J Comp Physiol A (1999) 185: 479–491

ORIGINAL PAPER

J. C. Hall

GABAergic inhibition shapes frequency tuning and modifies response properties in the auditory midbrain of the leopard frog

Accepted: 25 August 1999

Abstract The functional role of GABAergic inhibition in shaping the frequency tuning of 96 neurons in the torus semicircularis of the leopard frog, *Rana pipiens*, was studied using microiontophoresis of the $GABA_A$ receptor antagonist, bicuculline methiodide. Bicuculline application abolished, or reduced in size, the inhibitory tuning curves of 72 neurons. In each case, there was a concommitant broadening of the excitatory tuning curve such that frequency-intensity combinations that were inhibitory under control conditions, became excitatory during disinhibition with bicuculline methiodide. These effects were observed irrespective of the excitatory tuning curve configuration prior to bicuculline methiodide application. Results indicate an important role for GABA-mediated inhibition in shaping the frequency selectivity of neurons in the torus semicircularis of the leopard frog. Bicuculline application also affected several other response properties of neurons in the leopard frog torus. Disinhibition with bicuculline methiodide increased both the spontaneous firing rate (18 cells) and stimulus-evoked discharge rate (81 cells) of torus neurons, decreased the minimum excitatory threshold for 18 cells, and altered the temporal discharge pattern of 47 neurons. Additional roles for GABAergic inhibition in monaural signal analysis are discussed.

Key words Iontophoresis · Inferior colliculus · Amphibian · *Rana pipiens* · Bicuculline · GABA

Abbreviations *BIC* bicuculline methiodide · *CF* characteristic frequency · *DNLL* dorsal nucleus of the lateral lemniscus · *eFTC* excitatory frequency tuning curve · *GABA* γ-amino butyric acid · *IC* inferior colliculus · *iFTC* inhibitory frequency tuning curve · *PB* phasic burst · *PL-1* primary-like 1 · *PL-2* primary-like 2 · *PL-3* primary-like 3 · *PSTH* post-stimulus time histogram · *SPL* sound pressure level · *SRN* superficial reticular nucleus

J.C. Hall
University of Tennessee, Knoxville,
Department of Biochemistry and Cellular and Molecular Biology,
M 407 Walters Life Sciences Building,
Knoxville, TN 37996-0840, USA
e-mail: JHALL1@utk.edu, Fax: +1-423-974-0978

Introduction

A crucial role for GABAergic inhibition in information processing has been demonstrated for a number of sensory systems including vision (Crook and Eysel 1992; Sillito 1975, 1977), the somatosensory system (Dykes et al. 1984; Alloway et al. 1988, 1989; Alloway and Burton 1991), gustation (Smith and Li 1998), and the electrosensory system of gymnotiform fish (Shumway and Maler 1989). In the vertebrate auditory system, GABAergic mechanisms have been implicated in creating response selectivity for species-specific vocalizations, regulating response magnitude and latency, as well as shaping selectivity for sound level, duration, frequency, and interaural time and intensity differences (Muller and Scheich 1987, 1988; Faingold et al. 1991; Pollak et al. 1992; Vater et al. 1992; Yang et al. 1992; Pollak and Park 1993; Casseday et al. 1994; Park and Pollak 1994; Fuzessery and Hall 1996). However, information regarding GABAergic inhibition and its role in sound analysis is derived almost exclusively from studies of the mammalian auditory system. There is a paucity of information concerning GABAergic mechanisms and their function in the auditory system of non-mammalian vertebrate species. Indeed, relevant comparative data is derived from studies of just two avian species, barn owls (Fujita and Konishi 1991) and chickens (Muller and Scheich 1987, 1988). The present study addresses this problem by focusing on GABA-mediated inhibition and its role of in the analysis of acoustic signals at the level of the torus semicircularis of the leopard frog, Rana pipiens. Anuran amphibians, such as the leopard frog, are considered to be intermediate between fishes and amniote vertebrates and are among the first tetrapods to communicate acoustically. Hence, the frog auditory system serves as an

attractive model system for uncovering basic information about the evolution, structure, and function of the vertebrate auditory system.

The torus semicircularis of the leopard frog is the homolog of the mammalian inferior colliculus (Wilczynski 1988). It occupies a strategic position in the ascending auditory pathway, receiving input from multiple auditory centers in the lower brainstem (Wilczynski 1988). Single unit recordings in the torus have shown that excitatory tuning curves are flanked by lateral inhibitory sidebands (Fuzessery and Feng 1982). It has been hypothesized that these sidebands serve to shape the frequency selectivity of neurons in the torus including those that are highly selective for the bimodal spectrum of the species advertisement call (Fuzessery and Feng 1982; Fuzessery 1988).

Immunocytochemical data have revealed an extensive network of GABA-immunoreactive puncta in the torus (Hall 1993). These findings have led to the hypothesis that GABA mediates, at least in part, lateral inhibition at the level of the torus. The present study tests this hypothesis. Additionally, the role of lateral inhibition in shaping the frequency selectivity of single neurons in the torus is evaluated. Finally, the functional role of GABA in shaping other monaural response properties, including temporal discharge pattern and rate-level function, is examined. While GABA likely contributes to binaural processing, as has been shown in mammals, its role in this context is beyond the scope of the present study.

Findings of this study not only contribute to our understanding of the functional relevance of GABAergic mechanisms in the vertebrate auditory system, but also provide insight into those mechanisms that represent generalized solutions to common problems faced by all animals that communicate acoustically, as well as those that are specialized to meet the specific needs organisms communicating in a unique acoustical context. Parts of this study have been presented in abstract form (Hall 1993).

Materials and methods

Adult (~6.5–7.5 cm snout-vent) female leopard frogs, *R. pipiens*, were purchased from Kons Scientific. Animals were housed in Plexiglas terraria, fed crickets three times per week, and maintained on a 14 h:10 h light:dark cycle.

Surgical procedures

Frogs were anesthetized by immersion in 0.1% tricaine methanesulfonate (MS-222; Sigma). A dorsal surgical approach was used to expose the dura directly over the torus semicircularis. The frog was then allowed to recover overnight. The next day, the frog was anesthetized by hypothermia (Kaplan 1969) and the meningial membranes removed. The animal was then immobilized (*d*-tubocurarine chloride, 10 mg g^{-1} body weight), wrapped in moist gauze to aid cutaneous respiration, placed on a Styrofoam board in an audiometric chamber (IAC), and allowed to warm to room temperature (21–23 °C). Wound margins were swabbed repeatedly with 2% lidocaine during surgery and throughout the course of the experiment. Guidelines required by the Institutional Animal Care and Use Committee (assurance no. A366801, University Of Tennessee, Knoxville) were followed.

Stimulus presentation

Stimuli were generated using a combination of Tucker-Davis Technologies System II hardware and AP1/AP2 DSP operating system software. Custom software was used to control various signal parameters as well as data acquisition and display. Digitally generated waveforms were amplifed with a stereo amplifier and presented as closed-field, monaural stimuli through a Beyer DT-48 earphone enclosed in a brass housing filled with steel wool. The frequency response of the system was essentially flat (± 3 dB) from 100 Hz to 5 000 Hz, as measured with a Bruel and Kjaer 4134 condenser microphone. Monaural stimulation in frogs can be problematic due to acoustic coupling of the two ears via the closed mouth cavity. To ensure monaural stimulation, the mouth cavity was propped open effectively uncoupling the two ears (Eggermont 1988).

