GABAergic and Glycinergic Inhibition Sharpens Tuning for Frequency Modulations in the Inferior Colliculus of the Big Brown Bat

Koch, U. and B. Grothe. GABAergic and glycinergic inhibition sharpens tuning for frequency modulations in the inferior colliculus of the big brown bat. J. Neurophysiol. 80: 71–82, 1998. Discrimination of amplitude and frequency modulated sounds is an important task of auditory processing. Experiments have shown that tuning of neurons to sinusoidally frequency- and amplitude-modulated (SFM and SAM, respectively) sounds becomes successively narrower going from lower to higher auditory brain stem nuclei. In the inferior colliculus (IC), many neurons are sharply tuned to the modulation frequency of SFM sounds. The purpose of this study was to determine whether GABAergic or glycinergic inhibition is involved in shaping the tuning for the modulation frequency of SFM sounds in IC neurons of the big brown bat (Eptesicus fuscus). We recorded the response of 56 single units in the central nucleus of the IC to SFM stimuli before and during the application of the γ-aminobutyric acid-A (GABA_A) receptor antagonist bicuculline or the glycine receptor antagonist strychnine. To evaluate tuning to the modulation frequency, the normalized spike count (normalized according to the maximal response for each condition tested) was plotted versus the modulation frequency and the upper and lower 50% cutoff points were determined. Bicuculline increased the upper cutoff in 46% of the neurons by ±25%. The lower cutoff decreased in 48% of the neurons tested. In some neurons (~30%), a sharpening of the tuning by bicuculline was observed. Strychnine induced an increase of the upper cutoff in almost half of the neurons. Compared with bicuculline these changes were smaller. The lower cutoff decreased in 50% of the neurons with strychnine. The synchronization coefficient (SC) was calculated and compared for three modulation frequencies (50, 100, and 200 Hz) between predrug and drug condition. For all neurons, synchronization decreased (n = 36) or did not change (n = 26) during drug application. This was mainly an effect of the prolonged discharge in response to each cycle. Under predrug conditions, many neurons exhibited selectivity to the direction of the FM, hence they only responded once to each cycle. In a minority of neurons, direction selectivity was abolished by drug application. The main finding was that neuronal inhibition sharpens tuning to the modulation frequency in the majority of neurons. In general, changes induced by bicuculline or strychnine were comparable.

INTRODUCTION

Most natural sounds, including communication sounds and returning echoes from echolocation calls, contain frequency and amplitude modulations. These modulations create a temporal structure that is a major source of information for recognizing sounds, including speech (Shannon et al. 1995). It, therefore, is of great interest how the auditory system extracts temporal information like the periodicity of amplitude- or frequency-modulated sounds.

Most of our knowledge about temporal processing in the auditory system comes from experiments using periodic stimuli, e.g., sinusoidally frequency- and amplitude-modulated (SFM and SAM, respectively) sounds. These experiments have shown that the temporal structure of frequency and AM is reflected by neuronal responses in the auditory nerve and in lower auditory brain stem nuclei. At progressively higher levels of the auditory system, neurons become more and more selective for the periodicity of sounds (review: Langner 1992). For instance, neurons in the auditory nerve and the cochlear nucleus (CN) phase-lock to SAM and SFM sounds up to high modulation frequencies (~1 kHz) (Rhode and Greenberg 1992; Ruggero 1992; Vater 1982). In the superior olivary complex and the nuclei of the lateral lemniscus, many neurons do not respond to high-modulation frequencies anymore and thus exhibit low-pass filter characteristics for the modulation frequency (Grothe 1994; Huffman et al. 1995; Joris and Yin 1995; Yang and Pollak 1997). In the inferior colliculus (IC), additionally band-pass filter neurons that are tuned to narrow ranges of modulation frequencies are found (Casseday et al. 1997; Langner and Schreiner 1988; Rees and Moller 1983; Schuller 1979). This increasing selectivity for periodicity suggests active neuronal filter mechanisms.

Different principal mechanisms to create such neuronal filters have been proposed. One of them is based on the match of two excitatory inputs (Langner 1988). Another is based on a match of the periodicity of intrinsic oscillations of a filter neuron and the periodicity of the inputs (Peruzzi and Oliver 1997; Sarbaz and Rees 1996). Both mechanisms would not include inhibition as a main factor. However, recent experiments in the medial superior olive (MSO) and the dorsal nucleus of the lateral lemniscus (DNLL) of the mustached bat have shown that GABAergic and glycinergic inhibition is involved in increasing the selectivity of neurons for the modulation frequency of SAM sounds (Grothe 1994; Yang and Pollak 1997). The mechanism proposed in both of these studies acts via inhibition that follows the excitation and renders the neuron unresponsive for a certain period of time after the excitation phase of each cycle.

