Sharpening of Frequency Tuning by Inhibition in the Thalamic Auditory Nucleus of the Mustached Bat

Nobuo Suga, Yunfeng Zhang and Jun Yan


You might find this additional info useful...

This article cites 66 articles, 36 of which can be accessed free at:

http://jn.physiology.org/content/77/4/2098.full.html#ref-list-1

This article has been cited by 29 other HighWire hosted articles, the first 5 are:

**Spatial Profile of Excitatory and Inhibitory Synaptic Connectivity in Mouse Primary Auditory Cortex**
Robert B. Levy and Alex D. Reyes
[Abstract] [Full Text] [PDF]

**Fine frequency tuning in monkey auditory cortex and thalamus**
Edward L. Bartlett, Srivatsun Sadagopan and Xiaoqin Wang
[Abstract] [Full Text] [PDF]

**Correlation of neural response properties with auditory thalamus subdivisions in the awake marmoset**
Edward L. Bartlett and Xiaoqin Wang
[Abstract] [Full Text] [PDF]

**Contribution of Inhibition to Stimulus Selectivity in Primary Auditory Cortex of Awake Primates**
Srivatsun Sadagopan and Xiaoqin Wang
[Abstract] [Full Text] [PDF]

**Musical Experience Limits the Degradative Effects of Background Noise on the Neural Processing of Sound**
Alexandra Parbery-Clark, Erika Skoe and Nina Kraus
[Abstract] [Full Text] [PDF]

Updated information and services including high resolution figures, can be found at:

http://jn.physiology.org/content/77/4/2098.full.html

Additional material and information about *Journal of Neurophysiology* can be found at:

http://www.the-aps.org/publications/jn

This information is current as of May 15, 2012.
Sharpening of Frequency Tuning by Inhibition in the Thalamic Auditory Nucleus of the Mustached Bat

NOBUO SUGA, YUNFENG ZHANG, AND JUN YAN
Department of Biology, Washington University, St. Louis, Missouri 63130

Suga, N., Y. Zhang, and J. Yan. Sharpening of frequency tuning by inhibition in the thalamic auditory nucleus of the mustached bat. *J. Neurophysiol.* 77: 2098–2114, 1997. Unlike the quasitriangular frequency-tuning curves of peripheral neurons, pencil- or spindle-shaped frequency-tuning curves (excitatory areas) have been found in the central auditory systems of many species of animals belonging to different classes. Inhibitory tuning curves (areas) are commonly found on both sides of such “level-tolerant” sharp frequency-tuning curves. However, it has not yet been examined whether sharpening of frequency tuning takes place in the medial geniculate body (MGB). We injected an inhibitory transmitter antagonist, bicuculline methiodide (BMI), into the MGB of the mustached bat to examine whether frequency tuning is sharpened by inhibition in the MGB and whether this sharpening, if any, occurs in addition to that performed in prethalamic auditory nuclei. Thirty-seven percent of thalamic Doppler-shifted constant frequency (DSCF) neurons mostly showing a level-tolerant frequency-tuning curve had an inhibitory area or areas. BMI changed the inhibitory areas of these neurons into excitatory areas, so that their excitatory-frequency-tuning curves became broader. However, the BMI-broadened excitatory frequency-tuning curves were still much narrower than those of peripheral neurons. Our results indicate that level-tolerant frequency tuning of thalamic DSCF neurons is mostly created by prethalamic auditory nuclei and that it is further sharpened in 37% of thalamic DSCF neurons by lateral inhibition occurring in the MGB. The comparisons in sharpness (quality factors) of frequency-tuning curves between peripheral, thalamic, and cortical DSCF neurons indicate that the skirt portion of tuning curves is sharper in the above order, and that their tip portion is not significantly different between the peripheral and thalamic DSCF neurons, but significantly sharper in the cortical DSCF neurons than in the thalamic DSCF neurons. Therefore the central auditory system has inhibitory mechanisms for the progressive sharpening of frequency tuning. DSCF neurons in the primary auditory cortex were recently found to show facilitative responses to paired sounds. That is, they are combination sensitive. In the present studies, we found that thalamic DSCF neurons also showed facilitative responses to paired sounds. The responses of thalamic DSCF neurons to acoustic stimuli consisted of a slow and a fast component. BMI mainly increased the slow component and an excitatory transmitter antagonist, d-2-amino-5-phosphonovalerate mainly suppressed the slow component. Therefore the response pattern of these thalamic neurons is shaped by both γ-aminobutyric acid-mediated inhibition and N-methyl-d-aspartate-mediated facilitation.

**INTRODUCTION**

Because frequency analysis is the most fundamental function of the auditory system, the physical and physiological bases of frequency analysis and coding in the auditory periphery have been well studied. To extend the studies on the periphery, much research on the central auditory system has been performed and frequency-tuning curves of many single neurons have been measured. However, the question has only partially been answered as to where, how, and why the tuning curves change in shape from peripheral ones. Therefore the processing of auditory signals in the frequency domain by the central auditory system remains to be studied further.

In the central auditory system of the cat, Katsuki et al. (1958, 1959a) found that the higher the level in the auditory system, the sharper the frequency tuning of neurons, and that neurons in the medial geniculate body (MGB) of the thalamus showed the sharpest frequency tuning. However, Atkin and Webster (1972) reported that “in general, tuning curves [of MGB neurons of cats] are less sharp than those of cochlear nerve fibers” (page 376). Calford et al. (1983) reported that “no difference in sharpness of tuning was found between samples of units from nuclei in the lemniscal auditory pathway [of cats], although units from the anterior auditory field showed broader tuning than those in the lemniscal pathway” (page 395). The conclusion of Atkin and Webster (1972) and Calford et al. (1983), which is different from that of Katsuki et al., is probably due to differences in defining the sharpness of frequency-tuning curves of neurons and also in sampling neurons (Suga 1995; Suga and Manabe 1982).

Unlike quasitriangular tuning curves of peripheral neurons, the tuning curves of central auditory neurons show a large variation in shape. Among these, pencil- or spindle-shaped tuning curves (e.g., Fig. 2) have a very narrow bandwidth regardless of sound pressure levels, so that they are called “level-tolerant” sharp frequency-tuning curves, level-tolerant tuning curves, or level-tolerant excitatory areas (Suga 1977; Suga and Manabe 1982). For simplicity, neurons showing level-tolerant tuning are hereafter called level-tolerant neurons. (Here, level-tolerant means that the width of a frequency-tuning curve stays narrow regardless of sound pressure levels. This definition is not related to any physiological or psychological interpretation, but only to the shape of an excitatory frequency-tuning curve. The definition does not at all specify a rate-level function. If a level-tolerant neuron shows no excitatory responses to sounds at high sound pressure levels because of upper threshold, it simply means that the bandwidth of its tuning curve is infinitely narrow and that the neuron sends no frequency information about these sounds to other neurons.)