Recording procedures

Piggyback multibarrel microelectrodes (Havey and Caspary 1980) were used for single-unit recording and iontophoresis. Single-barrel micropipettes were pulled to give impedances of 10–30 MΩ when filled with 1 mol l^{-1} NaCl. A five-barrel, H-configuration micropipette was pulled and the tip broken back to a diameter of 10–15 μm. The single-barrel micropipette, used to record single-unit activity, was positioned at an angle of ~20° to the five-barrel micropipette and glued in place with cyanoacrylate such that its tip extended ~10 μm beyond that of the multibarrel micropipette. The central barrel of the multibarrel micropipette was filled with 1 mol·l^{-1} NaCl (pH 7.4) and served to balance iontophoretic currents. The remaining barrels were filled with GABA (500 mmol·l^{-1}, pH 3.0) or bicuculline methiodide (BIC; 10 mmol·l^{-1}, pH 3.0); BIC is a specific antagonist of $GABA_A$ receptors. The recording barrel was connected to a Dagan AC amplifier (model 2400) via a chlorided silver wire. Drug and balancing barrels were connected via chlorided silver wire to a Medical Systems Neurophore BH-2 used to generate and monitor iontophoretic currents. Positive currents of 5–60 nA were used for iontophoresis while negative currents of 15 nA were used to prevent leakage from the drug barrels. Data are presented only for those experiments in which the resistance of the drug barrels remained constant during iontophoresis.

Data collection

Search stimuli consisted of individual tones, tone combinations, or noise. Stimulus duration was typcially 200 ms (rise and fall time = 5 ms) though this was varied as some neurons in the torus semicircularis have been shown to respond selectivity to this stimulus feature (Gooler and Feng 1992). Stimuli were presented at a rate of one every 3–4 s.

Upon isolating the activity of a single neuron its level of spontaneous activity was measured for a 60-s stimulus-free period. Next, its minimum excitatory threshold (defined as that pressure level evoking $\geq$1 spike in response to each of five consecutive stimuli) to tones was determined. If the cell responded to tones, its characteristic frequency (CF) and excitatory frequency tuning curve (eFTC) was determined. Here, CF is defined as that frequency requiring the lowest sound pressure level to elicit a response to five consecutive stimuli.

Next, a two-tone inhibiton paradigm (Fuzessery and Feng 1982) was used to map the cell's inhibitory frequency tuning curve. Briefly, one tone was held constant at the cell's CF, 10 dB SPL above threshold, while a second tone was varied in intensity and frequency. The inhibitory frequency tuning curve (iFTC) was determined using total suppression of response to each of five consecutive stimulus presentations as criterion for inhibitory threshold. The two-tone inhibition paradigm was employed as neurons in the torus semicircularis typically exhibit very low rates of spontaneous firing. Unless noted otherwise, the flanks of both the eFTC(s) and

iFTC(s) of each cell was mapped at 10 dB SPL intervals over a frequency range of 100–2400 Hz.

Once the eFTC and iFTC had been mapped, the rate-intensity function was computed from post-stimulus time histograms (PSTHs) constructed (binwidth = 1 ms) from single-unit responses to 25 consecutive stimulus presentations, each of which consisted of a 200- to 300-ms tone burst (rise and fall time = 5 ms) presented at CF and at signal levels ranging from threshold to at least 50 dB SPL above.

Each of the procedures described above was then repeated while BIC or GABA was applied using a low (5–10 nA) iontophoretic current. At this point in time, the spontaneous firing rate, excitatory threshold at CF, eFTC, iFTC, and spike count function were collected in order to identify the specific effects of GABA-mediated inhibition. Dose-dependent changes in the cell's response properties were determined by increasing incrementally iontophoretic currents and reacquiring data. Iontophoresis was then halted and the neuron allowed to recover to its pre-drug condition.

To control for possible artifacts due to the level of iontophoretic current employed, a 100-nA depolarizing current was passed through the balance barrel while recording the sound-evoked activity of the neuron under study. A change in neuronal response properties due to the delivery of this current alone was never observed. Likewise, iontophoretic application of drug delivery vehicle alone never resulted in a change in the sound-evoked activity of neurons in the torus semicircularis indicating an absence of artifact due to pH.

Results

Sound-evoked activity was recorded from a total of 136 single neurons in 27 leopard frogs. The spontaneous firing rate, excitatory threshold at CF, eFTC, iFTC, and spike count function was acquired before and during BIC iontophoresis for 96 of these cells. BIC application had no effect on the responses of 15 cells, even at the highest level of iontophoretic current used. However, for 81 cells, the iontophoretic application of BIC did alter one or more response properties as reported below.

Frequency tuning curves in the torus semicircularis

Neurons in the torus semicircularis of leopard frogs were classified on the basis of the configuration of their eFTC as described in an earlier study by Fuzessery and Feng (1982). Six distinct neuronal classes were recognized. The eFTCs of *Class 1* (n = 36/96) neurons were symmetrical, typically having a rather uniform V shape (e.g., Fig. 1A, C, E). *Class 2* neurons (n = 18/96), on the other hand, had asymmetrical eFTCs with the high- or low-frequency flank being very steep relative to that of the opposite side (e.g., Fig. 2A, C, E). Fuzessery and Feng (1982) distinguished between neurons having asymmetrical (i.e., Class 2 cells) and sharply asymmetical eFTCs. Neurons of the latter group, designated as *Class 3*, exhibited eFTCs having a low-frequency flank that recurved towards higher frequencies. In the present study, neurons having sharply asymmetrical eFTCs were observed during, but not prior to, BIC application. The eFTCs of *Class 4* neurons (n = 18/96) had almost vertical high- and low-frequency flanks (e.g., Fig. 3A, C, E) while those of *Class 5* cells (n = 15/96) were unique in having closed eFTCs. That is, there was an upper threshold (typically 30–60 dB above CF threshold) with respect to their excitatory responses (e.g., Fig. 4A, C, E). Finally, *Class 6* neurons (n = 9/96) exhibited threshold minima at two different frequencies giving rise to bimodal eFTCs. In a few cases (n = 3/9) the excitatory frequency range was continuous giving the tuning curve a W-shaped appearance (Fig. 5A). In others (n = 6/9), there were two discontinuous excitatory frequency ranges (Fig. 5C, E). Tuning curves of the latter type have been considered to perform a logical "OR" function with respect to frequency filtering (Fuzessery and Feng 1982; Fuzessery 1988).

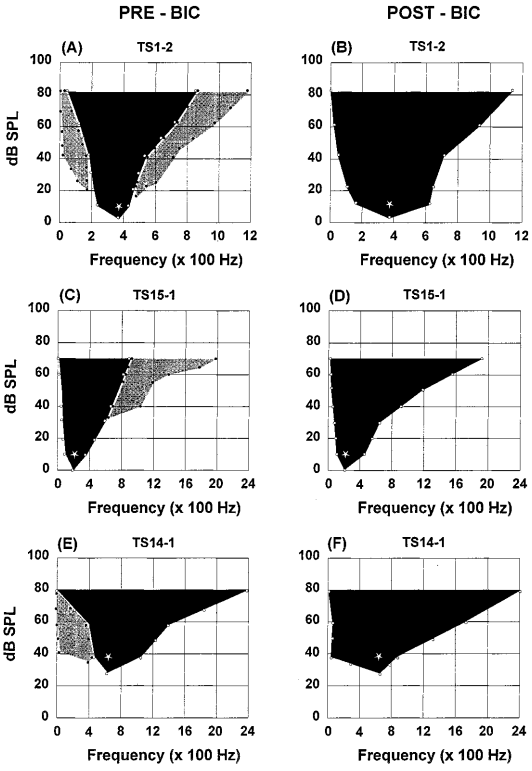

Fig. 1A–F Excitatory (*black areas*) and inhibitory (*gray areas*) frequency tuning curves of three class 1 neurons before and during bicuculline methiodide (BIC) application. *White stars* in the excitatory curves: frequency and intensity of the fixed excitatory tone when a second tone was varied to measure inhibitory tuning curves. Gaps between the excitatory and inhibitory tuning curves do not indicate regions that are not inhibitory, but rather that the inhibitory tuning curves were not mapped with sufficient precision at these points to provide conclusive evidence