Whether this mechanism might also apply to the processing of temporal information in the IC is unknown. However, neurons in the central nucleus of the IC receive a number of glycinergetic and GABAergic monaural and binaural projections from different lower auditory brain stem nuclei (for review: Oliver and Huerta 1992). The main GABAergic projections originate in both DNLL (Shneiderman and Oliver 1989; Shneiderman et al. 1993), the contra-lateral IC, and the ipsilateral ventral and intermediate nucleus of the lateral lemniscus (VNLL and INLL) (Gonzales Hernandez et al. 1996; Vater et al. 1997). The ipsilateral
was cemented onto the skull overlying the cortex with dental cement, the optimal center frequency, modulation depth, and threshold. The surface of the skull was cleared of tissue. A layer of cyno- and modulation frequency was used as a search stimulus. On entering the skull, an incision was made across the midsaggital line into the funnel of the bat's pinna.

The head was secured in a head holder with a bite bar. The local anesthetic bupivacain (H-configuration, Science Products) was pulsed and band-pass filtered (Tektronix, 5112), discriminated and fed into the TDT-system.

One barrel of the multibarrel electrode was used for balancing currents and was filled with 2 M Na-acetate. The other four barrels were filled with bicuculline methiodide (5 mM; pH: 3), strychnine (10 mM, pH: 3.5), GABA (0.5 M, pH: 3.5), and glycine (0.5 M, pH: 3.5) (all drugs were from Sigma). Adjustment of pH was achieved by titrating with 1 M HCl.

The drug electrodes and the balancing electrode were connected via silver chloride wires to a microiontophoresis system (Medical Systems, Neurophore BH-2) that was used to generate and monitor ejection (10–40 nA) and retention currents (–15 nA). The sum channel that was connected to the balancing electrode was used to balance currents and to eliminate current effects.

**Acoustic stimuli, data acquisition, data analysis**

Stimuli were digitally generated by using two DSP boards, 16 bit D/A converters (sampling rate 250 kHz), and attenuators from Tucker-Davis Technologies. Sounds were amplified (Toelner) and fed into a custom made earphone (Schlegel 1977) that was placed into the funnel of the bat’s pinna.

Stimuli were delivered monaurally to the ear contralateral to the IC we recorded from. A 100-ms sinusoidally frequency modulated signal with constantly varying center frequency, modulation depth, and modulation frequency was used as a search stimulus. On encountering activity of a single neuron in the central nucleus of the IC, the optimal center frequency, modulation depth, and threshold to drive the neuron was determined audiovisually. For all subsequent recordings, stimuli 20 dB above threshold were used. This sound intensity was chosen because many neurons in the IC have nonmonotonic rate-level functions and therefore do not show any significant response at higher intensities (Semple and Kitzes 1987). SFM signals first were presented to each neuron modulated over a range of different modulation frequencies (10–600 Hz).

To test the effects of GABAergic and glycinereric inhibition, the

**METHODS**

**Surgical procedure**

Seven big brown bats (*E. fuscus*) were used in this study. Before surgery animals were subcutaneously injected with 0.2 ml of the neuroleptic Thalamonal (Janssen) (0.05 mg Fentanyl plus 2.5 mg Droperidol/ml) and additionally anesthetized with Metofane (Janssen) by inhalation until no nociceptive response could be evoked. The head was secured in a head holder with a bite bar. The local anesthetic bupivacain (Curasan) was injected under the skin covering the skull. An incision was made across the midsagittal line of the skull, and muscles and skin were reflected from the skull. The surface of the skull was cleared of tissue. A layer of cyanoacrylate adhesive was applied to the surface of the skull and a rod was cemented onto the skull overlying the cortex with dental cement. Open wounds were treated with 1% H2O2. Recording sessions started 2 days after surgery.

Recording was performed every day for 8–14 days, and each session lasted for 2–6 h. Between recording sessions bats were housed in individual cages with free access to water and food. Before each recording session, the bat was injected subcutaneously with 0.15–0.2 ml of the neuroleptic Thalamonal. Thalamonal is equivalent to the neuroleptic Innovar-vet, which has been widely used in such experiments because it has little effects on neuronal response properties (Evans 1979). The bat was transferred to a heated, soundproof recording chamber where it was restrained in a cushioned holder molded to the animals body. The restraining cushion was attached to a custom made stereotaxic instrument. The rod that was mounted on the skull was secured to the stereotaxic instrument. Before the first recording session, a small hole was drilled in the skull above the inferior colliculus. The location of the IC was identified visually using landmarks on the skull. The ground electrode was placed between the reflected muscle and the skull. The recording electrode was advanced from outside of the experimental chamber using a hydraulic microdrive (Wells). To ensure positioning of the electrode within the central nucleus of the IC, neurons were only recorded at depths between 150 and 1,800 μm. The tonotopic organization of the central nucleus of the IC served as an indicator for the position of the electrode. Recording sessions lasted until the bat showed any sign of discomfort.

**Electrodes**

Piggy-back multibarrel electrodes (Havey and Caspary 1980) were used for recording and drug application. Single-barrel micropipettes were pulled to a tip diameter of <1 μm. A five-barrel micropipette (H-configuration, Science Products) was pulsed and the tip broken to a diameter of 15–20 μm. The single-barrel pipette was positioned so that the tip of the recording electrode protruded ~10 μm beyond the tip of the multibarrel electrode and attached with cyanoacrylate to the multibarrel electrode. The single-barrel electrode was filled with 2 M NaCl and used for recording the neuronal response. Action potentials were measured by an electrometer (Model Electro 705; World Precision Instruments), amplified and band-pass filtered (Tektronix, 5112), discriminated and fed into the TDT-system.