Level-tolerant neurons have been found in the central auditory systems of many different species of animals, such as little brown bats (Condon et al. 1994; Grinnell 1963; Suga 1964a,b, 1965a,b, 1971; Suga and Schlegel 1973),

Inhibitory” tuning curves or areas are commonly found on both sides of a level-tolerant tuning curve.
Yuma bats (Suga 19691), mustached bats (Olsen and Suga 1991; O’Neill 1985; Suga and Manabe 19822; Suga and Tsuzuki 19853; Suga et al. 1975, 1979; Yang et al. 19923), horseshoe bats (Metzner and Radtke-Schuller 1987; Rübسامen and Schäfer 1990; Vater et al. 19924), big brown bats (Casseday and Covey 19925), pallid bats (Fuzessery 19946), mice (Ehret and Moffat 19857), cats (Abeles and Goldberg 1970; Evans and Nelson 19738; Godfrey et al. 1975; Greenwood and Maruyama 19659; Phillips et al. 1985; Schreiner and Mendelson 1990; Schreiner and Sutter 1992; Spirou and Young 19910; Sutter and Schreiner 19911; Young and Brownell 197612), starlings (Leppesalack 197413), and leopard frogs (Fuzessery and Feng 198214). Iontophoretic injections of inhibitory transmitter antagonists broaden level-tolerant frequency-tuning curves (Sawaki et al. 1992; Vater et al. 1992; Yang et al. 1992). Therefore the central auditory system has inhibitory mechanisms for sharpening frequency tuning.

Level-tolerant neurons already exist in the cochlear nucleus of the mustached bat (Suga et al. 1975) and the cat (Young and Brownell 1976). In the little brown bat, neurons with level-tolerant tuning are rare in the cochlear nucleus, but are common in the inferior colliculus and the auditory cortex (Suga 1964a,b, 1965a,b). This is also true in the mustached bat (Olsen and Suga 1991; O’Neill 1985; Suga and Manabe 1982; Suga et al. 1975; Yang et al. 1992). Therefore it is almost certain that sharpening of frequency tuning takes place at different levels of the central auditory system, but may not always be progressive.

Katsuki et al. (1959a) speculated that sharpening of frequency tuning may be completed in the MGB. However, the research has not been performed to examine whether the sharpening by inhibition takes place in the MGB and whether this sharpening, if any, takes place in addition to what occurred in the prethalamic auditory nuclei. The aim of our present research is to obtain the answers to these two questions with the mustached bat. The reason we used the mustached bat instead of the cat in our research is described below.

If a particular species of animal frequently uses constant frequency (CF) and/or quasi-CF sounds for its very important acoustic behavior, it is expected that the neural mechanisms for fine frequency analysis of these sounds are enhanced in this species and can be more easily explored with this species than with a species that does not use or rarely explores such sounds. [a quasi-CF call is a slightly frequency-modulated (FM) “CF-like” call (Kanwal et al. 1994).] Domestic cats may communicate with quasi-CF sounds and may occasionally feed on rodents that emit CF and/or quasi-CF components (Ehret 1987; Sales and Pye 1974). It is, however, not yet known whether level-tolerant fine frequency analysis is important to cat’s auditory behavior in nature, although the cat’s central auditory system contains level-tolerant neurons. On the contrary, the mustached bat emits CF-FM biosonar pulses (orientation sounds). The CF components of the biosonar pulses are used for Doppler-shift measurements: relative target velocity and insect wing beats (Gaioni et al. 1990; Goldman and Henson 1977; Henson et al. 1987; Johnson et al. 1974; Schnitzler 1970). Doppler-shift measurements are based on frequency analysis. Its auditory system is highly specialized for the fine frequency analysis of the CF components in the biosonar signals, in particular the second harmonic CF component (CF2), which is ~61 kHz (Bodenhamer and Pollak 1983; Pollak et al. 1972; Suga and Jen 1976, 1977; Suga and Manabe 1982; Suga and Tsuzuki 1985; Suga et al. 1975).

In the mustached bat, iontophoretic injections of an inhibitory transmitter antagonist (bicuculline methiodide, BMI) broaden frequency-tuning curves of collicular (Yang et al. 1992) and cortical neurons tuned to ~61 kHz (Sawaki et al. 1992). The amount of broadening is very limited in the cortex compared with that observed in the inferior colliculus. In the MGB, neurons sharply tuned to ~61 kHz are located in its ventral division (MGBv) (Olsen 1986; Wentrup et al. 1994). Unlike that of the cat (Rinvik et al. 1987) and the rat (Winer and Larue 1988), the MGBv of the mustached bat contains an extremely small number of GABAergic neurons, but many GABAergic endings. These endings must predominantly originate from the prethalamic auditory nuclei and the thalamic reticular nucleus (Winer et al. 1992). In rats, glycinergic endings are practically nonexistent in the MGB (Aoki et al. 1988; Wenthold and Hunter 1990). This is presumably also the case in the mustached bat. In the present research, therefore, we iontophoretically applied bicuculline to single thalamic neurons and examined whether sharpening of frequency tuning by inhibition took place in the MGB; whether this sharpening, if any, was to cause further sharpening in the frequency tuning of prethalamic neurons; and whether sharpening was “practically” completed in the MGB. We obtained data that clearly indicate that additional sharpening by inhibition takes place in the MGB.

Neurons in the primary auditory cortex of the mustached bat had been thought to respond primarily to single frequencies, like those in other mammals. However, Fitzpatrick et al. (1993) found that cortical Doppler-shifted CF (DSCF) neurons are sensitive to a combination of two sounds, although they are undoubtedly neurons in the primary auditory cortex. These neurons are sharply tuned to a sound of ~61 kHz and their response to a ~61-kHz sound is facilitated by a sound at ~26 kHz. Because the frequency-tuning curves of cortical DSCF neurons at ~26 kHz are broad, they show a facilitative response to a combination of pulse CF (~30 kHz) or FM (sweeping ~30 to 24 kHz) and echo CF2 (~61 kHz) when an acoustic stimulus simulates the pulse-echo pair. The additional aim of the present paper is to explore whether thalamic DSCF neurons show a facilitative response to a pulse-echo pair, as cortical DSCF neurons do, and whether the facilitation, if any, is mediated by N-methyl-D-aspartate (NMDA). We obtained data indicating that thalamic DSCF neurons show the facilitative response to the pulse-echo pair and that NMDA is involved in evoking the facilitative response.

METHODS

The materials and methods in the present experiments were basically the same as those described in Suga et al. (1983) and Saitoh and Suga (1995). Therefore only the main parts of the methods are described below. The protocol of our research was approved by the animal study committee of Washington University (St. Louis, MO; Approval No.: 890486).

Animals and surgery

Seven Jamaican mustached bats, Pteronotus pellucens pellucens, weighing 11–14 g were used in this study. The CF2 resting fre-
frequency (RF) of each bat was measured before surgery and at the beginning of each experiment (Fig. 1). The CF2 RF is the frequency of the CF2 component of the biosonar pulse emitted by the bat at rest. The CF2 RF was used to normalize best frequencies (BFs) of individual neurons, as explained later. After an intramuscular injection of the neuroleptanalgesic Innovar-Vet (Fentanyl, 0.08 mg/kg body wt and Droperidol, 4 mg/kg body wt), the temporal muscles of the bat were reflected laterally to expose the dorsal part of the skull. The surface of the skull was scraped, and a 1.5-cm-long stainless steel post was attached to it with cyanoacrylate glue and dental cement. The surgical wound was treated with antibiotic ointment (Furacin) and local anesthetic (Lidocaine) and was sutured. Prednisolone (corticosteroid hormone) was administered before and 2 h after the surgery to reduce possible surgical shock. The bat was kept separated in a humidity- and temperature-controlled room during recovery and thereafter. Electrophysiological experiments began 3–4 days after the surgery.

