Neural Measurement of Sound Duration: Control by Excitatory-Inhibitory Interactions in the Inferior Colliculus

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Casseday, John H., Daphna Ehrlich, and Ellen Covey. Neural measurement of sound duration: control by excitatory-inhibitory interactions in the inferior colliculus. *J Neurophysiol* 84: 1475–1487, 2000. In the inferior colliculus (IC) of the big brown bat, a subpopulation of cells (~35%) are tuned to a narrow range of sound durations. Band-pass tuning for sound duration has not been seen at lower levels of the auditory pathway. Previous work suggests that it arises at the IC through the interaction of sound-evoked, temporally offset, excitatory and inhibitory inputs. To test this hypothesis, we recorded from duration-tuned neurons in the IC and examined duration tuning before and after iontophoretic infusion of antagonists to γ-aminobutyric acid-A ($GABA_A$) (bicuculline) or glycine (strychnine). The criterion for duration tuning was that the neuron's spike count as a function of duration had a peak value at one duration or a range of durations that was ≥2 times the lowest nonzero value at longer durations. Out of 21 units tested with bicuculline, duration tuning was eliminated in 15, broadened in two, and unaltered in four. Out of 10 units tested with strychnine, duration tuning was eliminated in four, broadened in one, and unaltered in five. For units tested with both bicuculline and strychnine, bicuculline had a greater effect on reducing or abolishing duration tuning than did strychnine. Bicuculline and strychnine both produced changes in discharge pattern. There was nearly always a shift from an offset response to an onset response, indicating that in the predrug condition, inhibition arrived simultaneously with excitation or preceded it. There was often an increase in the length of the spike train, indicating that in the predrug condition, inhibition also coincided with later parts of excitation. These findings support two hypotheses. First, duration tuning is created in the IC. Second, although the construction of duration tuning varies in some details among IC neurons, it follows three rules: *1*) an excitatory and an inhibitory event are temporally linked to the onset of sound but temporally offset from one another; *2*) the duration of some inhibitory event must be linked to the duration of the sound; *3*) an excitatory event must be linked to the offset of sound.

INTRODUCTION

Duration is an identifying feature of many biological sounds. For example, the duration of specific sound elements in a sequence is an important aspect of communication sounds such as speech (Miller and Liberman 1979; Shannon et al. 1995), frog calls (Narins and Capranica 1980), and echolocation calls (Neuweiler 1990). If sustained responses were the only neural representation of sound duration, then all neurons with sustained responses would represent all durations, and no neuron could represent any particular duration. Although this seems to be true in the periphery and lower brainstem, individual neurons in the auditory midbrain are tuned to different sound durations (Casseday et al. 1994; Ehrlich et al. 1997; Fuzessery 1994; Narins and Capranica 1980; Pinheiro et al. 1991). For many midbrain neurons, their response as a function of duration can be classified as band-pass in that the neuron responds to only a narrow range of sound durations and is unresponsive to sounds that are shorter or longer. Other midbrain neurons can be classified as short-pass, in that they respond to brief sounds but not long ones. We refer to these two types of specialized neurons as "duration-tuned neurons."

Two lines of evidence suggest that duration tuning is produced at neurons in the inferior colliculus (IC) through a specific temporal sequence of excitatory and inhibitory inputs (Casseday et al. 1994; Covey et al. 1996; Ehrlich et al. 1997). First, the general idea of temporal selectivity arose from the fact that the IC is the target of multiple excitatory and inhibitory inputs that respond with a broad range of latencies (review, Casseday and Covey 1995). For example, within the nuclei of the lateral lemniscus, neurons of the same or nearly the same best frequencies have latencies distributed over a range of at least 10 ms (Covey and Casseday 1991; Haplea et al. 1994). This range of latencies could provide IC neurons with the time delays necessary to measure sound duration, if we assume that the measurement of sound duration is accomplished by a coincidence detector that fires only when a marker for the end of the sound arrives at the same time as a delayed marker for the beginning of the sound. Second, direct evidence comes from observations on the time course of synaptic excitation and inhibition at the cell membrane of IC neurons. Using whole-cell patch-clamp methods, Covey et al. (1996) showed that a duration-tuned cell responds to sound with a net outward current, followed by a net inward current. The initial outward current is temporally locked to the onset of sound, and the inward current, with or without spikes, is temporally locked to the offset of sound. As sound duration is lengthened, the magnitude of the offset inward current increases until, at some intermediate duration, spiking occurs. With further increases in sound duration, the offset inward current diminishes; although some offset inward current remains, spikes do not occur.

These results gave rise to the following model, modified

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from one suggested by Narins and Capranica (1980) for creating duration-tuned neurons. The model is based on synaptic delay lines that provide a wide range in the latencies of the inputs to the duration-tuned cell. The model of a duration-tuned cell has three input components, as illustrated in Fig. 1 (Casseday and Covey 1995; Casseday et al. 1994; Ehrlich et al. 1997). These components are as follows.

1) Onset inhibition. Inhibitory input has the shortest latency and always leads or coincides with the excitatory input; it takes the form of a sustained inhibitory postsynaptic potential (IPSP) that lasts at least as long as the duration of the sound (middle traces, Fig. 1, *A* and *B*).

2) Onset excitation. Transient excitatory input is temporally locked to the onset of sound but has a latency that is at least as long as that of the onset inhibitory input (lower traces, Fig. 1, *A* and *B*); perhaps because of partial cancellation by the sustained inhibition, its magnitude is below spike threshold.

3) Offset excitation. A second excitatory event is correlated with the offset of sound; its magnitude is below the threshold for spike initiation (middle traces, Fig. 1, *A* and *B*). In earlier versions of the model, we raised the question of whether this offset depolarization was an intrinsic property of the IC cell, i.e., a rebound from inhibition, or whether it was an offset excitatory input from another source.

Evidence from extracellular recordings supports the three-component model of duration tuning (Ehrlich et al. 1997): The responses of duration-tuned cells are locked to the offset of sound throughout the range of durations to which they respond best. However, for neurons in which a small response persists at durations longer than the optimal durations, spike latency stops following sound offset and at the longer durations is locked to sound onset (Ehrlich et al. 1997). These results are consistent with the idea that onset excitation does not arrive at the cell before onset inhibition and that onset inhibition usually cancels onset excitation, resulting in a low probability of onset-evoked spikes. However, these studies left a number of questions unanswered. For example, is the excitatory input transient or sustained with respect to the stimulus? Is the inhibition glycinergic or GABAergic? If both are involved, do they have different actions, for example, different time courses?

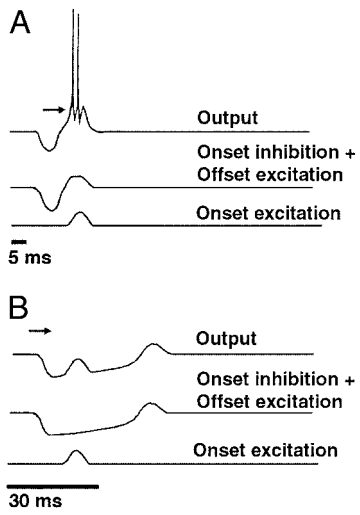

FIG. 1. A 3-component model for duration tuning with onset inhibition, delayed onset excitation, and offset excitation. Neither onset excitation nor offset excitation is in itself sufficient to generate spikes. The hypothetical neuron responds to a 5-ms tone but not to a 30-ms tone (upper traces in *A* and *B*). *A*: initial part of the response is dominated by onset inhibition. The inhibition is followed by an excitation at the offset of sound (middle traces). A second "delayed" excitation occurs in response to the onset of sound (lower traces). The duration of the 5-ms sound is such that the delayed onset excitation coincides with the offset excitation, allowing the neuron to respond. *B*: onset inhibition is sustained for the duration of the sound, and the offset excitation occurs later (middle trace) than in *A*. The onset excitation maintains the same latency for the 30-ms sound as for the 5-ms sound. Therefore the onset excitation does not coincide with the offset excitation, and the response remains below spike threshold (arrow, top trace). The best duration is determined by the latency of the delayed onset excitation. Band-pass duration tuning is generated by the coincidence of two excitatory events one of which is temporally locked to the offset of sound and the other to the onset of sound. In this model we proposed that the onset excitation was from excitatory input, whereas the offset excitation was a rebound from inhibition.

In the present study, we examined the role of neural inhibition in creating duration tuning by recording from duration-tuned cells before and after iontophoretic infusion of the area around the cell with antagonists to the inhibitory neurotransmitters, GABA, or glycine. The results support the hypothesis that duration tuning is generated in the IC by inhibitory control of response timing, but they also suggest some modifications to and extensions of the model.

METHODS

Surgical procedures

We used 35 big brown bats of both sexes. A day prior to recording, the bat was anesthetized with a combination of Metofane (methoxyflurane) and Innovar-Vet (fentanyl 0.4 mg/ml + droperidol 20 mg/ml; 0.125 ml/kg). Using an apparatus to fix the bat's head in a standard position, a metal post was attached to the skull with cyanoacrylate adhesive. The post was constructed so that it could be attached to a manipulator on a stereotaxic apparatus (Kopf, modified for bats). This method allowed the bat to be precisely repositioned in the stereotaxic apparatus from one recording session to the next. Each bat was used for one to six recording sessions (<6-h duration) on separate days. Between recording sessions, bats were housed in individual cages and were given food and water ad libitum. The cages were located in a temperature- and humidity-controlled room. All procedures were approved by the Duke University Institutional Animal Care and Use Committee.