Effects of BIC on the frequency tuning curves of neurons in the torus semicircularis

Class 1 neurons

Utilizing the two-tone inhibition paradigm, inhibitory areas (or iFTCs) were mapped for all 36 of the Class 1 neurons sampled. As illustrated in Fig. 1A, C, E,

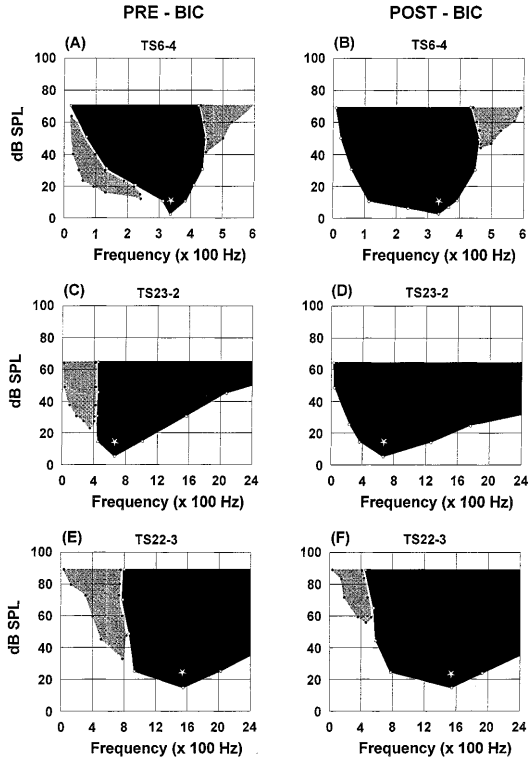

Fig. 2A–F Excitatory (*black areas*) and inhibitory (*gray areas*) frequency tuning curves of three Class two neurons before and during BIC application. See legend to Fig. 1 for additional information

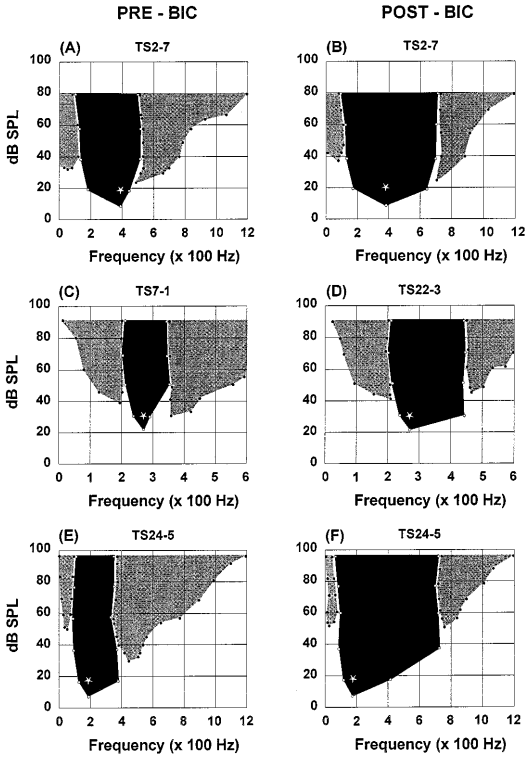

Fig. 3A–F Excitatory (*black areas*) and inhibitory (*gray areas*) frequency tuning curves of three class 4 neurons before and during BIC application. See legend to Fig. 1 for additional information

inhibitory areas were located along the low- and/or high-frequency flanks of the eFTC. It should be noted that, unlike other neurons in the torus, Class 1 neurons did not exhibit *total* two-tone inhibition. Rather, their maximum response to a CF tone was typically reduced by 85–90% during the addition of a second, inhibitory tone. Hence, for these cells, the mapped iFTCs did not represent areas giving rise to total two-tone inhibition, but frequency-intensity combinations that produced *maximal* inhibition instead.

During the iontophoretic application of BIC, inhibitory areas shown by Class 1 cells were either not affected ($n = 18$), or disappeared altogether ($n = 18$). In the latter case, frequency-intensity combinations comprising the inhibitory areas became excitatory. As a result, the eFTCs of these cells were broader while BIC was being applied than under control conditions.

The direction in which the eFTCs of Class 1 neurons broadened was dependent upon the position of their respective inhibitory areas prior to BIC application. For example, neurons whose eFTCs broadened towards lower frequencies ($n = 6$) always had a single inhibitory area adjacent to the low-frequency flank of the eFTC before BIC was applied (Fig. 1E, F). Conversely, cells whose eFTCs broadened towards higher frequencies ($n = 3$) always had a single inhibitory area adjacent to the high-frequency flank of the eFTC prior to BIC application (Fig. 1C, D). Regardless of the direction of broadening, the eFTCs of these cells always underwent a

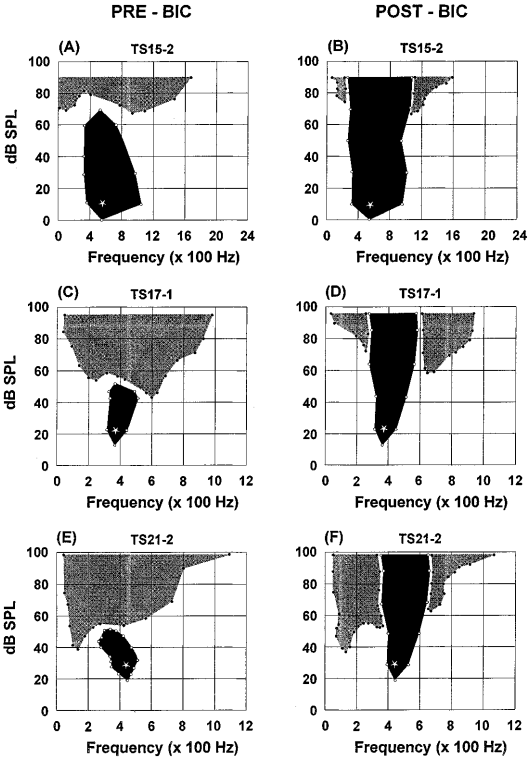

Fig. 4A–F Excitatory (*black areas*) and inhibitory (*gray areas*) frequency tuning curves of three class 5 neurons before and during BIC application. See legend to Fig. 1 for additional information

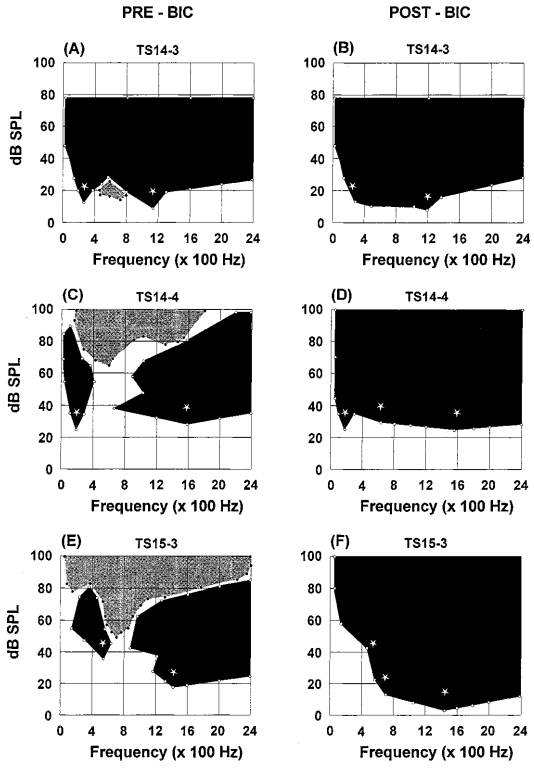

Fig. 5A–F Excitatory (*black areas*) and inhibitory (*gray areas*) frequency tuning curves of three class 6 neurons before and during BIC application. See legend to Fig. 1 for additional information

change in configuration; they were transformed from symmetrical to asymmetrical (i.e., Class 2). In contrast, cells having inhibitory areas adjacent to both the low- and high-frequency flanks of the eFTC ($n = 9$) did not undergo a change in configuration, as they broadened almost equally towards both higher and lower frequencies (Fig. 1A, B).