**Electrodes for recording of action potentials and for iontophoretic injections of drugs**

The reference electrode was a vinyl-lacquer-coated tungsten wire with a tip diameter of \(~20 \mu m\). It was placed on the dura mater at the dorsocaudal part of the cerebrum. A five-barreled carbon-fiber microelectrode (Armstrong-James and Millar 1979) was used for the recording of action potentials from single neurons in the MGB and also for the microiontophoretic injections of a \(\gamma\)-amino-butyric acid-A (GABA\(_A\)) receptor antagonist, BMI (5 mM, pH 3.0, Sigma) and/or an NMDA receptor antagonist, \(\alpha\)-2-amino-5-phosphonovalerate (APV, 50 mM, pH 7.7, Genosys Biotech). A glass tube at the center contained a 10-\(\mu m\)-diam carbon fiber. The carbon fiber was etched to be \(~3 \mu m\) at the tip and was used to record action potentials. This tube was surrounded by five glass tubes (barrels). The overall diameter of the multibarreled electrode was 15–20 \(\mu m\) at the tip of the barrels, which was 20–40 \(\mu m\) proximal to the tip of the carbon-fiber electrode. Three of the five barrels were filled with drugs: BMI and/or APV. The remaining two barrels were filled with isotonic saline solution (pH 7.0). These barrels were used as either a ground or a balance electrode.

**Recording of action potentials and iontophoretic injections of drugs**

An unanesthetized mustached bat was placed in a Styrofoam restraint that was suspended with an elastic band at the center of an echo-attenuated soundproof room maintained at 31–32°C. The metal post glued on the skull was locked into a metal rod to immobilize the bat’s head. Holes (100–200 \(\mu m\) diam) were made in the skull with a needle just before electrode placement.

The multibarreled electrode was inserted into the MGBv through the auditory cortex and then the hippocampus. Action potentials of single MGBv neurons were then recorded. The recording sites of thalamic DSCF neurons were determined to be within the MGBv on the basis of their positions on the frequency map of the MGB. Previous anatomic and physiological studies of the MGB have shown that the thalamic DSCF neurons are clustered in the dorsosanterior portion of the MGBv, a region that projects to the DSCF area of the primary auditory cortex (Olsen 1986) and that receives ascending input from the dorsoposterior division of the central nucleus of the inferior colliculus (Olsen 1986; Wenstrup et al. 1994). To avoid contamination of action potentials discharged by more than one neuron, a time-amplitude window discriminator (BAK Electronic, model DLS-1) was used.

For an iontophoretic injection of a drug, an electric current was applied to a drug-filled barrel with a constant current source (Medical Systems, model BH-2). The timing of the injection was controlled manually during monitoring of responses of a single thalamic DSCF neuron. Automatic current balancing was performed through the saline-filled barrel. Injection current was +50 nA for BMI and −40 to −50 nA for APV. Retention current was set at −10 nA for BMI and +10 nA for APV. The effect of BMI (increase in response magnitude and/or background discharges) was observed in \(~1\) min after the onset of the injection, and further change in neural activity was not observed during a 12-min BMI application. The drug effect apparently stabilized in \(~1\) min. The duration of the BMI injection during the frequency-amplitude (FA) scan was set at 12 min, because the drug effect required 1 min to reach a steady state and because the FA scan provided a frequency-tuning curve, required 11 min.

**Acoustic stimuli**

Two types of acoustic stimuli were delivered to the bats: 30-ms CF tones (tone bursts) to measure frequency-tuning curves and paired CF-FM sounds, similar to harmonics in a pulse-echo pair, to study facilitative responses (Fig. 1). The durations of the CF and FM components in the CF-FM sounds were 20 and 3.0 ms, respectively. The rise/fall time of the CF tones and CF-FM sounds was 0.5 ms. These acoustic stimuli were delivered by a condenser loudspeaker placed 74 cm in front of the bat. The repetition rate of these stimuli was either 5.0 or 6.7 per second, similar to that of biosonar pulses emitted by the bat in a cruising phase of echolocation.

Single CF tones were used to measure the BF, minimum threshold (MT), and frequency-tuning curve of a thalamic neuron. Paired CF-FM sounds were used to evoke the facilitative response of a neuron and to determine whether the neuron was qualified to be categorized as a thalamic DSCF neuron, i.e., a counterpart to a cortical DSCF neuron. Automatic current balancing was performed during the frequency-tuning (FA) scan to study facilitative responses (Fig. 1). The duration of the BMI injection during the frequency-amplitude (FA) scan was set at 12 min, because the drug effect required 1 min to reach a steady state and because the FA scan obtained a frequency-tuning curve, required 11 min.

**FIG. 1.** Schematized sound spectrograms of a biosonar pulse (solid lines) of the mustached bat and its echo (dashed lines). CF\(_1\)–CF\(_4\), 1st–4th of a constant-frequency (CF) component; DS, Doppler shift; FM\(_1\)–FM\(_4\), 1st–4th harmonics of a frequency-modulated (FM) component. Arrows: difference in frequency between the pulse CF\(_2\) and its Doppler-shifted echo CF\(_2\).
pulse and the second harmonic (H₂) of an echo (Fitzpatrick et al. 1993).

Sinusoidal waves were produced by a voltage-controlled function generator (Wavetek 134). The frequency of the waves was varied manually or by a computer. A CF tone was generated by shaping the output of the voltage-controlled function generator with a custom-built electronic switch. The FM component of a CF-FM sound was produced by applying a linear voltage ramp to the function generator. The voltage ramp was synchronized with the electronic switch. The amplitudes of these sounds were varied with a decade attenuator (Hewlett-Packard, model 350D) or a custom-built digital attenuator that was computer controlled. When the frequency and amplitude of a CF tone were controlled by an IBM 286 computer and a Modular Instruments hardware interface, these parameters were varied in a certain way called the FA scan. In the FA scan, frequency (F) scan consisted of 33 time blocks, in 32 blocks of which frequency was changed in 0.25-kHz steps from 56.00 to 63.75 kHz. In the 33rd (last) block, no stimulus was presented. The duration of each block was 200 ms, so that the duration of the F scan was 6,600 ms. The F scan was repeated five times at a given amplitude with a 50-ms silent period. After every five F scans, the amplitude of a CF tone was changed in 5-dB steps from 106 to 6 dB SPL [amplitude (A) scan], so that the FA scan consisted of 33 × 20 blocks. The duration of each FA scan was 11 min.

To study facilitative responses of a thalamic neuron to paired CF-FM sounds, the time interval between two CF-FM sounds in a pair was varied manually or by the computer. When it was computer controlled, it was varied in a certain way called a delay (D) scan. The D scan consisted of 13 time blocks: pulse alone (P), 10 pulse-echo pairs with different echo delays (0–36 ms in 4-ms steps), echo alone (E), and no stimulus (N) (Fig. 3). P and E stimuli, respectively, consisted of H₁ and H₂, the essential harmonics to evoke the facilitative response of thalamic DSCF neurons (Fig. 1). The N condition was used to count background discharges. The duration of each block was 150 ms, so that the duration of the D scan was 1,950 ms. The silent period between D scans was 50 ms. The silent period between the last E stimulus in the D scan and the first P stimulus in the next D scan was ≈314 ms.

Data acquisition

Once action potentials of a single thalamic DSCF neuron were recorded with a multiharreled electrode, the data acquisition was started. The data acquired consisted of 1) the measurements of BF, best amplitude (BA), upper threshold, and MT of the neuron; 2) recording of the neuron’s response to the D scan; and 3) continuous recording of the activity of the neuron while the FA scan was repeatedly delivered.