Stimuli

The auditory stimuli presented included pure tones, frequency-modulated (FM) sweeps, or broadband noise. Stimuli were generated by a D/A converter, which was controlled by a digital signal processor (Tucker-Davis Technologies). The sampling rate for generating signals was 347 kHz, and anti-aliasing filters were set to cutoff signals at frequencies above 120 kHz. The signal processor was controlled by custom software run on a Gateway 486 computer. Stimulus duration was varied from 1 to 100 ms. The sounds had a rise-fall time of 0.5 ms, except for sounds with a duration of 1 ms, where the rise-fall time was 0.4 ms. The waveforms always started at zero-phase angle. Sounds were presented at a rate of 3/s.

Sounds were delivered through Brüel and Kjaer ¼ in. condenser microphones, used as loudspeakers with a circuit to correct for nonlinearities in the frequency response (Frederiksen 1977). These transducers were placed as close as possible to the external ear, at a distance of ≤1 mm. The output of the loudspeaker was measured with a ⅛ in. Brüel and Kjaer microphone and found to be flat ±5 dB between 10 and 100 kHz. Using the frequency response curve of the microphones, attenuator settings used in the experiments were converted to sound pressure level (SPL, re 20 μPa). At the frequencies

used, the attenuation between the two ears was >30 dB, measured at the ear opposite the sound source.

Recording

At the beginning of the first day of recording, the bat was lightly anesthetized (Metofane) and tranquilized with Innovar-Vet. A small opening, <1 mm diameter, was made in the skull overlying the IC, which in bats is at the surface of the brain just under the skull. Between recording sessions, the opening was covered with sterile Vaseline. A waiting period of ≥30 min after administration of Metofane was sufficient for recovery of neural responses. During recording, local anesthetic (Lidocaine) was applied to the scalp incision. The bat was placed in a foam-lined holder, molded to restrain its body firmly but comfortably. The body holder was suspended by elastic bands to damp movements. During recording, the head was held in a fixed position by the post. If the bat showed signs of restlessness, the recording session was terminated.

Neural responses were recorded with glass micropipettes filled with one of the following solutions: 2 M KCl, a 5% solution of horseradish peroxidase in physiological saline, or a 2% solution of Chicago sky blue in 0.5 M sodium acetate. The recording electrode was mounted on a multibarrel electrode (see *Iontophoresis of drugs*) for microiontophoresis of pharmacological agents. The tip diameters of the recording electrodes were ~1.0 μm; impedances ranged from 10 to 40 MΩ. The electrode was placed over the IC and positioned stereotaxically. The electrode was advanced within the IC with a Kopf stepping hydraulic microdrive. Data were only collected when spikes had a signal-to-noise ratio of >3:1 and a biphasic shape, indicating that they were from cell bodies. Spikes were discriminated electronically, and spike times relative to stimulus onset were recorded on a computer.

When a single unit was isolated, we conducted routine tests to determine whether it responded best to pure tones, noise, or frequency sweeps. If the unit responded to pure tones, the best frequency (BF) and threshold were determined. If the unit responded to FM sweeps and not to pure tones, its best sweep depth, best center frequency, and threshold were determined. The best stimulus was then presented while varying sound duration in steps of 1 ms up to 10 ms and in steps of 2 or 5 ms thereafter. Data sets were collected for 20 or 50 stimulus presentations at the durations to which the unit responded. From the data sets, we obtained poststimulus-time histograms, spike counts, average latency of the first spike, variability of the first spike latency, temporal distribution of spikes, and rate-duration functions. A data set without presentation of sound was collected to determine spontaneous rate. For most units, spike times were collected at each duration increment. For a few units, at some increments, spike counts were recorded, but spike times were not collected. As many measures as possible were repeated during iontophoresis of drugs and after recovery.

Iontophoresis of drugs

Antagonists to inhibitory neurotransmitters were delivered by a five-barrel "piggy-back" electrode (Havey and Caspary 1980). The tip of the multibarrel pipette was broken off to an overall diameter of 10–15 μm. The recording electrode was affixed to the five-barrel electrode at an angle of about 20° with the tip of the recording electrode extending about 10 μm beyond the tip of the five-barrel electrode. Four of the barrels were filled with one of the following solutions: GABA (500 mM, pH 3.5–4.0), glycine (500 mM, pH 3.5–4.0), muscimol (10 mM, pH 6.0–6.5), bicuculline methiodide (20 mM, pH 3.0), or strychnine hydrochloride (20 mM, pH 5.8–6.0). One barrel was always filled with bicuculline, a GABA antagonist, and one was always filled with strychnine. The fifth barrel was filled with NaCl (165 mM) and was used for balancing the current. GABA and glycine were used to confirm that the cell had receptors for these neurotransmitters.

During an experiment, the drugs were retained in the electrode by applying a holding current of −15 nA. The drugs were ejected by applying positive current, usually less than +100 nA. While applying ejection current, the neural response to the best stimulus was monitored using several different sound levels. Current was increased in steps of about 10 nA until we determined that the drug affected the neuron's response. Because an increase in evoked spike count was an obvious and reliable consequence of drug application, we interpreted such an increase as evidence that the drug was being delivered to the neuron from which we were recording. We waited until the sound-evoked response stabilized at the new level, presented the stimulus set used before drug application, and recorded the neuron's responses. The ejection current was then turned off, and the response was monitored as before to determine when it returned to the predrug response rate. To document recovery, we waited an additional 10 min and then repeated at least part of the stimulus set. After recovery data were obtained, we repeated the sequence using the remaining antagonist. Recovery from bicuculline was usually complete by 20 min, but recovery from strychnine normally took ~45 min. For this reason, we usually administered bicuculline first. It was not always possible to obtain a complete set of data using both antagonists for each unit. However, for each of the neurons reported here, we did obtain a complete set of predrug, drug, and recovery data using at least one antagonist.

Criterion for duration tuning

In a previous paper (Ehrlich et al. 1997), we defined duration-tuned neurons as those in which spike count as a function of duration had a peak value at one duration or a range of durations that was ≥2 times the lowest nonzero value at longer durations. This criterion identifies neurons that have short-pass or band-pass characteristics for sound duration. It excludes neurons that simply require a minimum integration time and that might be described as having long-pass characteristics for sound duration. In this study, we considered duration tuning eliminated if the responses did not meet this criterion after drug application.

RESULTS

Antagonists of inhibitory neurotransmitters were applied to 51 single units in the IC (Table 1). For this sample of cells, 36 cells responded best to pure tones; 12 cells responded best to frequency sweeps, and three cells responded to noise. In general, strychnine had a smaller and less predictable effect on evoked spike counts than did bicuculline. Of the entire sample of neurons to which bicuculline or strychnine or both were applied, only one failed to show an increase in the number of spikes evoked under at least one of the drug conditions. For this neuron, no effects were seen on first spike latency, discharge pattern, or duration tuning. Although we did not test glycine, application of GABA also had no effect on this neuron's response rate. We concluded that it was likely that the drugs failed to reach the cell membrane, and the results of this

TABLE 1. *Number of all-pass and band-pass (including short-pass) neurons tested with Bic or Stry*

Tuning	Bic	Stry	Bic and Stry	Total
All pass	9	1	18	28
Band pass	12	1	9	22
Total	21	2	27	50

Bic, bicuculline; Stry, strychnine.

neuron were excluded from the tabulation of population statistics.

Of the 50 remaining cells, 22 had band-pass or short-pass tuning to stimulus duration (Table 1). To simplify the description, we refer to these units as duration-tuned. Duration-tuned units were defined as those in which spike count as a function of duration had a peak value that was ≥2 times the lowest nonzero spike count at longer durations or at shorter and longer durations. Duration tuning was considered to have been eliminated if, during drug application, spike count did not meet this criterion. As shown in Table 1, we tested 12 duration-tuned neurons with bicuculline only, one with strychnine only, and nine with bicuculline and strychnine. Of the duration-tuned neurons, 13 responded best to pure tones, and nine responded best to FM stimuli.

Effects of antagonists on duration tuning

As Table 2 indicates, application of bicuculline or strychnine eliminated duration tuning for most units. Not broken down in the table are nine units that were tested with both drugs. In four of these units, both bicuculline and strychnine eliminated duration tuning. In three of these units, bicuculline but not strychnine eliminated duration tuning. In one of the remaining two units, strychnine widened duration tuning while bicuculline had no effect, and in the other unit, neither drug had an effect. Within the population of duration-tuned neurons, there were individual differences in the effects of the two drugs. These differences, along with the effects of the drugs on discharge pattern and latency, are important for understanding the mechanisms underlying duration tuning.

The effects of both bicuculline and strychnine on duration tuning formed a continuum. At one extreme, some units completely lost their band-pass duration tuning by any criterion. Some units no longer met our criterion, peak spike count ≥2 times the count at other durations, but they retained a small peak of enhanced responsiveness at or near best duration. Other units retained band-pass tuning, but the range of durations that elicited spikes widened. Most of these effects are shown in Figs. 2–5, where spike counts are plotted as a function of sound duration, and representative response patterns are shown for four different units. Figures 2 and 3 are examples of units tuned to pure tones, and Figs. 4 and 5 are examples of units that responded to downward FM sweeps only. For most units on which both drugs were tested, bicuculline affected duration tuning to a greater degree than did strychnine.

Figure 2 shows an example of a neuron in which both bicuculline and strychnine clearly eliminated selectivity to sound duration. In the absence of drugs, this unit (1331-6) responded best to a duration of 1 ms. It discharged two or more spikes in response to each presentation of a 1-ms sound, but it virtually stopped responding to sounds >8 ms in duration. Figure 2*B* shows the response patterns evoked by a stimulus at best duration and two longer durations in the absence of drugs. Like most duration-tuned units, this unit's latency increased as duration increased, but it did so only within the duration range to which it responded best, 1–3 ms (Ehrlich et al. 1997). In the time scale of Fig. 2, this shift is not apparent. For sound durations of 1 and 2 ms, the average latencies were about the same, 15.7 and 15.5 ms. However, at sound durations of 3 and 4 ms, average first-spike latencies increased to 19.6 and 21.1 ms. We describe similar latency changes in more detail in a later section.