VNLL and INLL are considered to be one of the major sources of glycinergic projections to the IC along with neurons originating in the ipsilateral lateral superior olive (LSO) (Glendenning et al. 1992; Saint Marie and Baker 1990; Saint Marie et al. 1989; Vater 1997).

In the IC, GABAergic and glycinereric inhibition has been shown to affect the response selectivity of neurons for a number of stimulus parameters. For example, in many IC neurons GABAergic and glycinereric inhibition shapes interaural intensity difference functions (Klug et al. 1995; Park and Pollak 1993, 1994), influences duration tuning (Casseday et al. 1994; Covey et al. 1995), changes the response pattern, broadens frequency tuning curves, and abolishes nonmonotonic rate level functions (Faingold et al. 1989; Le Beau et al. 1996; Pollak and Park 1993; Vater et al. 1992).

Recently, Fuzessery and Hall (1999) demonstrated that selectivity of IC neurons for the direction of frequency-modulated sweeps is created mainly by GABAergic inhibition.

The question addressed in this study is whether inhibition sharpens the tuning to the periodicity of SFM sounds within the central nucleus of the IC. We used the big brown bat (*Eptesicus fuscus*) as experimental animal because the IC of these bats, which use frequency-modulated sweeps in echolocation calls, have been shown to contain many neurons that are sharply tuned to SFM sounds (Casseday et al. 1997). Moreover, repetitive FMs are an important component of bat communication sounds (Kanwal et al. 1994), and discrimination of modulation frequencies is an essential task within their repertoire of social behavior (Esser and Lud 1997).

We tested the effects of neuronal inhibition on tuning of single IC neurons to SFM sounds by recording the responses to the same range of modulation frequencies before and during the iontophoresetic application of the γ-aminobutyric acid-A (GABA_A) receptor antagonist bicuculline and the glycine receptor antagonist strychnine. We present evidence that both GABAergic and glycinereric inhibition have a profound impact on how IC neurons code stimulus periodicity.
antagonists bicuculline or strychnine were applied, respectively. Discharge rate evoked by a stimulus usually started to increase within seconds. The response of the neuron to a 50- or 100-Hz test tone was monitored continuously. Recordings under drug conditions did not start before discharge rate to the test stimulus had stabilized. Then the same range of modulation frequencies was presented. After each drug application, the retention current was turned on and the neuron was allowed to recover until the discharge rate to the test stimulus had dropped to the value of the predrug condition. Recovery usually took between 5 and 15 min. To limit the possibility of measuring artifacts, only those neurons were tested where GABA or glycine application stopped the response within seconds using low ejection currents (5–25 nA).

We based a neuron’s filter characteristics on two parameters. First, we determined the number of spikes evoked by each stimulus. Because there was a general increase in discharge rate during drug application the maximum was defined as 1 for each condition, and discharges were normalized according to it. Second, we calculated the synchronization coefficient (SC) for each neuron’s response to all modulation frequencies (Goldberg and Brown 1969). The SC is a measure of how precisely neuronal discharge locks to a certain phase of each modulation cycle. It ranges from 0 (no phase-locking) to 1 (perfect phase-locking). The on-response was excluded for the calculation of the SC.

By plotting normalized spike counts or the SC values versus the modulation frequency, we determined spike-count-based or SC-based modulation transfer functions (MTF), respectively. For spike-count-based MTFs, the cutoff points for responses to SFM sounds were defined as the modulation frequencies where the MTF dropped to 0.5. For the SC-based MTFs, a threshold of 0.5 was determined.

RESULTS

We recorded from 56 neurons in the central nucleus of the IC while presenting SFM sounds at various modulation frequencies.

First, filter characteristics of neurons for FM based on spike counts were determined according to the existence of upper and lower cutoff points in their spike count MTFs. Figure 1A shows MTFs of neurons that exhibited low-pass (only an upper cutoff point existed), band-pass (upper and lower cutoff points), or all-pass (no cutoff points) filter characteristics for FMs. Most of the neurons exhibited low-pass (33%) or band-pass (63%) filter characteristics for modulation frequency with upper cutoff points mostly <200 Hz (76% of the neurons).

Second, to characterize the precision of phase-locking to the stimulus envelope, the SC was calculated and plotted versus the modulation frequency to obtain a synchronization MTF (Fig. 1B). The degree of phase-locking varied considerably between neurons. At a modulation frequency of 50 Hz, the majority of neurons (41 of 52) showed good phase-locking with a SC >0.5. For only 11 of 52 neurons, the SC was <0.5. About two-thirds of the neurons (9 of 25) phase-locked well (SC ≥ 0.5) at a modulation frequency of 100 Hz. At modulation frequencies >100 Hz, phase-locking decreased rapidly for many neurons although spike count remained high (21 of 52) (e.g., the neuron shown in Fig. 1B). Other neurons phase-locked well until they ceased to respond (17 of 52) (the neuron shown in Fig. 1A). At a modulation frequency of ≥200 Hz, good phase-locking (SC > 0.5) was observed in only one neuron.