When the single-unit recording was stable and the bat was quiet, the response of the neuron to the FA or D scan was recorded before, during, and after BMI and/or APV injections. (BMI or APV was iontophoretically injected into the recording site, as already described.) The responses of a neuron to the acoustic stimuli of the FA or D scan were continuously monitored as rasters and an array of peristimulus time (PST) histograms displayed on a computer monitor screen. The shapes of action potentials were continuously monitored by comparing incoming action potentials with the template of an action potential recorded at the beginning of the continuous recording with a digital storage oscilloscope (Tektronix 2211). The data were used for off-line analysis as far as the shape of action potentials matched the template.

Data processing

Rasters and PST or PST cumulative (PSTC) histograms were plotted to display the responses of a single neuron to the D and/or FA scans. The binwidth of PST or PSTC histograms was 1.0 ms. Each histogram displayed the sum of the responses of a single neuron to an identical stimulus repeatedly delivered 50 times for the D scan or 5 times for the FA scan. A frequency-tuning curve was plotted with the use of the responses to the FA scans displayed as rasters.

The criterion for the determination of a threshold for an excitatory response was the boundary between a block showing one response (action potential) per five trials and a block showing no response per five trials. In unanesthetized animals, a response magnitude and a background discharge rate of thalamic neurons fluctuated to some extent. Therefore the threshold was determined with additional criteria as described below. If a given block showed at least one response per five trials, it was interpreted to be within an excitatory area. If a given block showed no response per five trials, but its adjacent blocks, away from the excitatory area, showed at least one response per five trials, the given block is interpreted to be within the excitatory area. Applying the above criteria, excitatory tuning curves were plotted by visually inspecting not only the response in a given block, but also the responses in its adjacent eight blocks. Excitatory tuning curves based on the data obtained by 0.25-kHz, 5.0-dB steps were then smoothed by hand (e.g., Fig. 4).

The criterion for the determination of a threshold for an inhibitory response was the boundary between a block showing a decrease of <70% in background discharges and a block showing a decrease of >70% in background discharges. However, additional criteria were also applied for determining a threshold for an inhibitory response for the following reasons. 1) The rate of background discharges was low, 0.8–6.8 per second (3.27 ± 1.52 per s, mean ± SD; N = 30 neurons), so that the number of background discharges per block (5 sweeps per block, 200 × 5 ms per block) was usually too small and widely fluctuated to obtain a reliable value for a decrease. 2) Like the excitatory response, the inhibitory response of a thalamic neuron fluctuated to some extent in unanesthetized animals. Therefore an inhibitory area was mapped by examining not only a given block, but also the adjacent eight blocks. If the mean number of discharges in a given block and the adjacent eight blocks was <70% of the background rate, the given block was interpreted as part of an inhibitory area. Inhibitory tuning curves were smoothed by hand, as were excitatory tuning curves (e.g., Fig. 4).

The effect of BMI on thalamic DSCF neurons was evaluated by comparing the frequency-tuning curves, PST histograms, and stimulus amplitude-impulse count functions (hereafter called amplitude-response functions) obtained before, during, and after the drug injection. If a tuning curve broadened by the drug application more than two blocks (i.e., >500 Hz) in the FA scan and returned (recovered) more than one block toward the original tuning curve, the broadening was considered to be significant. A difference in distribution of quality factors (Q values) or bandwidths of frequency-tuning curves between the peripheral, thalamic, and cortical neurons was statistically tested with the Kolmogorov-Smirnov test for goodness of fit.

RESULTS

Sharp frequency tuning and combination sensitivity of thalamic DSCF neurons

Stable recordings of action potentials before, during, and after a BMI injection were made from 30 thalamic DSCF neurons. Like cortical DSCF neurons (Suga and Manabe 1982), these thalamic DSCF neurons were easily identified because of their very sharp frequency tuning to a sound at ~61 kHz (i.e., CF₃ frequency of biosonar signals). The frequency-tuning curves of these thalamic DSCF neurons
FIG. 2. Inhibitory transmitter antagonist (bicuculline methiodide, BMI) broadens frequency-tuning curves of thalamic Doppler-shifted CF (DSCF) neurons. Excitatory and inhibitory frequency-tuning curves of 2 thalamic DSCF neurons (A and B) were measured before, during, and after a 50-nA, 12-min application of 5.0 mM BMI. Con.: control condition (——); excitatory frequency-tuning curve measured before the BMI application. Area inside the curve is the excitatory (response) area. Inh.-con. (shaded area): inhibitory (response) area measured before the BMI application. In this area, background discharges were inhibited by single tone stimuli. Curve surrounding the inhibitory area is the inhibitory tuning curve. BMI (● ●): excitatory frequency-tuning curve measured during BMI application. Rec.: recovery condition (---); excitatory frequency-tuning curve measured after BMI application. CN fiber (---): average excitatory frequency-tuning curve of 18 cochlear nerve fibers that were tuned to 60.5 ± 61.5 kHz. Crosses and open circles indicate the best amplitudes (BAs) to excite the neurons before and during the BMI application, respectively. Averaged frequency-tuning curve of 18 cochlear nerve fibers was obtained by Suga and Jen (1977). MGB, medial geniculate body.

had either a very sharp triangular shape (Fig. 2A), a pencil shape, or a spindle shape (Fig. 2B), and most of them were level tolerant. The frequency-tuning curves of peripheral neurons with a BF of ~61 kHz are narrow and triangular in shape, but not level tolerant (Fig. 2, CN fibers) (Suga and Jen 1977; Suga et al. 1975). The thalamic DSCF neurons were much more sharply tuned in frequency than those peripheral neurons, as further documented throughout the present paper.

Like cortical DSCF neurons (Fitzpatrick et al. 1993), these thalamic DSCF neurons showed a facilitative response to the combination of HI of a synthesized pulse and H2 of a synthesized echo. In other words, the interesting response properties of cortical DSCF neurons were due to those carried up by thalamic DSCF neurons. The essential combinations of signal elements in the HI and H2 to evoke the facilitative response of the thalamic DSCF neurons were pulse CF1 and echo CF2, or pulse FM1 and echo CF2. In Fig. 3A, a neuron shows a response to echo H2 stimulus (E) but not to pulse H1 stimulus (P). When these stimuli were paired and E was delayed from P, the neuron showed a facilitative response at a 24-ms E delay (Fig. 3AI), asterisk). At the 24-ms E delay, pulse FM1 was followed by echo CF2 with a 1.0-ms silent period. It was observed that the responses of thalamic DSCF neurons to acoustic stimuli often consisted of two components: fast and slow. The slow component was usually more facilitated than the fast component in the response to a paired P-E stimulus (Fig. 3), and was more affected by BMI and APV than the fast component (Figs. 9, 10, 12, and 13). The responses to P, E, and P-E pair with a 24-ms E delay are shown in Fig. 3, A2–A4, with a time resolution higher than those in Fig. 3AI. In Fig. 3A4, the fast and slow components are identifiable in the facilitative response.

Figure 3B shows another example of the responses of a thalamic DSCF neuron that did not respond to P stimulus but to E stimulus. As these two stimuli were paired, the neuron showed a facilitative response to the pair, in which E delayed from P by 0–4 or ~24 ms. The facilitation for 0- to 4-ms E delays was due to the combination of CF1 and CF2, and that for a 24-ms E delay was due to the combination of FM1 and CF2. Figure 3, B2–B4, shows the responses to P, E, and the simultaneous delivery of P and E with a time resolution higher than those in Fig. 3B1. The fast and slow components of the facilitative response are identifiable in Fig. 3, B3 and B4. Thalamic DSCF neurons were broadly tuned to echo delays, showing a single peak (Fig. 3AI) or two peaks (Fig. 3B1) in delay tuning curves measured with P-E pairs. Elimination of echo FM1 from the pairs had no effect on the facilitative response, but the elimination of pulse FM1 and echo CF2 abolished the facilitative response. Elimination of pulse CF1 reduced the facilitative response of most neurons. The essential elements of the P-E pair for the facilitation were pulse CF1, pulse FM1, and echo CF2.