TABLE 2. *Effects of Bic and Stry on band-pass tuning*

Effect	Bic	Stry
Complete abolition	15	4
Broadened	2	1
None	4	5
Not tested	1	12
Total	22	22

Bicuculline completely eliminated this unit's duration selectivity (Fig. 2, *A* and *C*) so that it responded vigorously to sounds of any duration, with spike counts increasing slightly as a function of duration. The response pattern remained transient, with the maximum number of spikes only slightly over six per stimulus even at long durations (Fig. 2*A*). Average first-spike latencies ranged from 14.3 to 14.9 ms at all durations. At long durations, there appeared to be a small offset response (Fig. 2*C*, *right*).

Application of strychnine also eliminated duration selectivity by our criterion (Fig. 2, *A* and *D*). The response pattern remained transient, and the spike count was about three per stimulus at all durations (Fig. 1*D*), which was only slightly more than the predrug spike count at 1-ms duration. This unit had low spontaneous activity (0.5–1.5 spikes/s) that did not increase during application of bicuculline or strychnine. Latencies ranged from 14.3 to 20.5 ms at all durations.

The fact that the responses to all durations remained transient after application of bicuculline or strychnine suggests that either the excitatory input to this neuron was transient or that one inhibitory transmitter alone was sufficient to inhibit sustained excitation. The unaltered latency at different durations during drug application indicates that the excitatory input was locked to sound onset but normally canceled by inhibition.

Figure 3 shows an example of a unit (1373-2) whose duration tuning was eliminated by bicuculline but not by strychnine. The unit had a best duration of 5 ms; it was broadly tuned, responding to sounds from ~3 to ~20 ms in duration (Fig. 3, *A* and *B*). Because of the broad tuning, the response latency could be followed at many durations, clearly showing that the unit responded at sound offset, as will be shown more fully in Fig. 7. During application of bicuculline, response latency became locked to sound onset (Fig. 3*C*). Application of bicuculline unmasked an excitatory discharge pattern that lasted for 50 ms or more at all stimulus durations and adapted slowly (Fig. 3*C*). Thus for this neuron, sound onset appeared to initiate a long-lasting excitation, the duration of which was largely independent of stimulus duration.

It appears that under normal conditions, when the sound was short, the excitatory response was inhibited for the duration of the sound, allowing the neuron to respond only after sound offset (Fig. 3*B*, 5 versus 20 ms). When the stimulus was long (≥50 ms), the excitatory response was completely inhibited. Even at short durations, when the neuron fired vigorously, the duration of the response was much shorter (Fig. 3*B*) than when GABAergic inhibition was blocked (Fig. 3*C*). There appears to have been a period of GABAergic inhibition that truncated the late portion of excitation [compare poststimulus time histogram (PSTHs) to 5 ms sound in Fig. 3, *B* and *C*]. These

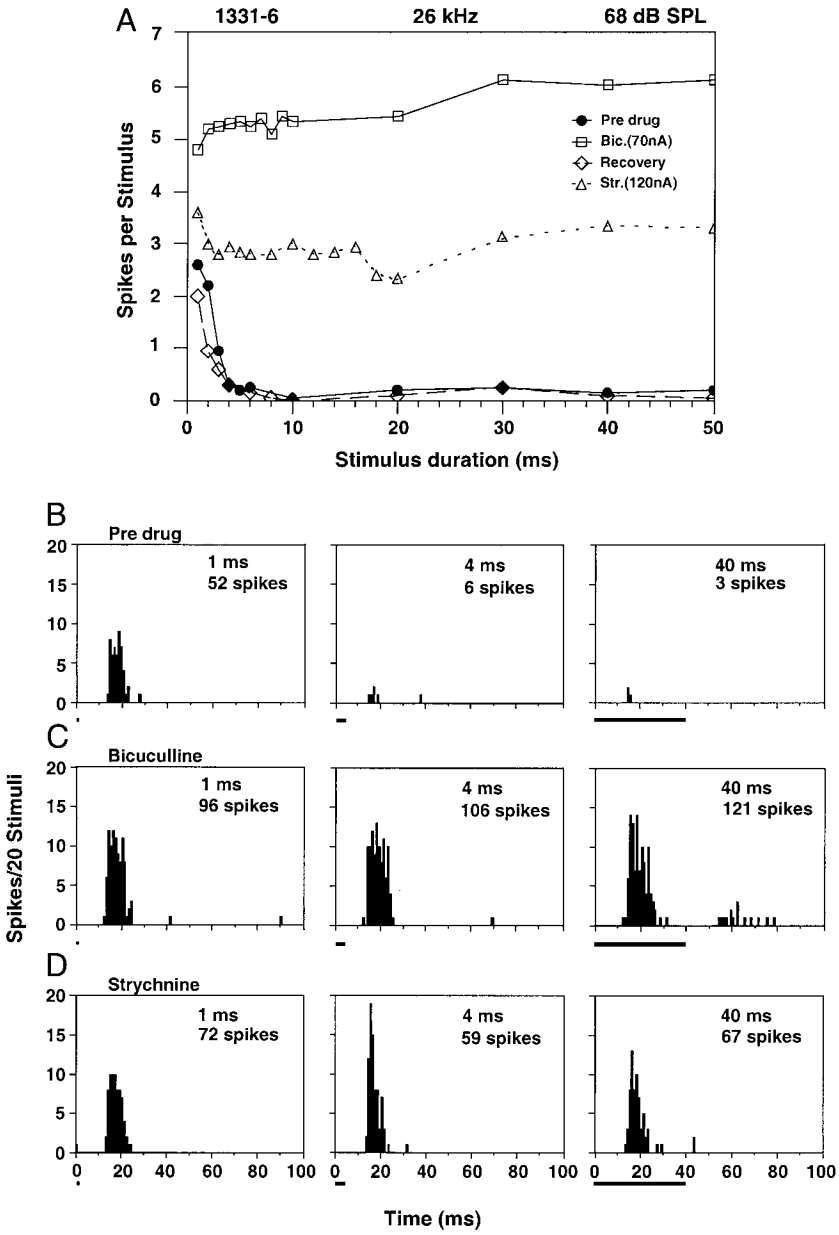

FIG. 2. Spike counts and poststimulus time histograms (PSTHs) for a unit with short-pass filter characteristics. Duration tuning was eliminated by application of bicuculline or strychnine. *A*: spike counts as a function of duration before and after application of drugs. In the absence of drugs (solid circles), the neuron responded best to a 1-ms pure tone and virtually failed to respond to sounds >8 ms. Selectivity to short sounds was eliminated by application of bicuculline (open squares). The neuron recovered duration tuning after bicuculline was discontinued (open diamonds). Application of strychnine (open triangles) also eliminated selectivity to short sounds although there was a slight peak in spike count at 1 ms. *B–D*: representative PSTHs for different durations before and during drug application. The response remained transient during application of the drugs. In this and other similar figures, the following conventions are used: the unit number, stimulus frequency, and sound pressure level are listed above panel *A*. Injection current levels are indicated within panel *A*. Bars under the X-axis of the histograms indicate stimulus duration, which is also stated in the histogram. Twenty stimuli were presented for each histogram, and the bin width was 1 ms.

observations indicate that for this neuron, unlike the previous example, a brief sound set up a long-lasting sequence of excitatory and inhibitory events.

The response latency during application of bicuculline (Fig. 3*C*) was shorter than the predrug response latency (Fig. 3, *B* and *C*). Therefore in this neuron GABAergic inhibition appears to have delayed spike onset as well as to have truncated the late period of the response. In the DISCUSSION, we shall show how our first model of duration tuning must be modified to account for this kind of response pattern.

After application of strychnine (Fig. 3, *A* and *D*), there was no effect on the range of the duration tuning. However, strychnine apparently was released from the pipette sufficiently to affect the cell's responses because the total spike count at 5 ms doubled, and the latency shortened (Fig. 3*A*). The decrease in response latency to the 5-ms sound (Fig. 3, *B* and *D*) indicates that glycinergic input normally canceled the earliest part of the excitatory input. Further, the few spikes that occur before the end of the 20-ms sound suggest that strychnine changed the neuron's discharge pattern so that an onset response appeared (compare middle graphs in Fig. 3, *B* and *D*). Thus the glycinergic input, although it was not sufficient to produce duration tuning, appeared to normally modulate the temporal characteristics of the excitatory response.