Class 2 neurons

Class 2 cells having eFTCs with steeply sloped high-frequency flanks ($n = 3$) had inhibitory areas adjacent to both the high- and low-frequency flank of the eFTC (Fig. 2A). In addition, the tip of the high-frequency inhibitory area was 20–25 dB SPL above its low-frequency counterpart. Iontophoretic application of BIC had very little, if any, effect on the high-frequency inhibitory area, but totally abolished that found adjacent to the low-frequency flank of the eFTC (Fig. 2B). Concomitantly, the eFTC of these cells broadened, but only towards lower frequencies. In so doing, the slope of the low-frequency flank of the eFTC also increased, becoming much more like that of the high-frequency flank. Thus, blocking GABAergic inhibition with BIC caused a transformation in the eFTCs of these cells such that they more closely resembled those exhibited by their Class 1 counterparts; i.e., the eFTC became more symmetrical.

Several ($n = 15$) of the Class 2 cells had a steeply sloped low-frequency flank (Fig. 2C, E). They differed in many respects from those having a steep high-frequency flank in that (1) they were more broadly tuned, with eFTCs extending well above 2400 Hz; (2) they had only one inhibitory area, that along the low-frequency flank of the eFTC; and (3) in 12 cases the low-frequency inhibitory area was either abolished by BIC application or reduced in size; BIC application had no effect on the eFTCs of the 3 remaining cells. The cell shown in Figs. 2C and 2D, for example, illustrates the broadening of the eFTC following total removal of the low-freqeuncy inhibitory area during BIC application. The cell depicted in Figs. 2E and 2F, on the other hand, illustrates how BIC application only reduced, but did not abolish, the inhibitory area along the low-frequency flank of the eFTC. Nonetheless, the eFTC still expanded into lower frequencies, though the broadening was less than that seen during the total removal of the adjacent inhibitory area.

Class 4 neurons

All ($n = 18$) of the Class 4, or level-tolerant, neurons had inhibitory areas adjacent to both the low- and high-frequency flanks of their eFTCs (Fig. 3). During BIC application, 15 of these cells expressed similar changes with respect to their frequency tuning: (1) the high-frequency inhibitory area was reduced in size, but never abolished; (2) there was little, if any, change in the low-frequency inhibitory area; and (3) the eFTC broadened towards higher, but not lower, frequencies. The latter is consistent with the observed reduction in the size of the high-frequency inhibitory area during BIC application. BIC application had no effect on the 3 remaining cells.

Class 5 neurons

Neurons with closed eFTCs (Class 5) showed high intensity inhibition that was partially eliminated when GABAergic input was blocked (Fig. 4). In every case ($n = 15$), the closed eFTCs were converted into a vertical, or level-tolerant, configuration characteristic of Class 4 neurons. Transformed eFTCs continued to be flanked by both high- and low-frequency inhibitory areas, another characteristic of Class 4 neurons. Indeed, for these cells, BIC application appeared to maximally influence inhibitory input only at frequencies near CF.

Class 5 neurons always exhibited non-monotonic rate-intensity functions (e.g., Fig. 8A). That is, their spike output increased with increasing signal level (for CF stimuli) up to some maximal value beyond which, a further increase led to a decline in the response. Blocking GABAergic input to these cells not only transformed the eFTC, but converted the non-monotonic spike-count function into a monotonic one. This is consistent with

the removal of high-intensity inhibition during BIC application and was shown by all Class 5 neurons examined.

Class 6 neurons

Inhibitory areas for neurons having bimodal eFTCs differed depending upon whether the eFTC was W shaped or discontinuous. Neurons having W shaped eFTCs ($n = 3$) had a single inhibitory area located in the "notch" of the W (Fig. 5A). This inhibitory area was typically small (only 200–400 Hz at its widest) and limited to intensities falling in the range of 5–25 dB SPL above the lowest excitatory threshold. Blocking GABAergic inhibition with BIC totally eliminated the inhibitory area as well as the notch of the W-shaped eFTC (Fig. 5B). That is, frequency-intensity combinations that were inhibitory under control conditions, became exciatatory when GABAergic inhibition was blocked.

Neurons having two discontinuous excitatory frequency ($n = 6$) areas also had only one inhibitory area (Fig. 5C, E). However, this inhibitory area was present only at high intensities (at least 30 dB SPL above the minimum excitatory threshold) and was extensive, typically exceeding 1200 Hz at its widest point. In every case, the tip portion of the inhibitory area was located at frequencies between the two excitatory regions of the neuron. Application of BIC always resulted in the total elimination of the inhibitory area producing an eFTC that almost spanned the entire audible range of the leopard frog. Moreover, the spike-count functions, which were non-monotonic for each of the excitatory areas, became monotonic when GABAergic inhibition was blocked (e.g., Fig. 9B).

Effects of BIC on other neuronal response properties in the torus semicircularis

Spontaneous firing rate

The spontaneous firing rate of 18 neurons was increased by 5–18 spikes s^{-1} (mean = 10.8 ± 5.27 spikes s^{-1}) while blocking GABAergic inhibition with BIC. There was no apparent relationship between BIC-induced changes in spontaneous firing rate and its effect on other neuronal response properties.

Threshold

For 18 neurons, the minimum excitatory response threshold at CF was lowered by 5–20 dB SPL (mean = 10.8 ± 5.37 dB SPL). There was no apparent relationship between a BIC-induced decrease in threshold and the eFTC configuration, presence of inhibitory sidebands, or the modification of either by BIC. For example, the neuron illustrated in Fig. 6A, B showed no inhibitory areas prior to, or during, BIC application. Yet, when GABAergic inhibition was blocked with BIC, there was a pronounced 17-dB SPL drop in the cell's excitatory response threshold at CF (955 Hz). BIC application also reduced the excitatory response threshold of cells having either one (Fig. 6C, D) or two (Fig. 6E, F) inhibitory areas flanking the eFTC. In the former, but not latter, case BIC application also abolished two-tone inhibition and broadened the eFTC.