Effect of BMI on thalamic DSCF neurons

Of the 30 thalamic DSCF neurons studied, BMI evoked no noticeable change in the frequency-tuning curves of 19 neurons, but produced broadening in the frequency-tuning
curves of the remaining 11 neurons: broadening toward both low and high frequencies in 6 neurons, broadening only toward high frequencies in 3 neurons, and broadening only toward low frequencies in 1 neuron. In the remaining one neuron, broadening was accompanied by a 10-dB lowering in an overall tuning curve, so that its sharpness (Q value) did not change.

The 11 thalamic DSCF neurons that showed a change in a frequency-tuning curve for a BMI application had an inhibitory frequency-tuning curve or curves (inhibitory area or areas). In Fig. 4A, for example, the neuron was tuned to 61.04 kHz and showed the best response to a 66- to 71-dB SPL, 60.25-kHz tone burst. The response to the tone burst at BF (61.04 kHz) was weak, although it was low in threshold, −1 dB SPL. It had a large inhibitory area that was located at frequencies >60.25 kHz and >66 dB SPL. In this inhibitory area, background discharges were inhibited by single tone stimuli. Because of this inhibition, the neuron showed upper thresholds for sounds between 60.75 and 62.50 kHz. A small inhibitory area was also located at 57−58 kHz and 66−86 dB SPL. When BMI was applied to this neuron, background discharges did not change significantly in rate, but excitatory responses to sounds, in particular at 66−96 dB SPL, increased. The best response was evoked by a 91-dB SPL, 60.50-kHz tone burst. The inhibitory areas disappeared and excitatory responses were evoked by the tone bursts that previously inhibited background discharges. As a result, the upper thresholds disappeared and the frequency-tuning curve became particularly broader toward frequencies higher than the BF of 61.04 kHz (Fig. 4B). The broadening in the frequency-tuning curve and the increase in the response magnitude to frequencies within the original inhibitory area indicate that the inhibitory areas were greatly overlapped with the excitatory area. The excitatory frequency-tuning curve became narrower 22 min after BMI application was stopped (Fig. 4C).

Figure 2 shows the frequency-tuning curves of two thalamic DSCF neurons; the curves showed broadening toward both low and high frequencies when BMI was applied to the neurons. The level-tolerant frequency-tuning curve of the neuron in Fig. 2A (Con.: control condition) is triangular and is sandwiched between inhibitory areas, whereas the level-tolerant frequency-tuning curve of the neuron in Fig. 2B (Con.) is spindle shaped and is engulfed by an inhibitory area. These tuning curves, including their tip portion, are much narrower than those of peripheral neurons. When BMI
FIG. 4. Effect of inhibitory transmitter antagonist (BMI) on the auditory response of a thalamic DSCF neuron. Rasters: action potentials discharged by a single thalamic DSCF neuron before (A), during (B), and after (C) a 50-nA, 12-min application of 5.0 mM BMI. The frequency and amplitude of a 30-ms tone burst were controlled by a computer (see METHODS).

was applied to these neurons, their inhibitory areas changed into the excitatory areas. As a result, the excitatory frequency-tuning curves became broader. The amount of broadening was small at the tip portion of the tuning curves, but large at high sound pressure levels. However, the tuning curves broadened by BMI were still much narrower than those of peripheral neurons. The shape of the tuning curves returned to those in the control condition ∼25 min after BMI application was stopped (Rec.: recovery condition). These data clearly indicate that the frequency-tuning curves sharpened by prethalamic auditory nuclei are further sharpened by lateral inhibition occurring in the MGB. BMI increased the magnitude of the responses of these neurons to tone bursts and their BAs.

Figure 5 shows the frequency-tuning curves of two thalamic DSCF neurons; the curves showed broadening toward either low or high frequencies when BMI was applied to the neurons. The tip portion of these curves (Con.) is similar to that of peripheral neurons, but their skirt portion is much narrower than that of peripheral neurons. An inhibitory area was located at frequencies either higher (Fig. 5A) or lower (Fig. 5B) than the neuron’s BF. When BMI was applied to these neurons, their inhibitory areas changed into excitatory areas, so that their excitatory frequency-tuning curves became broader. However, the BMI-broadened frequency-tuning curves were still much narrower than those of peripheral neurons. These data again indicate that the frequency-tuning curves sharpened by prethalamic auditory nuclei are further sharpened by lateral inhibition occurring in the MGB. BMI increased not only the magnitude of the response to tone bursts of these neurons, but also their BAs.

All the 19 neurons that showed no noticeable change in the frequency-tuning curve for a BMI application showed background discharges of 1.2–6.8 per second (3.78 ± 1.88
FIG. 5. Inhibitory transmitter antagonist (BMI) broadens frequency-tuning curves of thalamic DSCF neurons. Excitatory and inhibitory frequency-tuning curves of 2 thalamic DSCF neurons (A and B) were measured before, during, and after a 50-nA, 12-min application of 5.0 mM BMI. See legend to Fig. 2.

per s, N = 19) and ON responses. The neurons had no noticeable inhibitory area in which background discharges were inhibited by single tone stimuli. When BMI was applied to the neurons, their background discharges and/or magnitude of their responses increased by 18.8 ± 26.4% (N = 19) and 71.7 ± 46.2% (N = 19), respectively. These data indicate that the neurons' level-tolerant frequency tuning was created by prethalamic auditory nuclei and transmitted up to the neurons. Two examples of frequency-tuning curves that were unaffected by BMI are shown in Fig. 6.

The curves in Fig. 6 labeled “Con.” for control condition show the excitatory frequency-tuning curves of two thalamic DSCF neurons measured before a BMI application: one is opened at the upper portion (A) and the other is closed (B). Both of the curves, including their tip portion, are narrower than the tuning curves of peripheral neurons. The tuning curves measured during and after a BMI application are indicated by “BMI” and “Rec” (recovery condition), respectively. These three curves (Con., BMI, and Rec.) are identical. This indicates not only that the tuning curves were sharpened in prethalamic auditory nuclei, but also that our recording and measurement of tuning curves were stable and repeatable during the data acquisition. BMI increased the magnitude of the responses of those neurons to tone bursts, but did not change their BAs (Fig. 6, ⊖).

The broadening in a frequency-tuning curve evoked by BMI was usually small at low sound pressure levels, but large at high sound pressure levels (Figs. 2 and 5). This

FIG. 6. Inhibitory transmitter antagonist (BMI) had no effect on excitatory frequency-tuning curves of 2 thalamic DSCF neurons (A and B). See legend to Fig. 2.
was a general rule. However, the amount of change in a frequency-tuning curve varied from neuron to neuron, so that all the tuning curve data obtained from the 30 thalamic DSCF neurons were pooled, and changes in the bandwidths of the frequency-tuning curves were measured at 10, 30, 50, and 70 dB above MT (Fig. 7A). Broadening in a frequency-tuning curve at 10 dB above MT was very small and was >500 Hz only in 1 of the 30 neurons. However, broadening was noticeable at 30 dB above MT in six of the neurons. It was prominent at 50 and 70 dB above MT in 8 and 11 neurons, respectively. As shown in Figs. 2 and 5, BMI could not, however, broaden any of the frequency-tuning curves of the thalamic DSCF neurons to be the same as the tuning curves of peripheral neurons.

