 1992; Yang et al., 1992). Modification of response features by inhibition is universal and occurs not only in the ICc but in most, if not every nucleus of the auditory system (Backoff et al., 1997; Caspary et al., 1987; Grothe, 1994; Moore and Caspary, 1983; Suga et al., 1997; Wu and Oertel, 1986; Yang and Pollak, 1994a,b). In the ICc, inhibition creates phasic or onset response from a sustained excitatory drive in some ICc cells (Le Beau et al., 1996; Pollak and Park, 1993; Vater et al., 1992). The inhibition even affects ICc cells with sustained responses, reducing their discharge magnitude and duration so that the discharge train lasts only as long as the duration of the tone burst that generated it (Le Beau et al., 1996; Pollak and Park, 1993; Vater et al., 1992). Although we did not evaluate latency and rate-intensity functions in this study, previous studies of the ICc have shown that components of the early inhibition can also lengthen latencies and can create non-monotonic rate-intensity functions (Park and Pollak, 1993; Pollak and Park, 1993; Vater et al., 1992).

While the early contralaterally evoked inhibition shapes a number of response properties, the functional role of the persistent inhibition is puzzling. It presents a particular paradox for echolocating bats since the survival of these animals depends upon their ability to interpret echoes that reach their ears within a few ms after the emission of a loud echolocation call. For this

particular species, the Mexican free-tailed bat, persistent inhibition is greatly reduced with very brief signals, and thus persistent inhibition might not present as formidable a problem for echolocation as it would initially appear to be. The echolocation calls normally emitted by these bats are very brief and typically range from 2–3 ms to 0.5–1.0 ms, where the bat reduces the call duration as it first detects and then homes in on its target (Simmons et al., 1978, 1979). The reduced persistent inhibition with brief signals is consistent with the fast recovery times of some neurons in the ICc of Mexican free-tailed bats that were observed in earlier studies with brief signals that mimicked the bats' natural echolocation calls (Pollak et al., 1977a,b,c).

Fast recovery with brief signals, however, is not the rule and has not been seen in other studies of ICc neurons that were conducted in either anesthetized or awake animals (Friend et al., 1966; Lu et al., 1997; Suga, 1964; Yin, 1994). In some studies of anesthetized animals, the slower recovery may be partially attributed to anesthetics which are known to slow recovery times considerably. But anesthetic effects cannot be the entire explanation for the slow recovery since slow recovery was also observed in studies of the ICc in awake animals that employed brief stimuli (Casseday and Covey, 1995; Lu et al., 1997).

The functional significance of contralateral persistent inhibition becomes even more puzzling when one considers that it is *not* a feature of neurons in other lower auditory nuclei. The absence of contralaterally evoked persistent inhibition in lower nuclei is indicated by the fast recovery of responsiveness in the cochlear nucleus, the superior olivary complex and the nuclei of the lateral lemniscus (Backoff et al., 1997; Fitzpatrick et al., 1995; Grinnell, 1963; Grothe, 1994; Yang and Pollak, 1994a,b).

What appears to be occurring along the ascending auditory pathway is that a signal received at one ear evokes mixtures of excitation and inhibition at each synaptic station. The inhibitory innervation generates an early inhibition, an inhibition that acts during the duration of the signal, that, together with inherent membrane properties, modifies the magnitude and temporal patterns of the discharge trains. The modified or transformed discharge trains ultimately converge upon the ICc where they are further modified by the early inhibition, as discussed above. The situation changes dramatically in the ICc for signals that follow an initial signal within a few ms. Neurons in the nuclei below the ICc respond briskly to these following signals and apparently process them in a manner similar to the way they process the initial signal. However, the initial signal generates a new response at the ICc, a persistent inhibition, which prevents the vast majority of ICc cells from responding to the discharge trains of the following signal for tens of ms.

Thus persistent inhibition seems to temporally segregate the ICc population response to an acoustic signal from the responses evoked in lower nuclei by previous or later signals. Viewed in this way, such a temporal segregation of the response to a signal is reminiscent of one principle that operates in scene analysis (Bregman, 1990). The main idea of scene analysis is grouping, where features of a sound are assigned to a particular source. One principle of grouping is 'separation in time', where features that are temporally coincident tend to be grouped and assigned to a common source more strongly than are features that are temporally disparate. We do not know whether persistent inhibition is an underlying neural feature that contributes to the grouping of acoustic features, or whether it relates to other aspects of information processing. However, a neural role in 'grouping' provides at least one seemingly plausible explanation for the otherwise puzzling features of persistent inhibition and would further suggest that this process is an emergent property of the ICc.

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