Third, across the population of neurons that exhibited phase-locking (n = 44) two different patterns of phase-locking could be observed. At a modulation frequency of 50 Hz, one class of neurons responded only once to each modulation cycle (n = 32), hence these neurons only responded to the upward or to the downward portion of the SFM cycle. The other class of neurons (n = 12) phase-locked twice to each modulation cycle, i.e., they responded to the upward and to the downward part of each modulation cycle. However, at modulation frequencies of ≥100 Hz, all neurons responded once to each modulation cycle. Because the latencies of individual neurons differed greatly from each other (7–22 ms) (see latter paragraph), we could not determine whether for neurons that responded once, this response was to the upward or the downward part of the modulation cycle.
quencies before and during bicuculline application. At modulation frequencies of 30 and 50 Hz, this neuron responded robustly with a phase-locked discharge to each modulation cycle. At 70 Hz, a phase-locked response could only be evoked by the first two cycles of the stimulus. There was no response to the following SFM cycles. At ≥110 Hz, only a weak response, uncorrelated to the SFM cycles, could be elicited in this neuron.

Figure 2, right, shows the response of the same neuron during the application of bicuculline. For all modulation frequencies tested, the number of spikes increased considerably. At modulation frequencies of 30 and 50 Hz, the response of the neuron to each modulation cycle lengthened and, as a consequence to the prolonged response, phase-locking deteriorated. In contrast to the predrug condition, the response of the neuron at 110 Hz was even stronger than at lower modulation frequencies and a vigorous response could now be evoked at 250 Hz. The drug-induced changes in the spike-count–based MTFs for this neuron caused the upper cutoff point to shift from 60 to 260 Hz (333%) (Fig. 3A). However, synchronization decreased even more rapidly with bicuculline (Fig. 3B).

The population analysis, depicted in Fig. 4A, reveals that of 39 neurons, that exhibited low-pass or band-pass filter characteristics, 14 showed a large shift of their upper cutoff point to higher modulation frequencies (50%). Smaller

**Bicuculline changes spike-count–based filter characteristics to SFM sounds**

To characterize the effects of GABAergic inhibition on neuronal responses to SFM sounds, the GABA_A receptor antagonist bicuculline was applied. A prominent effect of bicuculline application was that the stimulus correlated discharge rate increased considerably (between 15 and 926%). For one third of the neurons this increase was >300%.

The comparison of spike counts before and during bicuculline application revealed that about half of the tested neurons changed their selectivity for FMs due to GABAergic inhibition. Figure 2 shows poststimulus time histograms (PSTHs) of a neuron’s response to various modulation frequencies before and during bicuculline application. At modulation frequencies of 30 and 50 Hz, this neuron responded robustly with a phase-locked discharge to each modulation cycle. At 70 Hz, a phase-locked response could only be evoked by the first two cycles of the stimulus. There was no response to the following SFM cycles. At ≥110 Hz, only a weak response, uncorrelated to the SFM cycles, could be elicited in this neuron.

Figure 2, right, shows the response of the same neuron during the application of bicuculline. For all modulation frequencies tested, the number of spikes increased considerably. At modulation frequencies of 30 and 50 Hz, the response of the neuron to each modulation cycle lengthened and, as a consequence to the prolonged response, phase-locking deteriorated. In contrast to the predrug condition, the response of the neuron at 110 Hz was even stronger than at lower modulation frequencies and a vigorous response could now be evoked at 250 Hz. The drug-induced changes in the spike-count–based MTFs for this neuron caused the upper cutoff point to shift from 60 to 260 Hz (333%) (Fig. 3A). However, synchronization decreased even more rapidly with bicuculline (Fig. 3B).

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FIG. 4. Distribution of changes of upper (A) and lower (B) cutoff points over the population of neurons tested. About 50% of the neurons showed an increase (≥25%) of their upper cutoff point during bicuculline application. Lower cutoff points also decreased in ~50% of the neurons. More than half of the neurons broadened their tuning for the modulation frequency of SFM sounds. Magnitude of changes are shown in percentages with the cutoff point under predrug conditions being 100%.

changes of upper cutoff points were observed in four neurons (between 25 and 50%). In nine neurons, shifts were <25%. Paradoxically, in 12 neurons, bicuculline shifted the upper cutoff point to lower modulation frequencies. There was, however, never a net decrease of absolute spike number observed during bicuculline or strychnine application for any modulation frequency.

The 27 neurons that under predrug conditions showed band-pass filter characteristics also were tested for changes of lower cutoff points during bicuculline application.

For example, under predrug conditions, the neuron in Fig. 5 did not respond at all to a stimulus modulated at 25 Hz and only very weakly at 50 Hz. However, during bicuculline application, this neuron exhibited a strong, phase-locked response at 25 and at 50 Hz (Fig. 5). The lower cutoff point for this neuron changed from 65 to 35 Hz (60%) during blocking of GABAergic inhibition (Fig. 6). Over the population of neurons tested (n = 27; see Fig. 4B), the lower cutoff point decreased in 13 neurons by >25%. Five of those showed downward shifts of >50%. More than one-third (n = 10) showed no or only minor shifts (<25%). As for the upper cutoff, bicuculline application shifted the lower cutoff point to unexpected, in this case higher modulation frequencies in some neurons (n = 4).