Many neurons in the IC of bats (Casseday and Covey 1992; Ehrlich et al. 1997; Fuzessery 1994; Suga 1964), rats (Poon et al. 1992), and mice (Brand and Grothe 2000) respond selectively to FM sweeps. In our sample of neurons, some FM sweep selective neurons also seemed to be tuned to duration. Figure 4 shows the effects of application of antagonists on a unit (1369-2) that responded selectively to downward FM sweeps (55–30 kHz), 3–15 ms duration, with a best duration of 9 ms. Application of bicuculline or strychnine completely eliminated this neuron's band-pass duration tuning. The predrug PSTHs for this unit show that the response latency tracked the end of the sweep, indicating that the neuron either re-

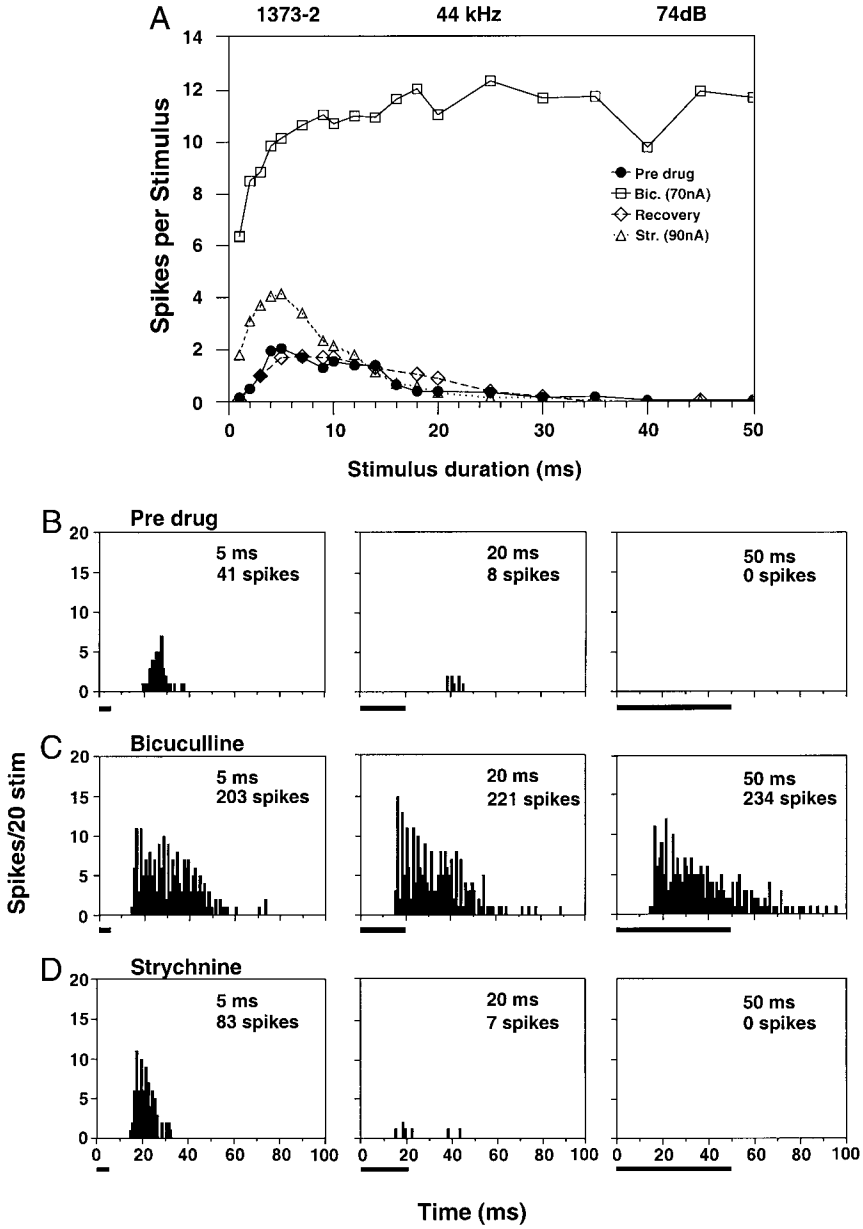

FIG. 3. Spike counts and PSTHs for a unit with band-pass filter characteristic. Duration tuning was eliminated by application of bicuculline but not strychnine. *A*: spike counts as a function of duration. In the absence of drugs (closed circles), the neuron responded best to a 5-ms pure tone and virtually failed to respond to sounds >18 ms. Selectivity to short sounds was eliminated by application of bicuculline (open squares). Strychnine (open triangles) resulted in an increase in spike count for sound durations at and around the predrug best duration but did not change the neuron's responsiveness to long durations. *B–D*: representative PSTHs for different durations before and during drug application. The response changed from transient to sustained during application of bicuculline. See caption to Fig. 2 for further explanation of this figure.

sponded to lower frequencies in the late part of the sweep or that it required the entire frequency sequence before responding.

After application of bicuculline, duration tuning was eliminated, and spike count simply increased to successive plateaus as duration increased (Fig. 4*A*). Duration tuning recovered to predrug levels after bicuculline application was stopped. Application of strychnine also eliminated band-pass tuning by our criterion. The spike counts at long durations were all greater than half the spike count at 9 ms. However, a slight peak in spike count remained at around 9 ms (Fig. 4*A*).

Application of bicuculline revealed a discontinuity in the neuron's temporal discharge pattern at sound durations of 20 ms or longer (Fig. 4*C*). For sounds below 20 ms, during application of bicuculline, the latency was shorter, spike counts were higher, and the response persisted far longer than before drug application. For sounds 20 ms in duration and longer, an early response was followed by a silent period and then by a large burst of spikes with latencies that appeared to track the late part of the sweep. This discontinuity in the spike train suggests that the two responses were elicited by separate parts of the sweep, possibly by separate frequency bands. A striking feature of the second response is that the temporal period of responding was not related to the duration of the sweep. The period of the second response, ~40 ms, was roughly the same for a 9-ms sweep as for a 50-ms sweep. The results suggest that under normal conditions any FM sweep elicited long-lasting excitation, the latter part of which was always inhibited. For a short sweep, the early part of the excitation remained, but when the sweep was longer than ~20 ms, most of the first part of the response also was inhibited. We will show in the DISCUSSION how the model can be modified to account for sustained excitatory input.

Like bicuculline, strychnine eliminated duration tuning in this neuron and unmasked two temporal components in its response. However, the change in spike count was smaller than

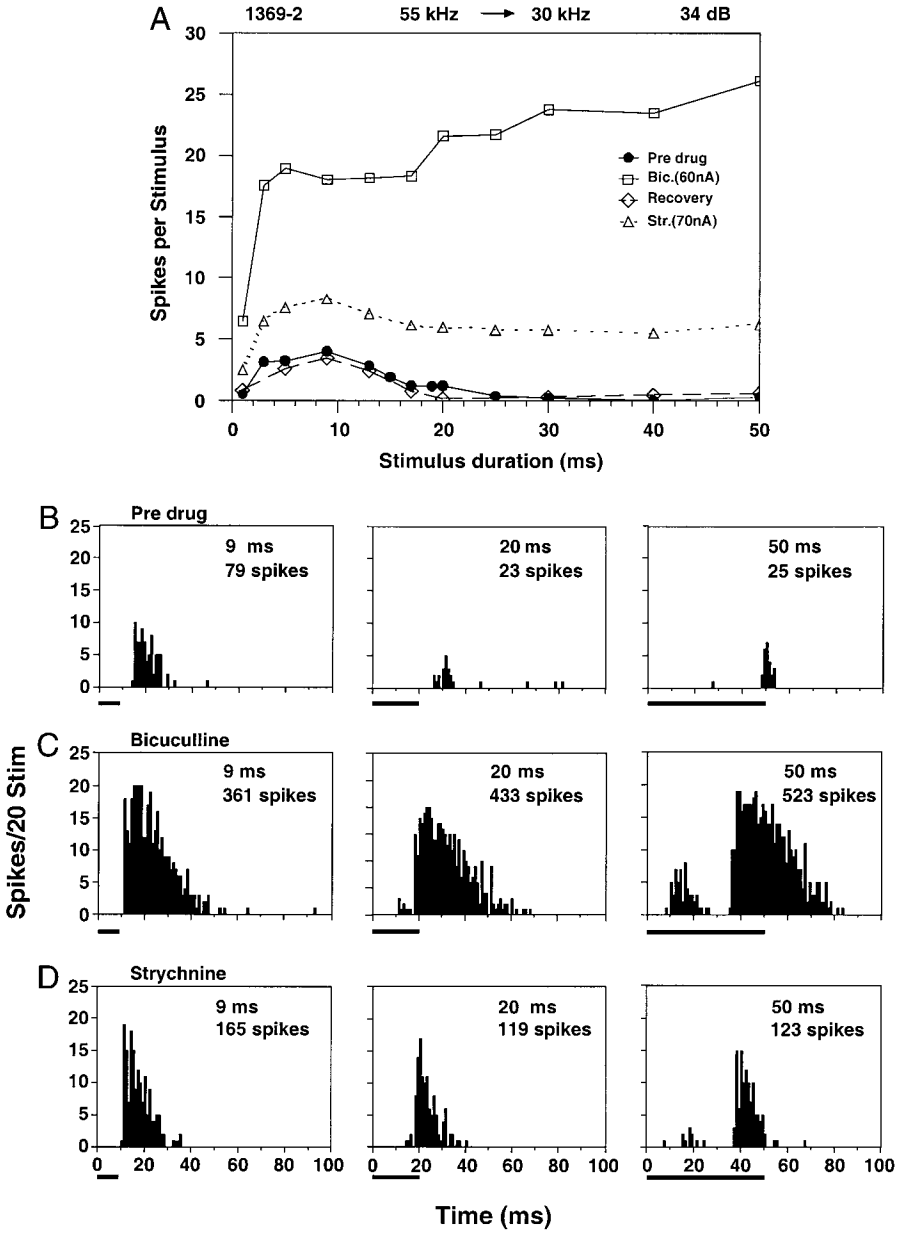

FIG. 4. Spike counts and PSTHs to an FM sweep 55–30 kHz. This unit had band-pass filter characteristics, and its duration tuning was eliminated by application of bicuculline or strychnine. *A*: spike counts as a function of duration. In the absence of drugs (filled circles), the neuron responded best to a 9-ms FM sweep and virtually failed to respond to sounds >20 ms. Selectivity to short sounds was eliminated by application of bicuculline (open squares). Application of strychnine (open triangles) also eliminated selectivity to short sounds, although there was a slight peak in spike counts at and around best duration. *B–D*: representative PSTHs for different durations before and during drug application. During application of bicuculline, the period of responding lengthened considerably at all durations. At long durations, both drugs appear to have unmasked two separate excitatory inputs, perhaps from spectrally different parts of the sweep. See caption to Fig. 2 for further explanation of the figure format.

with bicuculline. Most of this difference may be attributed to the fact that strychnine had only a small effect on the length of the spike train. This observation suggests that the glycinergic input was more transient than the GABAergic input, or, considering the results in Fig. 4*C*, the GABAergic inhibition exerted a greater effect on the late part of the excitatory response than did the glycinergic input.