Temporal discharge pattern

The predominant discharge patterns observed in this study included phasic ($n = 19$), phasic-burst (PB; $n = 23$), and tonic ($n = 54$). Phasic responses consisted of one or two spikes immediately following stimulus onset. PB responses consisted of three to six closely grouped spikes immediately following stimulus onset. Units having tonic responses produced action potentials throughout the duration of the stimulus. Tonic discharge patterns were of two types, primary-like 1 (PL-1) and primary-like 2 (PL-2), distinguishable on the basis of their average firing rates (Hall and Feng 1990; Gooler and Feng 1992). The mean firing rates for PL-1 and PL-2 cells were 10.8 ± 4.33 spikes s^{-1} ($n = 30$) and 34.1 ± 7.76 spikes s^{-1} ($n = 24$), respectively. Neurons with primary-like 3 (PL-3) discharge patterns, as seen at

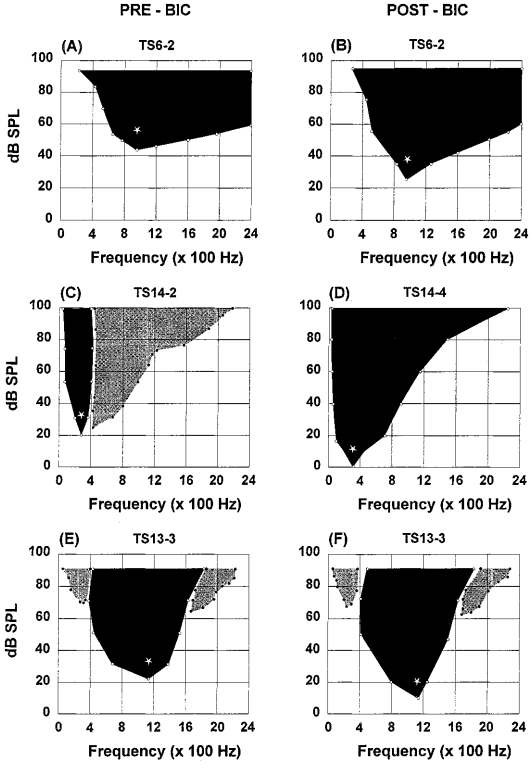

Fig. 6A–F Application of BIC frequently caused a decrease in the minimum excitatory threshold shown by neurons in the torus semicircularis. See legend to Fig. 1 for additional information

the level of the dorsolateral nucleus (Hall and Feng 1990), were not observed in the torus prior to BIC application.

The most commonly observed effect of BIC on neuronal discharge pattern was a transformation of PL-1 into PL-2 (Fig. 7A) or PL-3 (Fig. 7B), and PL-2 into PL-3 (Fig. 7C), discharge patterns. Indeed, the firing rate of the 30 cells having PL-1 discharge patterns was increased by BIC application resulting in their reclassification as PL-2 ($n = 18$) or PL-3 ($n = 12$) firing patterns. Cells of the latter type typically had a mean firing rate $\geq$80 spikes s^{-1} (see also Hall and Feng 1990). Of the cells having PL-2 discharge patterns, two-thirds ($n = 16$) showed PL-3 discharge patterns when GABAergic inhibition was blocked with BIC.

While all 23 of the neurons having PB discharge patterns showed substantial increases in firing rate when BIC was applied, the discharge pattern of only 1 underwent a transformation; specifically, it was changed from a PB to a PL-3 pattern (Fig. 7D). Application of BIC had no effect on the discharge pattern of the 19 phasic neurons, though all of these cells showed an increased spike count in response to tonal stimuli.

Discharge rate

The response property most frequently influenced by BIC application was discharge rate. Indeed, of the 96 neurons examined 81 showed a two- to sixfold increase in discharge rate to tonal stimuli presented at suprathreshold levels when GABAergic inhibition was blocked with BIC. This effect is readily apparent when comparing rate-intensity functions before and during BIC application.

In addition to converting the rate-intensity functions of Class 5 neurons from non-monotonic to monotonic as detailed above (Fig. 8A, B), BIC application also altered rate-intensity functions that were monotonic under control conditions. For example, the rate-intensity functions of 22 cells shifted to the left and upwards during BIC application (e.g. Fig. 8C) with maximal BIC effects occuring at SPLs within 20–40 dB of threshold. For the remaining cells, the maximum effect of BIC application on discharge rate was seen at higher signal levels, typically >40 dB SPL above threshold. Among this latter group however, the SPL at which BIC effects were first observed clearly varied. For example, in 16 cases, the effects of BIC application on discharge rate were readily apparent within 3–5 dB SPL of threshold (e.g. Fig. 8D). For 17 cells, the effects of BIC on discharge rate were not observed until signal levels were 13–20 dB SPL above threshold (e.g., Fig. 8E). Finally, for 17 neurons, BIC application had little, if any, effect on discharge rate at signal levels <30 dB SPL above threshold (e.g. Fig. 8F). Hence, the effective threshold of inhibitory input to these cells differed.

Discussion

The primary goal of the present study was to determine whether GABAergic inhibition is involved in shaping the frequency tuning of auditory neurons in the torus semicircularis of the leopard frog. Two questions were addressed; specifically, are inhibitory tuning curves mediated by GABAergic mechanisms at the level of the torus, and do these inhibitory areas sculpt the excitatory frequency tuning of torus neurons? Towards this end, the excitatory and inhibitory tuning curves of single neurons in the torus were measured before and during

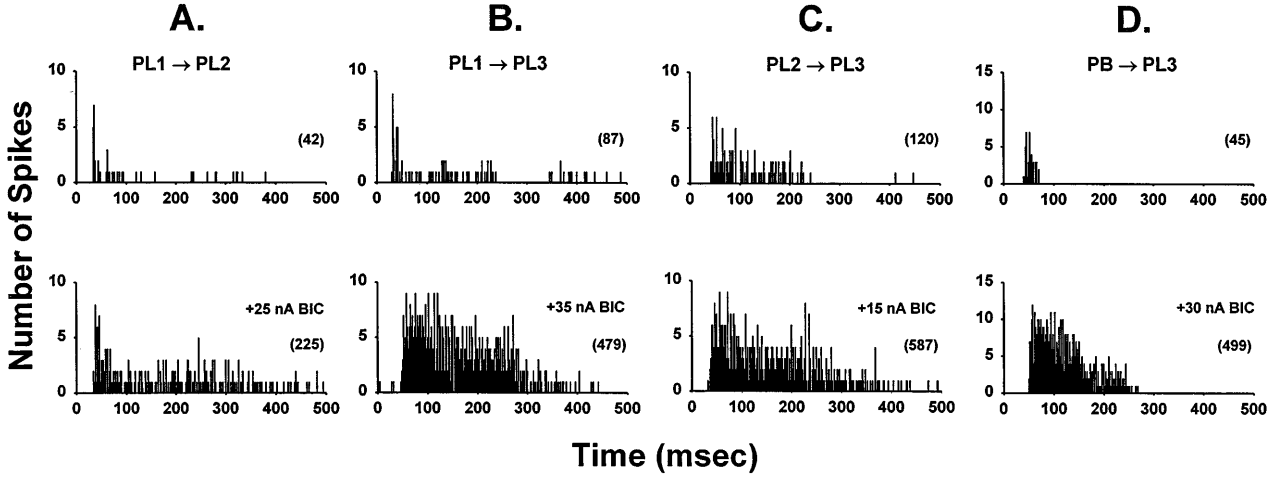

Fig. 7A–D Commonly observed effects of BIC on the temporal discharge patterns and discharge rates of single neurons in the torus semicircularis. For each cell, the top histogram represents the response pattern under control conditions, the bottom histogram, during the iontophoretic application of BIC (current parameters for iontophoresis as indicated). **A** Primary-like 1 (PL-1), control; primary-like 2 (PL-2), BIC. **B** Primary-like 1, control; primary-like 3 (PL-3), BIC. **C** PL-2, control; PL-3, BIC. **D** Phasic burst (PB), control; PL-3, BIC. In each case, there was a substantial increase in the number of spikes (in parentheses) elicited by an acoustic stimulus during BIC application relative to control conditions. Histograms compiled over 25 stimulus presentations, 1-ms bin-width. Stimuli were 300-ms duration tones (5-ms rise and fall) presented at each unit's characteristic frequency, 30 dB above the minimum excitatory threshold