Changes of both the lower and upper cutoffs were seen

FIG. 5. PSTHs of a neuron that, under predrug conditions, did not respond to modulation frequencies <50 Hz. During bicuculline application, this neuron started to respond robustly to modulation frequencies as low as 25 Hz.

FIG. 6. Spike-count–based MTF of the same neuron as in Fig. 6. Lower cutoff point shifted from 65 to 35 Hz.
in only four neurons. In all other neurons, bicuculline changed either the lower or the upper cutoff.

In many neurons, either the upper cutoff or the lower cutoff changed. In 13 of 41 neurons, bicuculline did not cause any shift (≥25%) in either the lower or the upper cutoff despite its profound impact on overall spike counts in all neurons. Figure 7 shows an example for these cases. This neuron exhibited a phase-locked response under predrug condition (at 50 Hz: SC = 0.99). At a modulation frequency of 130 Hz, the neuron stopped responding to each cycle of the stimulus so that only an onset response remained. During bicuculline application, the number of spikes evoked by each modulation cycle increased substantially, and the response to each modulation cycle lengthened. However, as in the predrug condition, at a modulation frequency of 130 Hz, the only significant response was that to the onset of the stimulus. After normalizing spike counts for this neuron, only a small change (17%) in filter characteristics for SFM sounds could be seen (Fig. 7B).

**Strychnine influences filter characteristics for SFM sounds based on spike counts**

Strychnine, like bicuculline, increased the maximal discharge rate of all neurons tested (between 22 and 281%). In contrast to bicuculline application, the increase in discharge rate never exceeded 300%.

An example of a neuron that changed its response to SFM sounds during strychnine application is shown in Fig. 8. This neuron phase-locked to modulation frequencies ≤50 Hz. At higher modulation frequencies, the response became very weak except for an onset response. During strychnine application, responses to all modulation frequencies became stronger (Fig. 8) and a robust response could be evoked ≤300 Hz. Phase-locking to each modulation cycle was almost equal with and without strychnine (Fig. 9B, bottom).

Changes of upper cutoff points during strychnine application were quantified over the population of neurons (n = 20) that exhibited low-pass or band-pass filter characteristics under predrug conditions (Fig. 9A). A significant shift (≥25%) of the upper cutoff point was observed in 10 neurons. In contrast to bicuculline application, in only half of these neurons (n = 5) was this shift >50%. For seven neurons, the increase of the upper cutoff point was <25%. For three neurons, strychnine shifted the upper cutoff point to lower modulation frequencies. The lower cutoff points were significantly decreased (≥25%) by strychnine in half of the tested neurons (Fig. 9B).

**Bicuculline and strychnine decreases the precision of phase-locking for all modulation frequencies**

SC-based MTFs were not linear for some neurons (n = 9) or exhibited all-pass filter characteristics in others (n = 13). Hence, it seemed inappropriate to determine upper and lower cutoff points to characterize the changes in the SC that occurred during drug application. Therefore, we quantified changes of SC induced by bicuculline or strychnine application for three standard modulation frequencies (50, 100, and 200 Hz).

Bicuculline caused the synchronization of the response to each modulation cycle to decrease in 26 neurons. In 16 neurons, synchronization did not change (change of SC < 0.1). Strychnine application decreased the SC in half of the tested neurons. For the other half, strychnine did not cause any change of SC. Phase-locking did not improve for any neurons during drug application. Interestingly, only 5 of the 18 neurons that increased their upper cutoff point in the spike-count-based MTF during bicuculline application exhibited good phase-locking (≥0.5) at those modulation frequencies they had not responded to under predrug condition.

Neurons that phase-locked to SFM sounds under predrug conditions changed their response during drug application in different ways. The different effects of bicuculline on SC are shown in Fig. 10. Before drug application, neuron A in Fig. 10 responded precisely to each modulation cycle (SC = 0.97). During drug application, the number of spikes increased fourfold but synchronization remained almost as high as before (SC = 0.94). Neuron B in Fig. 10 shows a similar response to a stimulus modulated at 50 Hz under predrug condition (SC = 0.93). Bicuculline increased the response rate threefold but synchronization to the stimulus became worse (SC = 0.42). Under the predrug condition, this neuron responded to each modulation cycle with one or two spikes within a time interval of 4 ms. Bicuculline lengthened the interval in which spikes could occur to 16
ms. In some neurons (see neuron C in Fig. 10), the phase-locked response became sustained during bicuculline application, hence, there was no synchronized response anymore (SC = 0.04).

In Fig. 11 the change in discharge rate caused by bicuculline application is plotted versus the change in synchronization. In general, for those neurons that increased their discharge rate more during drug application the decrease in phase-locking was larger (r = 0.61). In other words, the decrease in SC is mainly an effect of the prolonged period of response per cycle.