For the cell in Fig. 5 (1388-3), application of bicuculline did not eliminate band-pass duration tuning, but spike output greatly increased, and tuning to duration became considerably broader. For example, the range of durations at which the response was 50% of the maximum spike count changed from about 8 to about 20 ms. In this case, the increased breadth of duration tuning appears to be accounted for by a lower threshold for response (Fuzessery and Hall 1996).

Taken together, the units in Figs. 2–5 show the range of effects of antagonists to inhibitory neurotransmitters on duration tuning. For the 21 units tested with bicuculline, duration tuning was completely eliminated in 15, broadened in two, and unaffected in four units (Table 2). For two of these four, it was not possible to pass more than 35 nA current through the electrode, so it is possible that the amount of drug delivered was inadequate.

For the 10 units tested with strychnine, duration tuning was eliminated in four, broadened in one, and unaffected in five (Table 2). For one of these five, application of glycine failed to inhibit it. This unit was one in which bicuculline did eliminate duration tuning.

Effects of antagonists on spontaneous discharge

The IC receives GABAergic inhibitory input from nonauditory nuclei, such as the substantia nigra, which could provide tonic inhibition to IC neurons (review, Casseday and Covey 1996). Tonic inhibition raises a potential complication for interpretation of our results. That is, drug application might

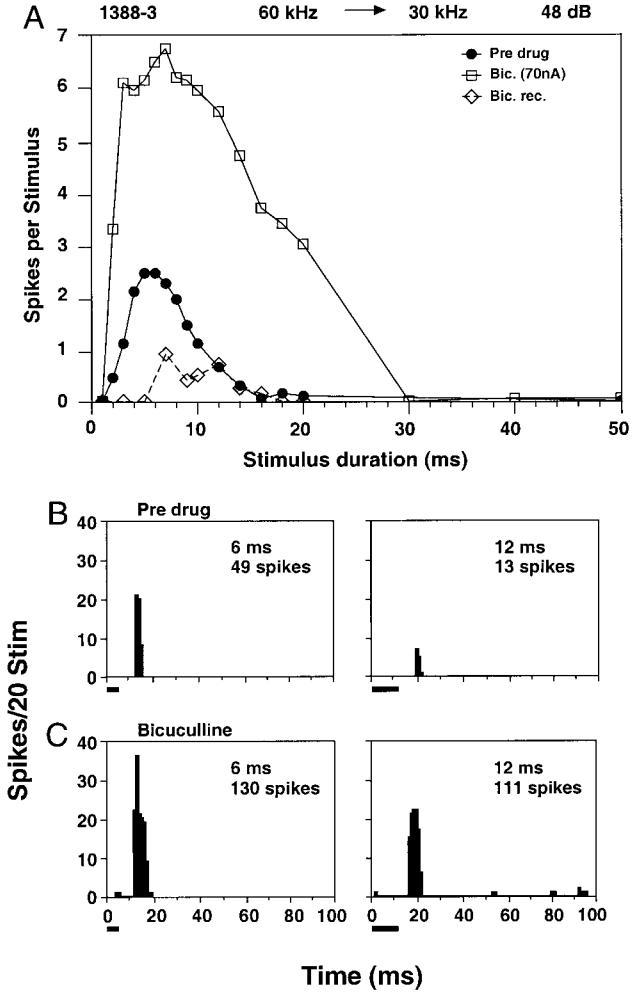

FIG. 5. Spike counts and PSTHs in response to a downward FM sweep (60–30 kHz). This unit had band-pass duration tuning that was not eliminated by application of bicuculline. *A*: spike counts as a function of duration. In the absence of drugs (filled circles), the neuron responded best to a 5-ms FM sweep and failed to respond to sounds >20 ms. Selectivity to short sounds remained intact during application of bicuculline (open squares), although spike count increased markedly. *B* and *C*: representative PSTHs for different durations before and during drug application. During application of bicuculline, the response pattern remained transient. Note that bicuculline decreased latency. See caption to Fig. 2 for further explanation of the figure format.

have raised the neurons' baseline activity so much that it masked the underlying duration tuning. Therefore we compared the effects of drug application on spontaneous activity with its effects on sound-evoked activity. We recorded spontaneous activity before and during drug application in 20 duration-tuned units. When bicuculline was applied, spontaneous activity increased in half of these units. The mean increase in spontaneous rate was 8 spikes/s. Application of strychnine caused spontaneous discharge to increase in 6/7 band-pass units tested. The mean increase for these units was 5.3 spikes/s.

An increase in spontaneous activity does not account for the increase in evoked spike count following application of bicuculline or strychnine. The unit (1358-8) shown in Fig. 6 was chosen to illustrate this point because it was the one that showed the largest increase in spontaneous activity. Before application of bicuculline, the unit had virtually no spontaneous activity and a transient PSTH (first and second panels, Fig. 6*A*). After bicuculline was applied, the spontaneous rate increased to 75 spikes/s. Nevertheless, comparing the responses evoked by 1 and 40 ms stimuli in Fig. 6*B* (*left* and *middle*), it is clear that the discharges in the presence of bicuculline are not simply the addition of the response before drug application to the baseline rate of spontaneous activity in the presence of bicuculline. For the 40-ms stimulus, less than one third of the total spike count can be accounted for by the increase in spontaneous discharge. Most of the increase in evoked response can be attributed to the unit's acquiring a sustained response. The most likely conclusion is that the conversion of a transient to a sustained response was due to antagonism of stimulus-evoked inhibition that normally suppressed the later part of the response. It might be argued that tonic inhibition, unrelated to the auditory stimulus, normally rendered subthreshold the sustained component of a primary-like excitatory input. Blocking the tonic inhibition would unmask the sustained component. However, to obtain a response pattern like that in Fig. 6*B* (*middle*), the sustained component would have to be enhanced more than the onset component.

Effects of antagonists on discharge pattern and latency

The previous figures showed that the discharge patterns seen during blocking of inhibitory neurotransmitters provide considerable information about the temporal properties of the excitatory input plus whatever class of inhibitory input remains. We suggested that most units in the IC receive inhibition that affects both the early and late parts of the excitatory input. For example, if latency decreases after inhibition is blocked, it suggests that inhibition normally truncates the early part of the excitatory response. Likewise, if the excitatory response lasts longer when inhibition is blocked, it suggests that inhibition normally truncates the late part of the excitatory response.

To assess the effects of inhibition on the early part of the response, we examined spike latencies before and after blocking inhibitory input. To assess inhibition on the late part of the response, we measured the temporal interval over which spikes occurred in response to sound. We shall refer to this measure as the "response period." For measurement of the response period, we selected units that had little or no spontaneous activity. We measured the time between the first and last evoked spike. Latency and response period were both measured at the unit's best duration.

RESPONSE PERIOD. For 9 of 12 duration-tuned units that responded to pure tones, the response period increased in the presence of bicuculline, with a mean increase of 7.6 ms. For 9 of 10 duration-tuned units that responded best to FM sweeps, the response period increased when bicuculline was applied, with a mean increase of 11.1 ms.

Strychnine caused smaller increases in response period than did bicuculline. In three of six duration-tuned units that responded to pure tones, the response period increased, with a mean increase of 1.0 ms. In all three duration-tuned units that responded best to FM sweeps, the response period increased, with a mean of 5.7 ms. Examples of these effects have been noted in Figs. 3–5.

We examined the response period to 5-ms pure tones of neurons insensitive to sound duration and found that the lengthening of the response period was not a special characteristic of duration-tuned neurons. Bicuculline caused the re-

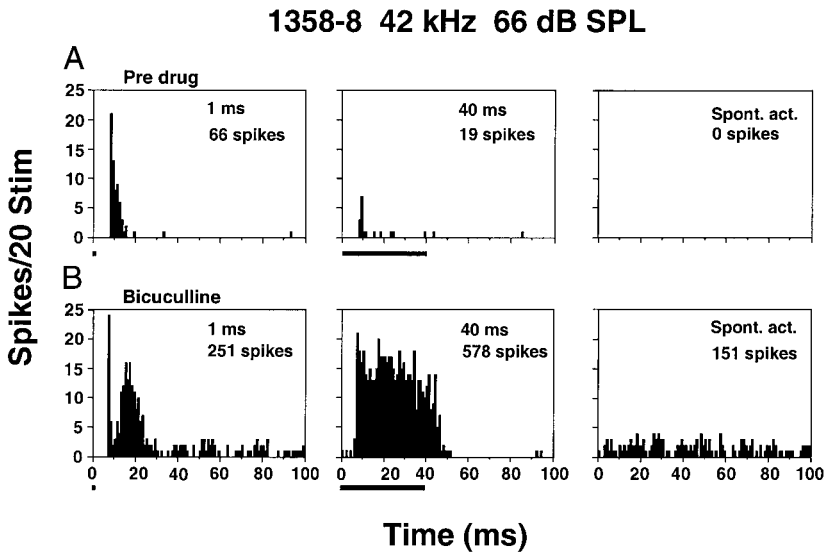

FIG. 6. PSTHs to show effects of bicuculline on response pattern and spontaneous activity. *A*: in the absence of drugs the response pattern was transient, and there was no spontaneous activity. *B*: after application of bicuculline, the response pattern became sustained, and spontaneous activity appeared. Of all units tested, this unit showed the largest increase in spontaneous activity. See caption to Fig. 2 for explanation of the figure format.

sponse periods of all 12 units tested to increase, with a mean increase of 13.2 ms. Strychnine caused the response period of six of seven units tested to increase, with a mean increase of 3.3 ms. These data reinforce the notion proposed earlier that the effective time course of glycinergic inhibition is shorter than that of GABAergic inhibition.