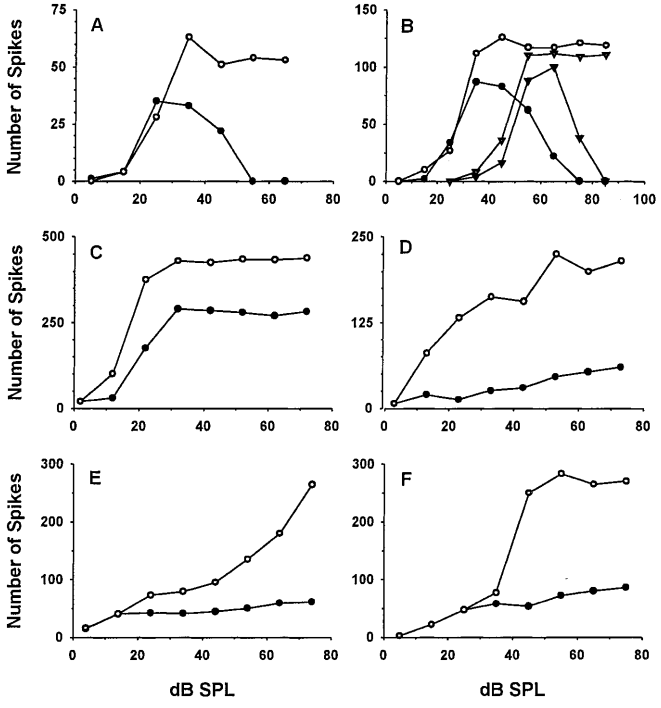

Fig. 8A–F Typical effects of BIC application on the rate intensity functions of single neurons in the torus semicircularis. The Y-axis refers to the total number of spikes in response to 25 consecutive stimulus presentations. Peaked rate-intensity functions (control), such as those shown for class 5 (**A**) and class 6 (**B**) neurons were converted to plateaued functions during BIC application. The class 6 neuron shown here had separate low- and high-frequency excitatory tuning curves with a characteristic frequency of 223 Hz and 1600 Hz, respectively. Panels **C–F** show the typical effects of BIC application on monotonic (control) rate-intensity functions (see text for details). Control rate-intensity functions are represented by *solid circles*. *Open circles* demarcate rate-intensity functions acquired during the iontophoretic application of BIC

the blockade of $GABA_A$ receptors with bicuculline. The underlying rationale was that blocking $GABA_A$ receptors with BIC should alter the configuration of inhibitory tuning curves if they are indeed mediated wholly, or in part, by GABAergic inhibition. Similarly, if the excitatory frequency tuning of neurons in the torus is shaped by the frequency tuning of their inhibitory input, then removal of that inhibition should be permissive, enabling the cell to respond to frequency-intensity combinations that were formerly inhibitory.

GABA mediates inhibitory frequency tuning

Results of this study provide direct evidence that inhibitory frequency tuning is mediated, at least in part, by GABAergic inhibition at the level of the leopard frog's torus semicircularis. Indeed, the iFTCs of 75% of the neurons examined were either abolished or reduced in size when GABAergic inhibition was blocked with BIC. Two other studies, one on the inferior colliculus (IC) of the pallid bat, *Antrozous p. pallidus* (Fuzessery and Hall 1996), the other on the medial geniculate of the mustached bat, *Pteronotus p. parnellii* (Suga et al. 1997), evaluated iFTCs both before, and during, disinhibition with BIC. These studies report results similar to those presented here, suggesting that GABAergic inhibition is a common mechanism mediating inhibitory frequency tuning in the vertebrate central auditory system.

Results of the present study permit an assessment of hypotheses concerning the actual frequency tuning of GABAergic input onto neurons in the torus. At present, there are two predominant hypotheses. The first, which shall be called "off-CF" inhibition, considers the inhibitory input to be tuned to frequencies higher and/or lower than that of the excitatory input (Fig. 9, right side); i.e., there is discontinuity between the best inhibitory frequencies and CF. The second, which shall be called "near-CF" inhibition (Palombi and Caspary 1996), states that the frequency tuning of the inhibitory input "matches" that of the excitatory input with the exception of being somewhat more broad (Fig. 9, left side). Off-CF inhibition was favored by Yang et al. (1992) as a means of explaining the creation of the different excitatory frequency tuning curve configurations shown by cells in the IC of the mustached bat. In contrast, models incorporating near-CF inhibition were presented by Palombi and Caspary (1996) to describe the shaping of the excitatory response area of neurons in the IC of the chinchilla. However, neither of these studies examined directly the frequency tuning of inhibition as was performed in the present study.

Several lines of evidence support the concept of near-CF inhibition in the torus semicircularis of leopard frogs. First, inhibitory best frequencies always occurred close to the flanks of the excitatory tuning curve such that there was no discontinuity with respect to CF. Indeed, if one extrapolated the inhibitory tuning curves to

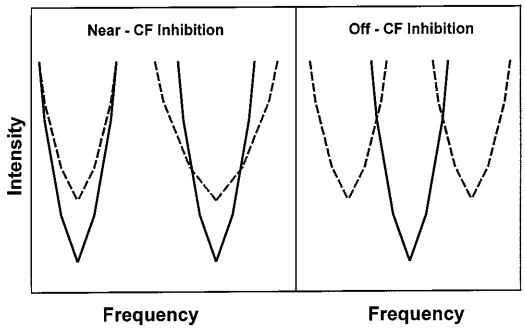

Fig. 9 Theoretical tuning curves depicting three possible relationships between the frequency tuning of excitatory (*solid lines*) and inhibitory (*dashed lines*) inputs to neurons in the torus semicircularis. *Near-CF Inhibition*: inhibitory and excitatory inputs are in tontopic alignment, though the frequency tuning curve of the inhibitory input may (*right*) or may not (*left*) be broader than that of the excitatory input. In either case, disinhibition with bicuculline would lead to an increase in discharge rate at CF and broader excitatory tuning (*right*). These were the most commonly observed effects. *Off-CF Inhibition*: inhibitory tuning curves flank the excitatory tuning curve. In this case, disinhibition with bicuculline would produce broader excitatory tuning, but no change in discharge rate. Modified from Palombi and Caspary 1996

lower intensities, the best inhibitory frequency would be at, or near CF for all of the neurons studied. Second, when neurons having closed tuning curves were disinhibited with BIC the major effect occurred near CF; specifically, the excitatory tuning curve extended through the center of the inhibitory curve at higher intensities. Again, there was no discontinuity between the best inhibitory frequencies and CF. Finally, all of the 81 neurons affected by the iontophoretic application of BIC showed an increase in discharge rate at CF. This finding is inconsistent with off-CF inhibition, except at very high signal levels where two disparate inhibitory tuning curves might overlap. It is, on the other hand, what one would expect if the tuning of both the inhibitory and excitatory input were in register. Similar results have been seen in the IC of the pallid bat (Fuzessery and Hall 1996) and the medial geniculate of the mustached bat (Suga et al. 1997). Taken together, these data suggest that GABAergic inhibiton generally arises from neurons with excitatory receptive fields in frequency alignment with their targets in the auditory midbrain.

For several neurons, some vestige of inhibitory frequency tuning remained during BIC application. The most extreme cases are represented by those cells whose iFTCs were not altered during the iontophoretic application of BIC, even though one or more of their other neuronal response properties were affected (e.g., Fig. 7A). In other cases, such as class 2 cells having both a low- and high-frequency inhibitory domain, BIC application abolished one inhibitory region, but had no effect on the other (e.g., Fig. 2A, B). Finally, for some cells, the iFTC was reduced in size, but not abolished, when GABAergic inhibition was blocked (e.g., Fig. 3E, F). These observations may be explained by one or more of the following: (1) the observed inhibition was glycinergic rather than GABAergic; originating in lower brainstem auditory centers where glycine-labeled somata have been reported (Hall 1993); (2) inhibition was imposed upon excitatory input at lower levels of the auditory brainstem and was, therefore, not affected by BIC application in the torus; (3) the nonlinear mechanical properties of the auditory periphery (Benedix et al. 1994), which would have been impervious to BIC application, produced suppressive effects that persisted at the midbrain level; (4) application of BIC did not effectively block all of the $GABA_A$ receptors present on a given neuron. Given the extensive nature of the dendritic arbors of some torus neurons (Feng 1983) this latter possibility cannot be excluded. In spite of these caveats, evidence indicates that GABAergic inhibition does indeed participate in the creation of iFTCs in the leopard frog torus semicircularis.