Figure 7B shows the distribution of the amounts of BMI-induced changes in the frequency-tuning curves of the 30 thalamic DSCF neurons (white bars) and that of differences in bandwidth between thalamic (without BMI) and peripheral frequency-tuning curves (black bars) at four stimulus levels: 10, 30, 50, and 70 dB above MT. The difference in distribution between the white and black bars indicates the amount of sharpening of frequency tuning that occurred in prethalamic auditory nuclei. The mean difference is 0.05, 0.25, 3.50, and 6.79 kHz, respectively, at 10, 30, 50, and 70 dB above MT. The bandwidths at 50 and 70 dB above MT are much narrower in all 30 thalamic neurons than those of peripheral neurons regardless of whether or not thalamic neurons were affected by BMI (Fig. 7, Bc and Bd). The difference in bandwidth at 30 dB above MT is small, but significant (P < 0.05; Fig. 7Bb). The difference in bandwidth at 10 dB above MT is not significant (P > 0.05; Fig. 7Ba).

Are background discharges different between neurons that showed broadening in frequency tuning with BMI and those that did not?

The rate of background discharges was 1.2−6.8 per second (3.78 ± 1.88 per s) for the 19 neurons showing no BMI broadening and 0.8−5.1 per second (2.45 ± 1.29 per s) for the 11 neurons showing BMI broadening. The difference in background discharge rate between the two groups of neurons is significant (t-test, P < 0.05). During BMI applica-

![Diagram showing changes in bandwidth of frequency-tuning curves](image-url)
tions, the rate of background discharges was 1.3–12.5 per second (4.40 ± 2.74 per s, N = 19) and 1.0–11.2 per second (3.51 ± 3.02 per s, N = 11), respectively, for the two groups of neurons. Percent change in discharge rate was -39–102% (18.8 ± 26.4%, N = 19) and -25–120% (24.2 ± 33.8%, N = 11), respectively, for the two groups of neurons. The change in background discharges evoked by BMI does not differ between the above two groups of neurons (t-test, P > 0.10). There was no clear link between a BMI-induced broadening in frequency tuning and a change in background discharges.

Differences in sharpness of frequency tuning between thalamic and cortical DSCF neurons

To examine whether the frequency-tuning curves of cortical DSCF neurons are narrower or broader than those of thalamic DSCF neurons, the quality factors (Q_{10dB} value) of the cortical DSCF neurons measured by Suga and Manabe (1982) are plotted together with those of thalamic DSCF neurons in Fig. 8. The quality factors of the ~61-kHz-tuned peripheral neurons measured by Suga and Jen (1977) were also plotted in the figure for comparison. A Q_{10dB} value is defined as a BF divided by a bandwidth at n decibels above MT. Here, n is either 10, 30, or 50. Q_{30dB} and Q_{50dB} values are respectively larger in the order of peripheral, thalamic, and cortical neurons (P < 0.01 and 0.05 for Fig. 8, B and C, respectively). Q_{10dB} values show no significant difference between peripheral and thalamic neurons (P > 0.05), but a significant difference between thalamic and cortical neurons: cortical neurons are sharper than thalamic neurons (Fig. 8A; P < 0.01). The above comparisons indicate that further sharpening of frequency tuning takes place even in the DSCF area of the auditory cortex, and that the sharpening in the DSCF area occurs even at the tip portion of frequency-tuning curves.

Changes in response pattern and BA evoked by BMI

As shown in Fig. 4, BMI evoked an increase in magnitude of an excitatory response (number of impulses per stimulus) and/or a change of an inhibitory response into an excitatory response. These changes were not due to an overall increase in discharge rate of a neuron, but specific to the responses to sound stimuli at particular frequencies and amplitudes.

Figure 9 shows the amplitude-response functions of a thalamic DSCF neuron obtained before, during, and after a BMI application. Before the BMI application, the neuron showed the best response to a 48-dB SPL, 60.50-kHz tone burst, a weak inhibitory response to the tone burst at 81 ± 100 dB SPL, and an upper threshold at 81 dB SPL. During the BMI application, the neuron showed almost no change in

**Fig. 8.** Distributions of quality factors (Q_{10dB}, Q_{30dB}, and Q_{50dB} values) of peripheral (AN, shaded bars), thalamic DSCF (MGB, black bars), and cortical DSCF (AC, white bars) neurons. Best frequencies of all these neurons were between 61.0 and 62.0 kHz. Infinity (∞): neuron had a closed tuning curve. [Peripheral and cortical data were obtained by Suga and Jen (1977) and Suga and Manabe (1982), respectively.]
FIG. 9. Effect of inhibitory transmitter antagonist (BMI) on the amplitude-response function (A) and auditory responses (B) of a thalamic DSCF neuron. Con., BMI, Rec.: amplitude-response functions obtained with a 30-ms, 60.50-kHz tone burst before, during, and after a 50-nA, 12-min application of 5.0 mM BMI. Shaded portion of “Con.”: decrease in background discharges evoked by a single tone stimulus. Auditory responses displayed by the PST histograms in B were evoked by the 30-dB SPL (Ba) or 70-dB SPL (Bb), 60.50-kHz tone bursts delivered 15 times. Note that the response to the 70-dB SPL tone burst consists of a fast and a slow component. Vertical dashed line: boundary between these two components.

background discharges, MT, and responses to 28- to 45-dB SPL tone bursts, but a drastic change in responses to 50- to 100-dB SPL tone bursts. The BA to excite the neuron became 65 dB SPL, and both the upper threshold and inhibitory responses disappeared. The prominent increase in response was due to multiple discharges. The PST histograms of Fig. 9B show the responses to 30-dB SPL (Ba) and 70-dB SPL (Bb) tone bursts of the same neuron as in Fig. 9A. The response to the 70-dB SPL sound consists of a fast component and a slow component. The vertical dashed lines in Fig. 9B indicate the boundary between these two components. BMI increased both the fast and slow components of the response, but its effect was much larger on the slow component than on the fast component (B2b). Therefore the large increase in impulse count shown in Fig. 9A was mainly due to the change in the slow component.

Figure 10 shows an additional example of the amplitude-response functions of a thalamic DSCF neuron obtained before, during, and after a BMI application. This neuron showed no sign of inhibitory response, but BMI prominently increased its excitatory response without changing both MT and upper threshold (Fig. 10A). This increase in response was mainly due to the change in the slow component of the response (Fig. 10B). The BA of the neuron increased by ~10 dB.

In Figs. 9 and 10, BMI increased the BAs of the neurons. The effect of BMI on BAs varied from neuron to neuron. In some neurons, a frequency-tuning curve broadened, but a BA did not change. In some others, a frequency-tuning curve did not change, but a BA changed. Figure 11 shows the relationship between BAs measured with and without BMI.

**Shaping response patterns by NMDA and GABA**

To obtain an insight into synaptic interactions shaping the auditory responses of thalamic neurons, BMI and/or APV was iontophoretically applied to thalamic DSCF neurons. The responses of all the 24 neurons studied were affected by BMI and APV without exception. However, there were some variations in the amount of the drug’s effects, as explained below.