**Bicuculline and strychnine removes direction selectivity for SFM sounds in only a minority of neurons**

As stated above, the majority of cells (73%) responded only once per SFM cycle, thus, exhibited a selectivity for the sweep direction of a 50-Hz SFM sound. In the majority of these neurons, this selectivity was maintained even during drug application. However, the direction selectivity was removed by blocking inhibition in some neurons, and these neurons responded twice instead of once to each modulation cycle. Figure 12 shows a PSTH of a neuron that responded with one discharge to each modulation cycle of the 50-Hz SFM sound. During strychnine application, this neuron started to respond twice to each modulation cycle (Fig. 12A). Across the population of neurons that exhibited phase-locked discharge at 50-Hz modulation frequency, 7 of 24 neurons lost their direction selectivity during blocking GABAergic inhibition. In 4 of 17 neurons, this could be seen during blocking glycineric inhibition. However, at higher modulation frequencies (>100 Hz), all neurons responded only to either the upward or the downward part of the modulation cycle even during bicuculline or strychnine application (Fig. 12B).

**Changes in latency by blocking inhibition are correlated to changes in upper cutoff point**

Many neurons in the IC exhibit inhibitory sidebands on one or on both sides of their tuning curve. The removal of these sidebands during blocking inhibition might be responsible for the effects of strychnine and bicuculline on the MTFs described above. If that is true, a decrease in latency during blocking inhibition should occur mostly in those neurons that also broaden their tuning for SFM sounds.

We analyzed the latency of the response of all tested neurons before and during drug application for a stimulus modulated at 50 Hz. During drug application, half of the tested neurons did not shorten their latency at all. The other half of the neurons shortened their latency <11 ms (average: 2.7 ms). A comparison of the drug-induced changes in latency and that of changes in upper cutoff points (Fig. 13) illustrates that there is a correlation (bicuculline: r = 0.47; strychnine: r = 0.56) between these features. In general, neurons that showed a larger decrease of latency increased...
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Their upper cutoff point more during drug application. Again, no functional difference could be seen between the effects of bicuculline and strychnine.

**DISCUSSION**

There were four main findings in this study. First, GABAergic and glycinergic inhibition sharpens tuning for the modulation frequency of SFM sounds significantly (≥25%) in 60% of the neurons. However, only in about one-third of the neurons, these changes were as large as ≥50%. Inhibition affected the upper as well as the lower cutoffs. Second, for two-thirds of the neurons, blocking inhibition caused phase-locking to deteriorate. Third, only a minority of neurons lost their selectivity for the direction of FM sounds during blocking inhibition. Fourth, we could not find any functional difference between GABAergic and glycinergic inhibition for SFM filtering. However, GABAergic inhibition was prominent in the entire IC, whereas glycinergic inhibition was more common in the ventral portion of the IC.

**Tuning to the periodicity of sounds in the auditory brain stem and midbrain**

Most neurons in lower auditory nuclei that project to the IC respond well to high modulation frequencies. For example, in the CN neurons show phase-locking ≤800 Hz (Vater 1982), and neurons in the nuclei of the lateral lemniscus phase-lock up to a maximum of 700 Hz (Huffman et al. 1995). No data published so far are available on processing of SFM signals by neurons in the superior olivary complex. However, using SAM sounds as a stimulus, it has been shown that neurons in the LSO and the superior paraolivary nucleus phase-lock up to high modulation frequencies (800 Hz) (Joris and Yin 1992; S. Kuwada, personal communication). Up to date, two exceptions are known, however: neurons in MSO...
membrane properties limit the response of many IC neurons to fast modulation frequencies. There is evidence in the fly visual system that motion sensitive neurons with fast Na⁺-channels respond to higher frequencies of moving gratings than do neurons without these channels (Haag and Borst 1997). Preliminary data from brain slice recordings of IC neurons suggest that intrinsic active and passive membrane properties contribute to the neurons’ ability to entrain to a series of current pulses and might play a role in determining their ability to phase-lock to temporally structured sounds (Peruzzi and Oliver 1997; Sarbaz and Rees 1996).

FIG. 11. Correlation between changes in spike number and changes in SC with bicuculline. Each dot represents 1 neuron. Graph shows that the neurons where spike count increased more during bicuculline application showed a larger decrease of their SC.

and subpopulation of neurons in the DNLL act as low-pass filters for SAM sounds, i.e., most upper cutoff points are <200 Hz. There, blocking glycinergic or GABAergic inhibition broadens or even abolishes low-pass filter characteristic of neurons for the modulation frequency of SAM sounds (Grothe 1994; Grothe and Sanes 1994; Grothe et al. 1997; Yang and Pollak 1997). Taken together, these data from lower auditory brain stem nuclei suggest that at least some excitatory inputs to the IC respond well to high modulation frequencies of both SFM and SAM, and we might expect there to be some active neuronal filter mechanisms to increase selectivity for modulation frequency at the level of the IC.

In our sample, the response of about three-quarters of the neurons fell to 50% of the maximum at modulation frequencies <200 Hz. None of the neurons tested exhibited good phase-locking at a modulation frequency >200 Hz. Similar observations have been made previously in IC neurons of the same species (Casseday et al. 1997), the horseshoe bat (Schuller 1979), and the rat (Rees and Moller 1983).