GABAergic networks shape excitatory frequency tuning

Results of the present study show that the eFTCs of neurons in the leopard frog torus are shaped by the frequency tuning of their inhibitory input. Typically, frequency-intensity combinations that were inhibitory under control conditions became excitatory during disinhibition with BIC. These effects are not incongruous with the concept of near-CF inhibition and, in fact, are consistent with studies of orientation preference in the visual cortex of cats (Blakemore and Tobin 1972; Ferster 1986) demonstrating that the orientation tuning of both the excitatory and inhibitory input to neurons in area 17 were in register. In some cases the tuning of the inhibitory input overlapped that of the excitatory input, while in other cases it was broader. In the latter cases, the orientation tuning of cortical neurons was sharpened, due presumably, to the relatively greater strength of inhibitory input that effectively blocked the weaker excitatory input at non-optimal orientations. The effects of BIC application on neurons having disparate bimodal excitatory tuning curves (Fig. 5) provides a dramatic example of this phenomenon. Prior to BIC application, these cells showed two discrete excitatory receptive fields separated by an area of inhibition. During BIC application, inhibitory frequency tuning was totally abolished such that the cells became responsive over almost the entire audible range of the leopard frog. Broadening of the excitatory frequency receptive field during the blockade of GABAergic inhibition with BIC has been reported for auditory neurons in a number of species including chickens (Muller and Scheich 1988), chinchillas (Palombi and Caspary 1996), mustache bats (Yang et al. 1992; Suga et al. 1997), horseshoe bats (Vater et al. 1992), and pallid bats (Fuzessery and Hall 1996). While evaluation of the frequency tuning of inhibitory input before and during BIC application was performed in only a few of these studies, data nonetheless support a role for GABA-mediated inhibition in shaping the excitatory frequency tuning of neurons in the vertebrate auditory system.

GABAergic inhibition sharpens excitatory frequency tuning

What purpose could be served by the sculpting of the excitatory frequency receptive field by inhibition? It has been proposed that an important function of inhibition in the vertebrate auditory system is to "sharpen" the frequency tuning of central auditory neurons (Katsuki et al. 1958; Sug 1995). Results of the present study corroborate this proposal. For example, class 4 neurons showed substantially broader eFTCs during disinhibition with BIC than under control conditions (Fig. 3). Indeed, regardless of eFTC configuration, when iFTCs were reduced in size or abolished during BIC application, there was always a corresponding "broadening" of the eFTC, particularly at high levels of stimulation. These findings are consistent with those reported in studies of the IC of the pallid bat (Fuzessery and Hall 1996) and auditory thalamus of the mustached bat (Suga et al. 1997). Thus, the specialized filter capacities resulting from the mechanical properties of the auditory

periphery can be enhanced by neural mechanisms in the central auditory system.

GABAergic inhibition creates selectivity for complex acoustic features

Enhanced selectivity for pure tone frequency is but one advantage that might be offered by the neural sharpening of the eFTC. Another, and perhaps more vital, role might be the creation of selectivity for the behaviorally relevant features of sounds that are, in general, not pure tones but temporally and spectrally complex. This issue was addressed by Fuzessery and Hall (1996) in their study of the pallid bat IC, describing neurons that responded exclusively to the animal's biosonar pulse, a brief downward FM sweep used for general orientation during flight. Blocking GABAergic inhibition with BIC abolished these highly selective responses, rendering the cells sensitive to an array of stimuli including tones. Here, the primary role of GABAergic inhibition appears to be the creation of selectivity for a behaviorally relevant acoustic signal, the biosonar pulse, and not the sharpening of the frequency tuning curves per se.

Among frogs, the advertisement call often mediates male-female sexual encounters (Rand 1988). The advertisement call, which is produced only by males, provides females with temporal and/or spectral cues that facilitate species recognition (Gerhardt 1988) and may serve as a mechanism to prevent crossbreeding, the production of inviable offspring, and subsequent loss of biological fitness. Hence, like the biosonar pulse of the pallid bat, the advertisement call is a critical component of frog acoustic behavior.

Fuzessery and Feng (1983) identified putative "mating-call detectors" in the rostral torus and caudal thalamus of *R. pipiens*. These cells responded only to acoustic stimuli containing both low- and high-frequency energy matching that of the species advertisement call; neither low- nor high-frequency stimuli alone were effective in activating these neurons. Moreover, stimuli containing mid-frequency energy were inhibitory. Class 6 neurons having bimodal tuning curves with separate low- and high-frequency excitatory areas, as well as an inhibitory mid-frequency region, are believed to provide the foundation upon which putative mating-call detectors are built (Fuzessery and Feng 1983; Hall 1994). In the present study, the mid-frequency inhibitory region of class 6 neurons was abolished during the blockade of GABAergic inhibition (Fig. 5). Moreover, frequencies within this region became excitatory, such that the cells responded over most of the audible range of the leopard frog. Thus, these cells receive a very broadly tuned excitatory input that is sculpted by GABAergic inhibition to enhance selectivity for a behaviorally important acoustic signal, the advertisement call. The creation of selectivity for sound duration in the IC of the big brown bat (Casseday et al. 1994), velocity information in the cortex of the mustached bat (Suga et al. 1979; Suga and Manabe 1982; Suga and Tsuzuki 1985), and species vocalizations in the forebrain of chickens (Muller and Scheich 1987, 1988) are additional examples suggesting that a major role for GABAergic inhibition is to create, or enhance, neuronal selectivity for behaviorally relevant sounds, and not the sharpening of frequency tuning curves alone.

GABAergic inhibition functions in gain control

The acoustic communication signals produced by males of some anurans can reach 120 dB SPL at a distance of 50 cm (Narins and Zelick 1988). Moreover, these signals are typically produced in an environment where background noise levels as high as 85 dB SPL have been measured at a distance of 3.0 m from the calling animal (Narins and Zelick 1988). Hence, a mechanism that would not only change the dynamic range over which communication signals could be coded, but also enhance their detection in noise, would be of great adaptive value. Data from the present study suggest that GABAergic inhibition might provide such a mechanism in the torus of the leopard frog. First, BIC application increased the stimulus-evoked discharge rate of the majority (about 84%) of neurons examined. Second, for approximately 20% of these cells, disinhibition with BIC not only decreased substantially the excitatory response threshold but also increased each cell's dynamic range. Finally, about 36% of these cells showed a shift in dynamic range that was coupled with an increased discharge rate to suprathreshold stimuli. Thus, GABAergic inhibition provides a basis for the dynamic control of sensitivity and/or gain whereby the discharge of neurons in the torus semicircularis can be regulated to accommodate changes in stimulus intensity and/or level of background noise.

GABAergic inhibition has also been shown to regulate discharge rate and excitatory threshold in the auditory system of cats (Walsh et al. 1990), rats (Ebert and Ostwald 1995a; Ebert and Ostwald 1995b; Faingold et al. 1989, 1991), several species of bats (Vater et al. 1992; Yang et al. 1992; Pollak and Park 1993; Yang and Pollak 1994; Fuzessery and Hall 1996; Suga et al. 1997), guinea pigs (Le Beau et al. 1996), chinchillas (Caspary et al. 1994; Palombi and Caspary 1996), barn owls (Fujita and Konishi 1991) and chickens (Muller and Scheich 1988). Most, if not all, of these animals, like frogs, face the task of communicating in naturally noisy environments and may utilize GABAergic inhibition in accomplishing this task.