To gain some idea of whether the excitation that is suppressed by GABAergic input is usually transient or usually sustained, 10 duration-tuned units were tested with a 40- or 50-ms-long stimulus during application of bicuculline. Eight of these units continued to respond transiently (e.g., Fig. 2), indicating that either the excitatory input was transient or glycine alone was sufficient to truncate the late part of the response. Of the other two units, one had a long, slowly adapting response to all durations (Fig. 3), and the other had a sustained nonadapting response to long durations (Fig. 6). For both of these neurons, bicuculline revealed excitatory input, evoked by the shortest stimulus, that lasted many times longer than the duration of that stimulus (Figs. 3*C* and 6*B*). Apparently for such neurons, GABAergic inhibition normally truncates the later part of the response. For five duration-tuned units, application of strychnine had little or no effect on the transient discharge pattern (e.g., Fig. 2).

Inhibitory shortening of the response period is not unique to duration-tuned neurons. In the IC units sampled, the proportion of nonduration-tuned units that received sustained or prolonged excitatory input was about the same as for duration-tuned units. In two of eight nonduration-tuned units, application of bicuculline produced a sustained response to a 50-ms tone. For the other six, the response remained transient.

LATENCY. For some units, blocking inhibition brought about a clear decrease in latency (e.g., Fig. 3). Two further examples with tests at several durations show that much of the latency decrease can be accounted for by a change in the response from offset to onset (Fig. 7). Unit 1373-2 was tested with bicuculline (Fig. 7*A*) and unit 1380-8 with strychnine (Fig. 7*B*). The results are similar for both. The predrug latency increased with stimulus duration. However, during drug application, the latency remained constant as sound duration increased.

Whether or not a unit's latency changed is important evidence concerning the effects of inhibition on the early part of the response of an IC cell. Units that responded to sound offset under predrug conditions started responding to sound onset when inhibition was blocked. This finding raises a problem in measuring and interpreting latency. Obviously, for these units, the quantitative decrease in latency is somewhat arbitrary, because it depended on the duration of the sound, and as just shown (Fig. 7), the difference can be made larger by increasing the sound duration. However, for the purpose of this analysis, the important question is not the exact amount by which

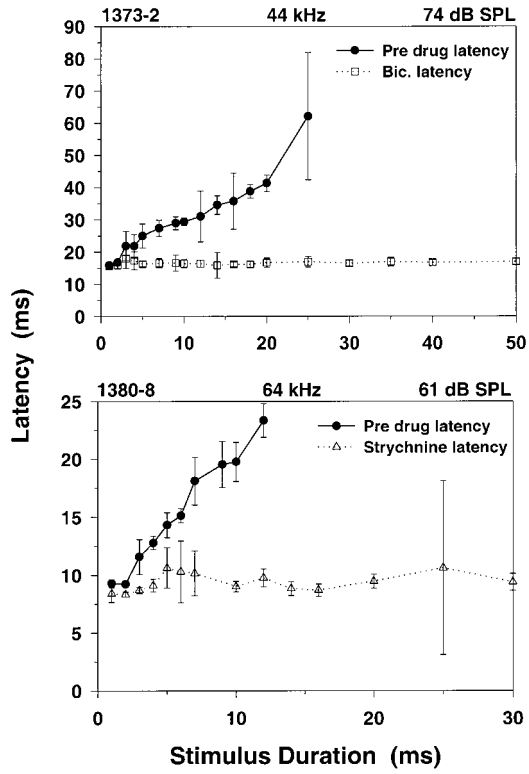

FIG. 7. Examples of changes from an "offset" response pattern to an "onset" response pattern during blocking of inhibitory neurotransmitters. In the absence of drugs, the latency of both units tracked the end of the stimulus. After application of bicuculline (*A*) or strychnine (*B*), response latency became locked to stimulus onset and remained constant. Bars: SD.

latency changed, but whether or not it did. To obtain accurate latency measurements for duration-tuned units, we measured latency to sounds that produced the peak spike count, i.e., at best duration. For nonduration-tuned units, latency was measured in response to a 5-ms sound.

Figure 8 shows the changes in latency for the entire sample of units tested with bicuculline or strychnine. The general tendency is for latency to decrease during drug application regardless of whether or not the cell exhibits band-pass tuning to duration. For each unit, we performed *t*-tests on first spike latencies, at best duration, to determine whether the drug produced a significant shift in latency. When bicuculline was applied, 50% (10/20) of duration-tuned units and 18% (5/28) of nonduration-tuned units had a highly significant shift in latency ($P < 0.0001$; filled symbols in Fig. 8*A*). When strychnine was applied, 75% (6/8) of duration-tuned units and 35% (7/20) of nonduration-tuned units had a highly significant shift ($P < 0.0001$; filled symbols in Fig. 8*B*). The few units that shifted significantly in the longer direction were not tuned to duration.

DISCUSSION

The finding that bicuculline and strychnine eliminated or drastically altered the band-pass duration tuning of IC neurons supports two general ideas. First, duration tuning appears to be an emergent property that is established in the IC. Second, duration tuning is generated by a coincidence mechanism that requires specific temporal sequences of inhibition and excitation. Among our sample of neurons, there were minor variations in the patterns of inhibition and excitation. The DISCUSSION will be concerned with the details of how duration tuning could be created in the IC using these different patterns. We begin with a description of the different sources of excitatory and inhibitory inputs to duration-tuned neurons. We then examine how the results reported here fit a model proposed previously to account for band-pass duration tuning. Finally, we show an addition to the model that takes into account different response patterns of the inputs to the duration-tuned cell.

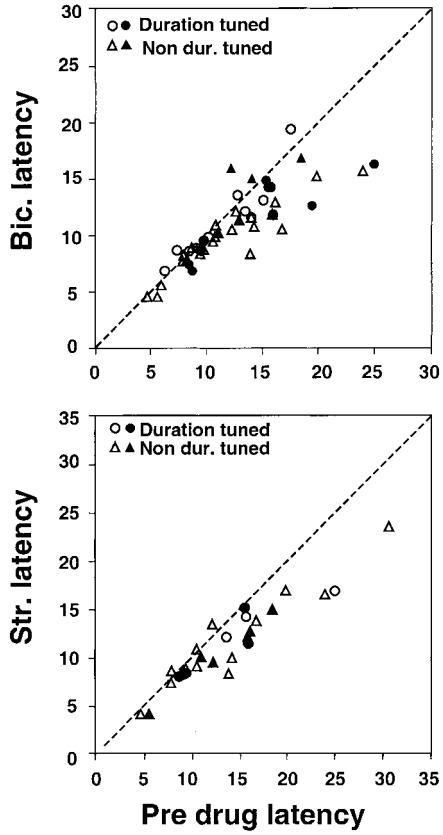

FIG. 8. Effects of bicuculline and strychnine on response latencies of a population of inferior colliculus (IC) cells. For duration-tuned cells, all measurements were taken at best duration. For nonduration-tuned cells, measurements were taken at a stimulus duration of 5 ms. Values below the diagonal represent units in which latency decreased following application of bicuculline (*A*) or strychnine (*B*). The decrease was seen in units that had band-pass tuning to duration as well as those that did not. The filled symbols represent highly significant differences ($P \leq 0.0001$; *t*-test) between predrug values and values during drug application. No units with band-pass duration tuning had a significant shift to a longer latency.

Excitatory and inhibitory inputs to duration-tuned neurons

RESPONSE PATTERNS. Although duration tuning appears to first emerge at the level of the IC, it depends in part on the response properties of neurons that project to the IC. Of course, we have no way of identifying the entire population of neurons that project to each duration-tuned neuron; nevertheless, lower brainstem neurons that have response patterns relevant to our findings are putative sources of input to duration-tuned neurons in the IC. The relevant response patterns are transient-on, sustained-on, and transient-off. Neurons with each pattern could be either excitatory or inhibitory.

Although the majority of neurons at levels below the IC have a sustained response pattern in *Eptesicus*, the conversion from sustained to transient discharge pattern occurs as early as the cochlear nucleus (Haplea et al. 1994). In several species of bats, transient-on responses are also seen in the medial superior olive (MSO) and in the nuclei of the lateral lemniscus (Covey and Casseday 1991; Grothe 1994; Grothe et al. 1992, 1997). This type of response pattern is relevant to the finding that for most neurons (8/10 tested with bicuculline and 5/5 tested with strychnine) the response remained transient even after blocking one class of inhibition. This finding could be interpreted in several different ways. First, it is possible that either GABAergic or glycinergic input alone is sufficient to maintain a transient response even though the excitatory input is sustained. Second, the excitatory input to these duration-tuned neurons might have already been transient. Third, the membrane properties of the cell might have rendered the response transient even though the excitatory input was sustained. Further experiments will be necessary to determine the extent to which each of these factors contributes to the transient response of IC neurons.

Another response category that is important for creating duration tuning is transient onset inhibition. One possible candidate for the source of onset glycinergic inhibition is the ventral nucleus of the lateral lemniscus (VNLL). In *Eptesicus*, these nuclei include the columnar division (VNLLc), which is a homogeneous set of small spherical cells (Covey and Casseday 1986) that are broadly tuned to frequency and respond to the onset of sound with a single, constant latency spike (Covey and Casseday 1991). Recently it has been shown that these neurons are glycinergic (Vater et al. 1997). Because these neurons have short-latency transient responses, they presumably could only provide the initial segment of onset inhibition and would not be a source of sustained inhibition or late

inhibition. This idea is consistent with the observation that strychnine revealed only a fairly brief transient response in the same neurons that had a sustained response under the influence of bicuculline. Thus VNLLc neurons are a putative source of early-onset inhibition. However, there is a limitation to this idea. Many of the columnar cells have high thresholds for pure tones, so they would not serve duration-tuned cells with low thresholds. A possible function of these high-threshold cells might be to provide an input that would endow duration-tuned cells with tolerance to high sound levels.