Also, many neurons we recorded from exhibited band-pass filter characteristics. The existence of band-pass filter characteristics for modulation frequencies has been described for IC neurons in a number of mammals (Casseday et al. 1997; Felsheim and Ostwald 1996; Langner and Schreiner 1988). However, neurons in lower auditory nuclei like the CN, the SO, and the nuclei of the lateral lemniscus usually exhibit all-pass or low-pass filter characteristics. Therefore, it is likely that band-pass filter characteristics are created in the IC itself.

Previously, Langner and Schreiner (1988) proposed a model to explain tuning of IC neurons to the modulation frequency of SAM sounds. This model includes an oscillator tuned to a specific modulation frequency that originates in lower auditory nuclei. Through a coincidence, detection mechanism the oscillations are compared with inputs that phase-lock to the envelope of the stimulus. In this model, neurons are supposed to respond well to stimuli with modulation frequencies that match the frequency of the oscillation. There are several reasons why it is unlikely that this mechanism is exclusively responsible for tuning of IC neurons to modulation frequencies. One reason is that according to this model, neurons should respond well to several harmonics of the best modulation frequency. But such tuning has never been described in IC neurons.

Another possible mechanism is that active and passive membrane properties limit the response of many IC neurons to fast modulation frequencies. There is evidence in the fly visual system that motion sensitive neurons with fast Na⁺-channels respond to higher frequencies of moving gratings than do neurons without these channels (Haag and Borst 1997). Preliminary data from brain slice recordings of IC neurons suggest that intrinsic active and passive membrane properties contribute to the neurons’ ability to entrain to a series of current pulses and might play a role in determining their ability to phase-lock to temporally structured sounds (Peruzzi and Oliver 1997; Sarbaz and Rees 1996).

FIG. 12. PSTHs of 1 neuron before and during strychnine application. A: at a modulation frequency of 50 Hz, this neuron responded only to 1, i.e., either the up or the downward, part of the modulation cycle. During strychnine application, the neuron started to respond to the upward and the downward part of the modulation cycle. B: at a modulation frequency of 70 Hz, this neuron responded only once per modulation cycle also during strychnine application.
In principle, there are two different ways in which inhibition might influence the response of IC neurons to modulation frequencies. First, the inhibition could be locked to a certain phase of the modulation cycle. Then the relative timing of excitation and inhibition would determine to which modulation frequencies the neuron responds to or not. A model like this has been proposed by Grothe (1994) for tuning of MSO neurons to the modulation frequency of SAM sounds. In this model, an excitatory input that is phase-locked to the stimulus envelope is followed by an inhibitory input that also is phase-locked to the stimulus envelope but delayed by a certain amount. At faster modulation frequencies, depending on the duration and the timing of the delayed inhibition, the excitation will occur at the same time as the inhibition of the preceding cycle. For a SFM sound, it would be either possible that inhibition is tuned to the same frequency than the excitation. Then the MSO model could be directly applied to tuning of IC neurons to SFM sounds in the IC. Or, inhibition could be tuned to lower or higher frequencies than the excitation like the inhibitory sidebands that have been described in a number of publications (Fuzessery and Hall 1996; Kuwada et al. 1997; Vater et al. 1992; Yang et al. 1992). The reduction in latency (on average by 2–3 ms) and the lengthening of the response to each cycle of the stimulus suggest at least a partial removal of inhibitory sidebands by blocking inhibition.

A second possibility, shown in in vitro preparations, is that phase-locked inhibition builds up at high repetition rates through postsynaptic temporal summation. Therefore this inhibition acts like sustained inhibition (Grothe and Sanes 1994).

Both ways of how inhibition could act on sharpening filter characteristics for stimulus periodicity would only apply for the upper cutoff. A possible reason for shifts of lower cutoffs by blocking inhibition might include temporal summation of excitatory postsynaptic potentials that is required to reach threshold. Temporal summation is small at low rates when the stimulus moves slowly through the excitatory frequency band. An underlying weak inhibition could then easily block the response at low rates.

*Why is the effect of blocking inhibition in most neurons much smaller than expected?*

Almost two-thirds of the neurons showed a clear change in their response to SFM when inhibition was blocked. However, the filter shifts observed were mostly smaller than might have been expected when filter cutoffs shown for lower brain stem nuclei, and those found for IC cells were compared (see earlier). Only 33% of the neurons showed upper cutoff shifts of ≥50%. Interestingly, the same percentage of neurons was affected by bicuculline application in the IC of the mustached bat when these neurons were tested either for changes in interaural intensity difference functions (Park and Pollak 1993) or broadening of tuning curves (Yang et al. 1992). The reasons for a lack of dramatic effects might be similar in all three cases.

The first possible reason is that the drugs only reached the terminals at the cell soma but did not reach more remote synapses along the cells’ dendrites. Many inhibitory synapses, like the GABAergic terminals originating in the lateral lemniscus, terminate on dendrites (Oliver and Beckius 1992). This suggestion is supported by the findings of Poon et al. (1992), who showed that rat IC neurons that are sensitive to FM sweeps have dendritic trees arranged orthogonal to the frequency laminae, spanning areas ≈500 μm. The arrangement of excitatory and inhibitory synapses on the neuron and their activation pattern has been demonstrated to affect temporal filter properties of neurons (Carr and Boudreau 1993; for review see Segev 1992).