Responses of single thalamic DSCF neurons were recorded to H1 of a pulse (P stimulus) and/or H2 of an echo (E stimulus). Each stimulus consisted of the CF and FM components (See Fig. 1). The PST histograms in Fig. 12,
A–C, display the responses of a thalamic DSCF neuron to P, E, and P-E stimuli, respectively. The response of the neuron to E stimulus consisted of a fast and a slow component (B1). The point of overlap between the 2 components is indicated by a vertical dashed line. When BMI was applied to the neuron, the slow component increased, but the fast component was unchanged (B2). When APV was applied during the BMI application, this increased portion of the slow component disappeared (B3). After termination of the APV application, but continuation of the BMI application, only the slow component increased (B4). When the BMI application was terminated, the slow component became very small (B5). These observations indicate that the slow component of the excitatory response is mediated by NMDA, and that the NMDA-mediated response is controlled (suppressed) by GABA. The drug effects described above were more prominent on the facilitative response to a P-E stimulus than on the response to the E stimulus alone. In Fig. 12C, the slow component of the facilitative response of the neuron to P-E stimulus (C1) was dramatically increased by BMI (C2). This increased portion of the facilitative response was eliminated by APV (C3).

The PST histograms of Fig. 13 show the responses of three thalamic DSCF neurons studied with APV and BMI. In A and B, BMI dramatically enhanced the slow component of the auditory responses of the neurons with little effect on its fast component, and APV reduced the BMI-enhanced slow component with little effect on the fast component. (The boundary or the point of overlap between the fast and slow components is indicated by the arrows in A and B.) In C, however, BMI enhanced the whole response of the neuron, and APV reduced the whole BMI-enhanced response. The boundary between the fast and slow components, if any, was unclear in the response of this neuron.

The differential effect of APV on the fast and slow components of the BMI-enhanced auditory responses of thalamic DSCF neurons was found in 17 neurons of the 24 studied. In these neurons, the reduction of the response evoked by APV was 3–32% (16.7 ± 10.4%) for the fast component, but was 38–71% (52.6 ± 10.6%) for the slow component. The reduction of the slow component was 2.0–16.3 (5.09 ± 4.97) times larger than that of the fast component. The data obtained with the drugs clearly indicate that the response properties of thalamic DSCF neurons are shaped in the MGB through the interaction between inhibition mediated by GABA and excitation/facilitation mediated by non-NMDA and NMDA.

DISCUSSION

How to compare the sharpness of frequency tuning of central auditory neurons with that of peripheral auditory neurons

The sharpness of a tuning curve has been expressed by a Qmax value that is a BF divided by a bandwidth at n decibels above MT (n dB bandwidth). When Kiang et al. (1965)
FIG. 12. Effects of an inhibitory transmitter antagonist (BMI) and an excitatory transmitter antagonist (APV, n-2-amino-5-phosphonovalerate) on auditory responses of a thalamic DSCF neuron. Responses to acoustic stimuli are displayed as PST histograms. Vertical dashed lines in B and C: point of overlap between the fast and slow components in the response. The acoustic stimuli were a pulse alone (A), echo alone (B), and pulse-echo pair (C). The pulse (P) and echo (E) stimuli were H1 (A1, ——) of the pulse and H2 (B1, ——) of the echo, respectively. Each harmonic consisted of a 20-ms CF and a 3.0-ms FM component. CF1: 29.47 kHz, 42 dB SPL; CF2: 61.89 kHz, 29 dB SPL. In FM1 and FM2, frequency swept downward by 6.0 and 12 kHz from the frequencies of CF1 and CF2, respectively. 1 ± 5 in A–C: control condition, BMI application, BMI and APV applications, BMI application, and recovery condition, respectively.

Suga and Tsuzuki (1985) pointed out that the choice of parameters characterizing tuning curves should be contingent on the problem to be discussed. To discuss sharpening, a change in the skirt portion of a tuning curve (e.g., $Q_{30\text{dB}}$) should be mainly considered, not the tip portion, because the tip portion is sharp at the periphery without inhibition. To discuss broadening, on the other hand, the tip portion of a tuning curve (e.g., $Q_{10\text{dB}}$) should be mainly considered, not the skirt portion, because the skirt portion is broad at the periphery without excitatory integration.

The frequency-tuning curves of peripheral neurons commonly show a deflection point at $\sim 40$ dB above MT where the slopes of the passive (1st) and active (2nd) filters join (e.g., Evans 1972). Therefore $Q_{50\text{dB}}$ and $Q_{30\text{dB}}$ values or 30- and 50-dB bandwidths may be used to determine whether the passive and/or active portions of a tuning curve are sharpened by inhibition in the central auditory system.

In cats, frequency-tuning curves with $Q_{50\text{dB}}$ and/or $Q_{30\text{dB}}$ values much larger than those of peripheral neurons have been obtained at different levels of the central auditory system (Abeles and Goldstein 1970; Evans and Nelson 1973; Godfrey et al. 1975; Greenwood and Maruyama 1965; Katsuki 1958, 1959a; Phillips et al. 1985; Schreiner and Mendelson 1990; Schreiner and Sutter 1992; Spirou and Young 1991; Sutter and Schreiner 1991; Young and Brownell 1976). Therefore the cat’s central auditory system has a mechanism for the sharpening of frequency tuning, and this mechanism drastically sharpens the skirt of a tuning curve. The functional significance of the sharpening of frequency tuning is discussed later.
Schematized sound spectrogram of the paired stimulus. BMI was 1st applied rons. The frequency-tuning curves of 37% of these thalamic the tuning curves with high Q 10dB values. Different from upper thresholds of 39% of the neurons (Sawaki et al. 1992).

De®nition of level tolerance

The frequency-tuning curves of peripheral neurons commonly tory system, and that progressive sharpening by inhibition may be concluded that sharpening of frequency tuning by inhibition applies for a tuning curve that has a bandwidth narrower between inhibitory tuning curves was found (Suga et al. 1992). In the inferior colliculus of the mustached bat, 42% of cortical DSCF neurons studied had frequency-tuning curves that were much sharper than those of peripheral neurons. The frequency-tuning curves of 37% of these thalamic neurons became broader when BMI was iontophoretically applied to the neurons, but the BMI-broadened curves were still much narrower than those of peripheral neurons (Figs. 2, 5A, and 6). The quality factors of cortical DSCF neurons are slightly higher than those of thalamic DSCF neurons (Fig. 8). BMI injections into the DSCF area of the primary auditory cortex broadened the frequency-tuning curves of 58% of cortical DSCF neurons studied and eliminated the upper thresholds of 39% of the neurons (Sawaki et al. 1992).

Sharpening of frequency tuning by inhibition occurs at different levels

Auditory nuclei and the auditory cortex consist of several subdivisions, and each subdivision contains neurons with different response properties (e.g., Suga 1990). Therefore neurons with identical BF should be pooled for a comparison of sharpness of frequency tuning between peripheral and central neurons, and a division of a neuron or the cortex studied for the comparison should be identi®ed. Therefore in the present study we focused on neurons that are directly involved in ®ne frequency analysis to extract velocity information carried by Doppler-shifted echoes at ~61 kHz.