Transient off-responses have been seen in the MSO of several species of bats (Covey et al. 1991; Grothe 1994; Grothe et al. 1992, 1997; Grothe and Sanes 1994). A recent study of the MSO in the big brown bat (Grothe and Casseday 1998) showed, in a sample of over 90 units, that 23% responded at the offset of sound. Therefore these MSO neurons are a putative source of offset excitation to duration-tuned neurons.

Finally, there is no shortage of sources of sustained responses, many of which are presumably excitatory and include cochlear nucleus, superior olive, and nuclei of the lateral lemniscus. A potential source of sustained inhibition is in the nuclei of the lateral lemniscus. Many neurons in the intermediate and ventral nuclei of the lateral lemniscus respond with a sustained discharge pattern; a large number of neurons in these nuclei stain for glycine or GABA or both (Vater et al. 1997), indicating that they are candidates for providing sustained inhibitory input.

DELAY LINES. The pathways ascending to the IC are assembled in a way that could create synaptic delay lines, in the range of several milliseconds, as they converge at an IC neuron. For example, the range of latencies in the nuclei of the lateral lemniscus is considerably greater than the range in the cochlear nucleus (Haplea et al. 1994). Neurons in the nuclei of the lateral lemniscus have a spread of latencies of ~10 ms for a given frequency (Covey and Casseday 1991). Thus a compelling hypothesis is that these ascending inputs provide IC neurons with specific temporal sequences of excitation and inhibition (review, Casseday and Covey 1995; Covey and Casseday 1999). Moreover, GABAergic cells are present in the IC, providing a further possibility for feed-forward inhibition that could alter the responses of other IC neurons to temporal components of sound. In addition to these synaptic inputs, it is highly probable that intrinsic membrane properties contribute to shaping the time course of excitation and inhibition at the IC cell.

In addressing the question of whether duration tuning arises at the IC exclusively or whether it exists at lower levels, we have to consider that there may be species differences. Fuzessery and Hall (1996) examined duration-tuned neurons in the IC of the pallid bat (*Antrozous pallidus*) and found that the duration tuning of many neurons was not affected by application of bicuculline. They concluded that duration tuning either originated at some level below the IC, or in a population of IC neurons that projected to the ones from which they recorded. They did not examine the effects of strychnine on these cells. Thus another possibility is that in the IC of *Antrozous*, glycinergic inhibition plays a more important role in creating duration tuning than it does in the IC of *Eptesicus*.

A model for duration tuning

As described in the introduction, our previous model for duration tuning had three main components: onset inhibition, onset excitation, and offset excitation (Fig. 1).

ONSET INHIBITION. For many neurons in the IC, blocking inhibition reduces spike latency. This observation indicates that these neurons receive stimulus-evoked inhibitory input that precedes or arrives simultaneously with onset-evoked excitatory input. There is direct evidence for onset inhibition (Covey et al. 1996; Kuwada et al. 1997). For example, intracellular recordings from 15 IC neurons showed that for six of these, the initial sound-evoked synaptic input was hyperpolarizing (Covey et al. 1996). Although band-pass duration-tuned neurons clearly do receive onset inhibition, it is difficult to know whether this inhibition is sustained or transient or perhaps differs among neurons. For the two neurons that produced a sustained response after inhibition was blocked, we can conclude that, under normal conditions, inhibition must have been sustained to partially or completely counteract the sustained excitatory input. For neurons whose response remained transient after inhibition was blocked (8/10 tested with bicuculline and 5/5 tested with strychnine), we have no way of estimating the temporal properties of inhibition, except to say that the inhibition must last at least long enough to cancel the excitatory input at nonoptimal stimulus durations. To create band-pass duration tuning, inhibition must arrive before excitation. If it did not, there would be no mechanism to counteract suprathreshold onset or offset excitation at short durations; the result would be short-pass filtering. The only units in our sample that appeared to have short-pass tuning also had best durations of $\leq$1 ms. However, because we could not test durations below 1 ms, it was not possible to determine whether these units were actually short-pass filters.

For many units (Table 2), application of either bicuculline or strychnine eliminated duration tuning. These results suggest that both glycinergic and GABAergic inhibition are necessary for duration tuning, but that neither alone is sufficient to produce it. Therefore the question arises of whether the two inhibitory neurotransmitters serve different roles in creating duration tuning. If either glycine or GABA contributed more than the other to onset inhibition, then we would expect that that there would be a clear difference between the effects of strychnine and bicuculline on response latency. We saw no such difference (Fig. 8). Of course, for neurons tuned to very short durations, the range of possible latency decrease is accordingly short. In this case it would be difficult to detect a difference. One way in which the effects of bicuculline and strychnine did differ was that there was a greater lengthening of the response period when GABAergic inhibition was blocked than when glycinergic inhibition was blocked (e.g., Fig. 4). This finding indicates that GABAergic inhibition dominates over the sustained period of the response, while glycinergic inhibition is most effective in the early part of the response. A possible candidate for the source of onset glycinergic inhibition is VNLLc.

Although glycine is not the only source of onset inhibition for all IC neurons, it could be the main source for neurons with very short best durations. For these neurons, it would have been very difficult to see any latency shift when tested at best duration. For example, if tested with a 1-ms tone, the duration

of the onset inhibition might not be more than 1 ms, making any latency shift difficult to see.

TRANSIENT ONSET EXCITATION. The original model of duration tuning (Fig. 1) proposes that there is an excitatory input locked to sound onset, with a fixed latency and a transient discharge pattern. The responses of most duration-tuned neurons maintained a transient discharge pattern even when inhibition was blocked. However, rather than being locked to the offset of sound, the response became locked to the onset, so that latency remained relatively constant across duration. Our interpretation of this observation is that these duration-tuned neurons received transient excitatory input evoked by sound onset, but that this input was normally canceled by inhibition at nonoptimal durations. What this interpretation means for the model is that the onset excitatory input is not simply a subthreshold EPSP that only produces a spike when it coincides with another excitatory event; instead, under normal conditions the onset excitatory input is actively inhibited at nonoptimal durations. When sound duration is longer than the unit's best duration, the onset inhibition must last long enough to override the entire period of excitation. Evidence consistent with this idea is seen when there is partial "failure" of the onset inhibition. In the absence of drugs, some units fired a few spikes even at nonoptimal durations. These spikes had about the same latency as the spikes at best duration and approximately the same latency as the spikes revealed when inhibition was blocked (Fig. 6). Presumably, the onset inhibition incompletely canceled the onset excitation in these neurons, making it evident that an excitatory input arrived at a fixed delay relative to stimulus onset. These observations suggest that duration tuning can be controlled by only two main components, onset-evoked, sustained inhibition, and a suprathreshold excitatory input. Of course, this model would only create short-pass duration tuning.

OFFSET EXCITATION. The model calls for excitatory input or some other depolarizing influence, the latency of which is correlated with the offset of sound. Offset excitation could be generated by an intrinsic mechanism such as rebound from inhibition, as originally suggested (Casseday et al. 1994), or it could result from offset excitation via an afferent pathway. The fact that blocking inhibition for most neurons converted the offset response to an onset response indicates that inhibition normally suppressed onset excitation (Figs. 3 and 6). In cases where no offset response remained after blocking inhibition, there was either no extrinsic offset excitation or it was blocked by the remaining inhibition. The idea that extrinsic offset excitation is absent in some cells is supported by intracellular recordings showing that the size of the inward current that is correlated with stimulus offset decreases as stimulus duration increases. That is, when inhibition has more time to decay before sound offset, the inward current at sound offset is smaller (Covey et al. 1996). Nevertheless, in cases where an excitatory response remained at the offset of sound after blocking one source of inhibition (Fig. 2), we may have evidence for extrinsic excitatory input that occurs at sound offset. As pointed out previously, many neurons in MSO respond only at the offset of sound. The MSO is a major source of projections to the IC, so this finding raises the possibility that its offset responders provide offset excitatory input to duration-tuned cells. However, it is possible that the remaining source of inhibition contributed a rebound phenomenon.

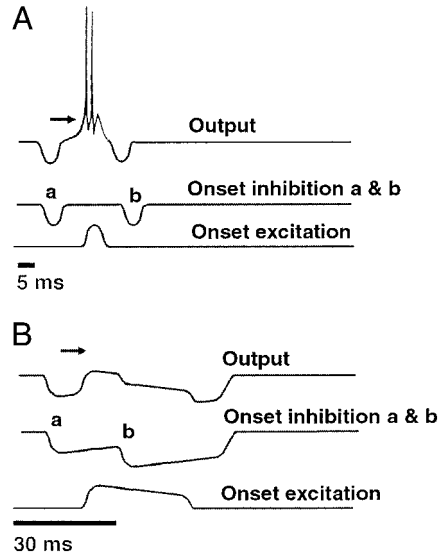

FIG. 9. A model for duration tuning with three sustained inputs: early onset-inhibition (*a*), late onset-inhibition (*b*), and delayed onset-excitation. *A*: for short sounds the early (*a*) and late (*b*) inhibition (*middle trace*) are correspondingly short. The delayed onset excitation (*bottom trace*), which is suprathreshold, falls in the gap between the two inhibitory events, and spike threshold is reached (arrow, *top trace*). *B*: for long sounds the gap between the early and delayed inhibition is closed (*middle trace*). The onset excitation (*bottom trace*) is over-ridden by inhibition and the output (*top trace*) remains below spike threshold (arrow). This model could create band-pass duration tuning. Longer best durations would require increased latency of excitation and a corresponding increased latency of the late inhibition.