Another possible explanation is that the neurons that were unaffected by drug application received the majority of their excitatory inputs from neurons in regions of the lower auditory brain stem that respond only poorly to high modulation frequencies. The MSO, for example, sends excitatory projections to the IC and its neurons respond poorly to SAM sounds at high modulation frequencies (Grothe 1994; Grothe et al. 1997). However, the existence of band-pass filter neurons for periodic stimuli seems to be a unique feature of IC neurons and has not been reported for neurons in more peripheral auditory nuclei (for review see Langner 1992). Therefore, some filter mechanisms at the level of the IC have to be active. Intrinsic IC connections might be another source of excitatory inputs that respond poorly to high modulation frequencies or exhibit band-pass filter properties. In this case, the IC would represent a network of microcircuits that successively filters auditory information.

Because broadening of SFM tuning induced by blocking inhibition as observed in this study is relatively small, there is most likely not a single mechanism for limiting SFM...
response in IC cells but a variety of different mechanisms including inhibition. Moreover, because response properties of individual IC neurons differ so much, diverse combinations of mechanisms are likely to act on different neurons.

Pattern of phase-locking and its influence by inhibition

A large proportion of the neurons responded only once to each modulation cycle, presumably selectively to the upward or to the downward part. Such a selectivity for the direction of the FM has also been described for IC neurons in other mammals, like bats and rats (Fuzessery 1994; Fuzessery and Hall 1996; Poon et al. 1991; Schuller 1979; Vater and Schlegel 1979). We found that only a minority of cells lost this direction selectivity during blocking inhibition. In contrast, Fuzessery and Hall reported that blocking GABAergic inhibition reduced selectivity for the direction of FM sweeps in all of the neurons they tested (Fuzessery and Hall 1996). However, the stimuli used in the two experiments differed profoundly. Fuzessery used single, linearly modulated FM sweeps, whereas we used SFM sounds with a duration of 100 ms that contained many modulation cycles. These stimulus paradigms suggest that the response pattern to FMs is also interactive with the repetition rate of the modulation. This idea is supported by the observation that at low modulation frequencies, neurons responded to both the upward and the downward part of the modulation cycle, whereas at higher modulation frequencies, the same neurons responded only once per modulation cycle. Therefore, a mere sensitivity to the direction of the frequency change as traditionally defined might underlie the observed direction selectivity in our experiment. However, it is also possible that the observed direction selectivity is an interaction between responses to the downward and upward part of the modulation or to different cycles of the SFM sound.

It is possible that much of the direction selectivity we observed is conveyed from neuronal inputs that originate in lower nuclei. For instance, a large proportion of neurons in the lateral lemniscus only respond to either the upward or the downward part of an SFM cycle (Gordon and O’Neill 1996; Huffman et al. 1995). A recent model of selectivity of striate cortex neurons for the direction of a moving visual stimulus proposes a completely different mechanism. It suggests a small bias toward a certain direction is enormously increased by feedback recurrent excitation (Suarez et al. 1995). This so called “canonical microcircuit,” first proposed by Douglas and Martin (1991) also might apply in some parts to information processing in the inferior colliculus.

Inhibition preserves good phase-locking of IC neurons

For many neurons (64%), phase-locking deteriorated during bicuculline or strychnine application, suggesting that GABAergic and glycinergic inhibition preserves or enhances precise phase-locking for IC neurons. The same effect of GABAergic inhibition has been shown in a large subpopulation of neurons in the DNLL. These neurons respond to pure tone stimuli with a sustained response. They phase-lock to SAM sounds up to high modulation frequencies. Iontophoretic application of bicuculline lengthens the response of these neurons to each modulation cycle, thus causing phase-locking to deteriorate (Yang and Pollak 1997). The observed lengthening of the response and deterioration of phase-locking to SFM during application of bicuculline could be due to removal of the inhibitory sidebands, or removal of a sustained inhibitory input.

No qualitative differences could be observed between GABAergic and glycinergic inhibition in shaping SFM filter properties

Although changes caused by blocking glycine were usually quantitatively smaller than those changes caused by blocking GABA, no functional difference between GABAergic and glycinergic inhibition on SFM response properties could be observed. Similarly, Klug et al. (1995) did not observe any functional difference between the two inhibitory transmitters GABA and glycine on binaural processing of spatial information. However, the location of the neurons on which bicuculline or strychnine was effective differed. These findings suggest that GABAergic and glycinergic inhibition do not serve two different functions but rather reflect two different ontogenetic origins (for review: Oliver and Huerta 1992).

Conclusions

Inhibition plays a fundamental role in processing of frequency modulated signals of some IC neurons. It is also likely that several mechanisms on the cellular as well as on the systems level are simultaneously involved in processing temporal features. However, our data indicate that synaptic inhibition, whether conveyed over long projection neurons or within small intranuclear circuits, performs an important role in making neurons selective for the temporal structure of sounds.

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