All thalamic DSCF neurons studied had frequency-tuning curves that were much sharper than those of peripheral neurons. The frequency-tuning curves of 37% of these thalamic neurons became broader when BMI was iontophoretically applied to the neurons, but the BMI-broadened curves were still much narrower than those of peripheral neurons (Figs. 2, 5, and 7). The quality factors of cortical DSCF neurons are slightly higher than those of thalamic DSCF neurons (Fig. 8). BMI injections into the DSCF area of the primary auditory cortex broadened the frequency-tuning curves of 58% of cortical DSCF neurons studied and eliminated the upper thresholds of 39% of the neurons (Sawaki et al. 1992). These data indicate the following three important facts. 1) Sharpening of frequency tuning is extensively performed in prethalamic auditory nuclei. 2) Additional sharpening of frequency tuning takes place in 37% of thalamic neurons by GABA-mediated inhibition. 3) Sharpening of frequency tuning by GABA-mediated inhibition also takes place in the auditory cortex.

In the inferior colliculus of the mustached bat, 42% of neurons studied showed broadening of a frequency-tuning curve for BMI (Yang et al. 1992). In the cochlear nucleus of the mustached bat, a level-tolerant tuning curve sandwiched between inhibitory tuning curves was found (Suga et al. 1975). On the basis of all the data summarized above, it may be concluded that sharpening of frequency tuning by inhibition takes place at different levels of the central auditory system, and that progressive sharpening by inhibition creates very sharp level-tolerant frequency tuning of some neurons, as Katsuki et al. (1958) described. This does not mean that all frequency-tuning curves are progressively sharpened. It is possible that level-tolerant tuning created at one level is not further sharpened. Perhaps it is worth
mentioning that the central auditory system also creates broadly frequency-tuned neurons, as reported in numerous papers (e.g., in cats, Aitkin and Webster 1972; in little brown bats, Suga 1965a,b).

Three types of frequency-domain processing of auditory signals

The frequency-domain processing of auditory signals in the central auditory system may be divided “neuroethologically” into three types: 1) processing of CF and quasi-CF sounds by neurons with level-tolerant sharp frequency tuning, 2) processing of spectral patterns of broadband sounds such as complex sounds with many harmonics and noise bursts by neurons with excitatory (or facilitatory) and inhibitory areas and by neurons particularly sensitive to broadband sounds, and 3) processing of sounds changing in spectrum such as FM sounds by neurons with excitatory (or facilitatory) and inhibitory areas and by neurons particularly sensitive to FM sweeps. These three types of frequency-domain processing are mutually related and overlap. Which type of signal processing is more prominent than the others depends on the properties of biologically important sounds such as those produced by a given species and by its prey and predators (e.g., Casseday and Covey 1992; Fuzessery 1994; Suga and Manabe 1982; Suga et al. 1983). The processing of CF or quasi-CF sounds is directly related to the problem of whether the central auditory system has a mechanism for the sharpening of frequency tuning of neurons.

Level-tolerant neurons and level-tolerant fine frequency discrimination

The acoustic behavior of mustached and horseshoe bats indicates that they are highly specialized for processing velocity information that is carried by the long CF components of a biosonar pulse and its Doppler-shifted echo (Gaioni et al. 1990; Henson et al. 1987; Schantz et al. 1968, 1970; Schuller et al. 1974). In the DSCF and CF/CF areas of the auditory cortex of the mustached bat, the great majority of neurons tuned to the frequencies of these CF components shows level-tolerant tuning that is created by lateral inhibition (Suga and Manabe 1982; Suga and Tsuzuki 1985; Suga et al. 1979). The sharper the level-tolerant tuning curve, the closer the best inhibitory frequencies to the best excitatory or facilitatory frequencies (Suga and Tsuzuki 1985). Fine analysis of velocity information (Doppler shifts evoked by relative target motion and insect wing beats) is directly related to fine frequency analysis, so that level-tolerant tuning is directly related to level-tolerant fine frequency discrimination. The DSCF area of the primary auditory cortex shows a very fine frequency representation (Suga and Jen 1976; Suga and Manabe 1982; Suga et al. 1987). Inactivation of this area with muscimol evokes a clear deficit of the bat’s ability to perform a fine frequency discrimination (Riquimaroux et al. 1991).

Big brown bats frequently emit long “shallow FM” sounds in the search phase of echolocation. In these sounds, frequency slowly sweeps from 30 to 20 kHz (Master et al. 1991). The great majority of collicular neurons tuned to these frequencies shows level-tolerant tuning (Casseday and Covey 1992). The long shallow FM sound is suited for target detection at long distances. The sharper the tuning, the larger the signal-to-noise ratio, i.e., the better the target detection ability.

Rodents emit long CF and quasi-CF sounds. When a baby mouse is isolated from its mother, for example, it emits such sounds (Sales and Pye 1974). In the inferior colliculus of mice, many level-tolerant neurons have been found (Ehret and Moffat 1985). The long CF or long quasi-CF sounds are suited to detecting and discriminating the individuals producing them. Level-tolerant tuning is ideal for this purpose.

The data obtained from animals that frequently use CF or quasi-CF sounds for the detection and discrimination of a signal source and/or target motion indicate that level-tolerant neurons are directly related to the properties of species-specific CF or quasi-CF sounds and species-specific behavior utilizing these sounds. Therefore it is our contention that one of the important functions of level-tolerant neurons is level-tolerant fine frequency discrimination.

Many central auditory neurons have both excitatory and inhibitory frequency-tuning curves. Their responses to complex sounds depend on the balance between excitation and inhibition. Therefore, “the analysis of complex sounds is not performed in such a way that single neurons with narrow excitatory areas always respond when certain components of complex sounds fall into the excitatory areas. Many neurons require a certain structure in the complex sound to be excited by it” (Suga 1968). Level-tolerant neurons are not exceptional in this respect. They would also be involved in the processing of complex sounds. It is also our contention that the processing of auditory signals by central auditory neurons should be evaluated in relation to biologically important sounds.

Inhibition plays different roles in processing auditory information in the frequency, amplitude, time, and binaural domains. Therefore inhibition does not necessarily create level-tolerant tuning (e.g., Caspary et al. 1994; Evans and Zhao 1993; Palombi and Caspary 1992; Vater et al. 1992; Yang et al. 1992).

Fast and slow components of auditory responses of thalamic DSCF neurons

In rats, neurons in the dorsal division of the MGB show a large NMDA-mediated depolarization to an electrical stimulation of the brachium of the inferior colliculus, whereas neurons in the MGBv seldom show it. However, some MGBv neurons show a noticeable NMDA response when GABA-mediated inhibition is eliminated by picrotoxin (Hu et al. 1994). In the mustached bat, thalamic DSCF neurons are located in the MGBv (Olsen 1986; Wenstrup et al. 1994). These thalamic DSCF neurons are combination sensitive (Fig. 3) and show an NMDA-mediated auditory response that is mostly inhibited by GABA. When BMI is applied to the neurons, the NMDA-mediated response (i.e., the slow component of the response) becomes prominent (Figs. 12 and 13). Therefore the data obtained from mustached bats and rats are similar to each other. However, there appears to be a quantitative difference between them, because the auditory responses of thalamic DSCF neurons were reduced by APV without exception. This difference may be due to the combination sensitivity of the thalamic DSCF neurons.
We are grateful to the Jamaican Ministry of Agriculture for permitting the export of stachted bats from Jamaica. We also thank J. F. Olsen and A. Kadir for comments on this article.

Our research on bats has been supported by research grants from the National Institute on Deafness and Other Communicative Disorders (DC-00175), Office of Naval Research (N00014-90-J-1068), and Human Frontier Science Program.

Address for reprint requests: N. Suga, Dept. of Biology, Washington University, 1 Brookings Dr., St. Louis, MO 63130.

Received 26 December 1995; accepted in final form 18 December 1996.

REFERENCES