LATE ONSET-EVOKED INHIBITION AND SUSTAINED ONSET EXCITATION. The results raised the possibility of a type of inhibitory input not proposed in the earlier model. In two neurons, blocking inhibition revealed sustained excitatory input (Figs. 3 and 6). Thus sustained inhibition must have contributed to their duration tuning by canceling the entire period of excitation at long durations. The model has to be adapted to accommodate sustained, onset-correlated, excitation in units such as these.

One possible mechanism employs a combination of early inhibition from one source and late inhibition from another (Fig. 9). For example, assume that early inhibition and late inhibition are both evoked by sound onset and that both are sustained for a time equivalent to the sound duration. For short-duration sounds, there would be a temporal gap or window during which excitation could depolarize the cell. As sound duration increased, so would the duration of the early inhibition. Therefore as sound duration increased, the end of the early inhibition would eventually overlap with the beginning of the late inhibition, closing the window during which excitation could occur. As pointed out earlier, a potential source for sustained inhibition of this type is in the nuclei of the lateral lemniscus.

Conclusion

Given the important role inhibition plays in shaping duration-tuning, future studies will be aimed at questions of whether duration-tuned cells are also restricted in their response to other stimulus parameters. Do duration tuned cells have limited frequency response profiles, such as closed or

very narrow frequency tuning curves? Is duration tuning tolerant to changes in sound level? Do duration-tuned cells respond to other temporally modulated stimuli, such as sinusoidal modulations of frequency or amplitude, and if so are these responses consistent with their duration tuning characteristics?

The results of this study indicate that our previous model can account for duration tuning of all but two of the neurons in this study. These two neurons had a sustained excitatory input, which requires a variation of the model, the addition of a delayed (late) inhibitory input. This addition is a rather minor modification of the general principle that duration tuning requires a specific temporal sequence of inhibition and excitation. Considering the rich variety of inputs to neurons in the IC, it does not seem surprising to find variation in the sequence, relative strengths, and durations of the excitatory and inhibitory inputs that produce duration tuning. Although the inputs to each IC neuron may vary in their properties, three principles for construction of duration tuning emerge. First, an excitatory component and an inhibitory component are temporally linked to the onset of sound but arrive at slightly different latencies. Second, the duration of one or more inhibitory components must be linked to the duration of the stimulus. Third, there must be excitation that is temporally linked to the offset of the sound so that it coincides with onset excitation at optimal durations. Tuning to other temporal parameters may be brought about by variations on the same theme (Casseday et al. 1997; Grothe 1994). Moreover, these mechanisms might operate in other sensory systems or other neural systems in which it is important to encode the duration of sensation or action.

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NOTE ADDED IN PROOF

A recent study (Bauer et al. 2000) has provided important evidence concerning the time course of GABAergic inhibition in IC cells in another species of bat. This study showed that inhibition persists for tens of ms after the excitatory response and is eliminated by application of bicuculline. Thus the long-lasting GABAergic inhibition seen in our duration-tuned cells is also found in other types of IC cells, apparently providing the system with other types of temporal filtering.

REFERENCES

BAUER EE, KLUG A, AND POLLAK GD. Features of contralaterally evoked inhibition in the inferior colliculus. *Hear Res* 141: 80–90, 2000.

BRAND A AND GROTHE B. Duration-tuned neurons in the mouse inferior colliculus (Abstract). *Assoc Res Otolaryngol* 23: 255, 2000.

CASSEDAY JH AND COVEY E. Frequency tuning properties of neurons in the inferior colliculus of an FM bat. *J Comp Neurol* 319: 34–50, 1992.

CASSEDAY JH AND COVEY E. Mechanisms for analysis of auditory temporal patterns in the brainstem of echolocating bats. In: *Neural Representation of Temporal Patterns*, edited by Covey E, Hawkins H, and Port R. New York: Plenum, 1995, p. 25–51.

CASSEDAY JH AND COVEY E. A neuroethological theory of the operation of the inferior colliculus. *Brain Behav Evol* 47: 311–336, 1996.

CASSEDAY JH, COVEY E, AND GROTHE B. Neural selectivity and tuning for sinusoidal frequency modulations in the inferior colliculus of the big brown bat. *Eptesicus fuscus. J Neurophysiol* 77: 1595–1605, 1997.

CASSEDAY JH, EHRLICH D, AND COVEY E. Neural tuning for sound duration: role of inhibitory mechanisms in the inferior colliculus. *Science* 264: 847–850, 1994.

COVEY E AND CASSEDAY JH. Connectional basis for frequency representation in the nuclei of the lateral lemniscus of the bat Eptesicus fuscus. *J Neurosci* 6: 2926–2940, 1986.

COVEY E AND CASSEDAY JH. The monaural nuclei of the lateral lemniscus in an echolocating bat: parallel pathways for analyzing temporal features of sound. *J Neurosci* 11: 3456–3470, 1991.

COVEY E AND CASSEDAY JH. Timing in the auditory system of the bat. In: *Ann Rev Physiol*, edited by Hoffman JF and De Weer P. Palo Alto, CA: Annual Reviews, 1999, p. 457–476.

COVEY E, KAUER JA, AND CASSEDAY JH. Whole-cell patch-clamp recording reveals subthreshold sound-evoked postsynaptic currents in the inferior colliculus of awake bats. *J Neurosci* 16: 3009–3018, 1996.

COVEY E, VATER M, AND CASSEDAY JH. Binaural properties of single units in the superior olivary complex of the mustached bat. *J Neurophysiol* 66: 1080–1094, 1991.

EHRLICH D, CASSEDAY JH, AND COVEY E. Neural tuning to sound duration in the inferior colliculus of the big brown bat, *Eptesicus fuscus. J Neurophysiol* 77: 2360–2372, 1997.

FREDERIKSEN E. Condenser microphones used as sound sources. Brüel and Kjaer Technical Review, 3, 1977.

FUZESSERY ZM. Response selectivity for multiple dimensions of frequency sweeps in the pallid bat inferior colliculus. *J Neurophysiol* 72: 1061–1079, 1994.

FUZESSERY ZM AND HALL JC. Role of GABA in shaping frequency tuning and creating FM sweep selectivity in the inferior colliculus. *J Neurophysiol* 76: 1059–1073, 1996.

GROTHE B. Interaction of excitation and inhibition in processing of pure tone and amplitude-modulated stimuli in the medial superior olive of the mustached bat. *J Neurophysiol* 71: 706–721, 1994.

GROTHE B AND CASSEDAY JH. The medial superior olive of the big brown bat, *Eptesicus fuscus*—a possible source for off-responses to duration tuned neurons in the auditory midbrain (Abstract). *Assoc Res Otolaryngol* 21: 3, 1998.

GROTHE B, PARK TJ, AND SCHULLER G. Medial superior olive in the free-tailed bat: response to pure tones and amplitude-modulated tones. *J Neurophysiol* 77: 1553–1565, 1997.

GROTHE B AND SANES DH. Synaptic inhibition influences the temporal coding properties of medial superior olivary neurons: an in vitro study. *J Neurosci* 14: 1701–1709, 1994.

GROTHE B, VATER M, CASSEDAY JH, AND COVEY E. Monaural interaction of excitation and inhibition in the medial superior olive of the mustached bat: an adaptation for biosonar. *Proc Natl Acad Sci USA* 89: 5108–5112, 1992.

HAPLEA S, COVEY E, AND CASSEDAY JH. Frequency tuning and response latencies at three levels in the brainstem of the echolocating bat, *Eptesicus fuscus. J Comp Physiol* [*A*] 174: 671–683, 1994.

HAVEY DC AND CASPARY DM. A simple technique for constructing "piggyback" multibarrel microelectrodes. *Electroencephalogr Clin Neurophysiol* 48: 249–251, 1980.

KUWADA S, BATRA R, YIN TC, OLIVER DL, HABERLY LB, AND STANFORD TR. Intracellular recordings in response to monaural and binaural stimulation of neurons in the inferior colliculus of the cat. *J Neurosci* 17: 7565–7581, 1997.

MILLER JL AND LIBERMAN AM. Some effects of later-occurring information on the perception of stop consonant and semivowel. *Percept Psychophys* 25: 457–465, 1979.

NARINS PM AND CAPRANICA RR. Neural adaptations for processing the two-note call of the Puerto Rican treefrog, *Eleutherodactylus coqui. Brain Behav Evol* 17: 48–66, 1980.

NEUWEILER G. Auditory adaptations for prey capture in echolocating bats. *Physiol Rev* 70: 615–641, 1990.

PINHEIRO AD, WU M, AND JEN PH. Encoding repetition rate and duration in the inferior colliculus of the big brown bat, *Eptesicus fuscus. J Comp Physiol* [*A*] 169: 69–85, 1991.

POON PW, CHEN X, AND CHEUNG YM. Differences in FM response correlate with morphology of neurons in the rat inferior colliculus. *Exp Brain Res* 91: 94–104, 1992.

SHANNON RV, ZENG FG, KAMATH V, WYGONSKI J, AND EKELID M. Speech recognition with primarily temporal cues. *Science* 270: 303–304, 1995.

SUGA N. Recovery cycles and responses to frequency modulated tone pulses in auditory neurons of echolocating bats. *J Physiol (Lond)* 175: 50–80, 1964.

VATER M, COVEY E, AND CASSEDAY JH. The columnar region of the ventral nucleus of the lateral lemniscus in the big brown bat (*Eptesicus fuscus*): synaptic arrangements and structural correlates of feedforward inhibitory function. *Cell Tissue Res* 289: 223–233, 1997.