Additional roles for GABAergic inhibition

Temporal response pattern

Results of the present study support a role for GABAergic inhibition in shaping the temporal discharge

pattern of neurons in the torus of the leopard frog. The most common effect was the transformation of PL-1 into PL-2 or PL-3, and PL-2 into PL-3, discharge patterns. However, it should be noted that these different response categories are distinguised exclusively on the basis of differences in their mean discharge rate to tonal stimuli. Since GABAergic inhibition regulates discharge rate, it is likely that these transformations are an epiphenomenon resulting from a disruption of the gain control mechanism during BIC application, and not the disruption of a mechanism shaping neuronal discharge pattern per se. These results also emphasize that the different response categories that have been identified (i.e., PL-1, PL-2, and PL-3) may not necessarily correspond to distinct neuronal types.

The transformation of a PB into a PL-3 discharge pattern during BIC application, provides the most compelling evidence that GABAergic inhibition shapes temporal discharge pattern in the torus of the leopard frog. This effect can be explained if the PB pattern results from a tonic excitatory input whose late component is suppressed by an additional, GABAergic inhibitory input. Blocking inhibition with BIC would unmask the excitatory input giving rise to a pronounced tonic response.

The PB-like discharge patterns of neurons in the IC of guinea pigs (Le Beau et al. 1996) and horshoe bats (Vater et al. 1992) are also transformed into sustained responses during disinhibition with BIC. However, the number of cells having PB-like discharge patterns that are influenced in this manner is substantially lower in frogs, about 4%, as compared to guinea pigs and horshoe bats where approximately 75% and 38% of the cells, respectively, show comparable changes. Numerous cases of onset responses becoming sustained during BIC application are well documented in other species as well (Watanabe and Simada 1973; Faingold et al. 1989, 1991; Yang et al. 1992; Pollak and Park 1993; Park and Pollak 1994). This, coupled with findings of the present study indicating that phasic discharge patterns are unaltered by BIC application in the torus, implies a lesser role for GABAergic inhibition in the creation of temporal discharge pattern in the auditory midbrain of leopard frogs as compared to other vertebrates.

Spontaneous activity

About 19% of the units in frog torus showed an increase in spontaneous activity during BIC application. This is consistent with the increase in spontaneous firing with BIC observed in the IC of bats (Vater et al. 1992; Pollak and Park 1993). A large increase in the spontaneous firing rate of IC neurons in the rat was observed when input from the dorsal nucleus of the lateral lemniscus to the IC was blocked by microinjection of the GABA agonist 4,5,6,7-tetrahydroisoxazolo-(4.5-c)pyridin-3-ol (THIP) into the contralateral dorsal nucleus of the lateral limniscus (DNLL) (Faingold et al. 1993). It was concluded that the DNLL provides a tonic inhibitory input to the IC. In frogs, the superficial reticular nucleus (SRN) is considered to be the homolog of the mammalian lateral lemniscal nuclei (Rose and Wilczynski 1984). The SRN projects heavily to the torus (Feng and Lin 1991). In an earlier immunocytochemical study, Hall (1993) described a large population of GABAergic neurons in the SRN. Hence, GABAergic neurons in the SRN may be the source of tonic inhibitory input onto a subset of neurons in the torus semicircularis of frogs. If so, then this would be very similar to what has been observed in mammals, strengthening arguments supporting homology between the SRN and the nuclei of the lateral lemniscus.

Possible sources of GABAergic innervation in the torus semicircularis

The substrate for GABAergic inhibition in the torus of the leopard frog could include intrinsic as well as extrinsic connections. The torus of leopard frogs contains a large proportion of GABA-immunoreactive cells (Hall 1993). Whether or not these cells participate in local inhibitory interactions remains to be determined. Extrinsic sources of GABAergic innervation could arise from the superficial reticular nucleus, as discussed above, or the superior olivary nucleus. Not only do both of these auditory centers project heavily to the torus (Feng 1986; Feng and Lin 1991), but a number of GABA-immunopositive cells are found in each (Hall 1993). Whether inhibitory connections between these structures and the torus exist, is an issue yet to be resolved.

In summary, the results show that GABAergic inhibition plays a major role in shaping the response properties of auditory neurons in the torus of the leopard frog. It mediates inhibitory freqeuncy tuning and participates in the creation of response selectivity in the frequency domain. GABAergic circuits also play a crucial role in adjusting the dynamic range of neurons in the frog's auditory midbrain and hence the extraction of signals from noise. Similar findings in birds and mammals suggest that these functions are common to all animals that communicate acoustically.

Acknowledgements This work was supported in part by NSF DUE – 9751615. Experiments complied with the "Principles of animal care", publication No. 86-23, revised 1985 of the National Institute of Health an with the current laws of the United States of America in which the experiments were performed.

References

Alloway KD, Burton H (1991) Differential effects of GABA and bicuculline on rapidly- and slowly-adapting neurons in primary somatosensory cortex of primates. Exp Brain Res 85: 598–610

Alloway KD, Rosenthal P, Burton H (1989) Quantitative measurements of receptive field changes during antagonism of GABAergic transmission in primary somatosensory cortex of cats. Exp Brain Res 78: 514–532

Alloway KD, Sinclair RJ, Burton H (1988) Responses of neurons in somatosensory cortical area II of cats to high-frequency vibratory stimuli during iontophoresis of a GABA antagonist and glutamate. Somatosens Res 6: 109–140

Benedix JH, Pedemonte M, Velluti R, Narins PM (1994) Temperature dependence of two-tone rate suppression in the northern leopard frog, *Rana pipiens pipiens*. J Acoust Soc Am 96: 2738–2745

Blakemore C, Tobin EA (1972) Lateral inhibition between orientation detectors in the cat's visual cortex. Exp Brain Res 15: 439–440

Caspary DM, Backoff PM, Finlayson PG, Palombi PS (1994) Inhibitory inputs modulate discharge rate within frequency receptive fields of anteroventral cochlear nucleus neurons. J Neurophysiol 72: 2124–2133

Casseday JH, Ehrlich D, Covey E (1994) Neural tuning for sound duration: role of inhibitory mechanisms in the inferior colliculus. Science 264: 850

Crook JM, Eysel UT (1992) GABA-induced inactivation of functionally characterized sites in cat visual cortex (area 18): effects on orientation tuning. J Neurosci 12: 1816–1825

Dykes RW, Landry P, Metherate R, Hicks TP (1984) Functional role of GABA in cat primary somatosensory cortex: shaping receptive fields of cortical neurons. J Neurophysiol 52: 1066–1093

Ebert U, Ostwald J (1995a) GABA alters the discharge pattern of chopper neurons in the rat ventral cochlear nucleus. Hear Res 91: 160–166

Ebert U, Ostwald J (1995b) GABA can improve acoustic contrast in the rat ventral cochlear nucleus. Exp Brain Res 104: 310–322

Eggermont JJ (1988) Mechanisms of sound localization in anurans. In: Fritzsch B, Ryan MJ, Wilczynski W, Hetherington TE, Walkowiak W (eds) The evolution of the amphibian auditory system. Wiley, New York, pp 307–336

Faingold CL, Gehlbach G, Caspary DM (1989) On the role of GABA as an inhibitory neurotransmitter in inferior colliculus neurons: iontophoretic studies. Brain Res 500: 302–312

Faingold CL, Anderson CAB, Caspary DM (1991) Involvement of GABA in acoustically-evoked inhibition in inferior colliculus neurons. Hear Res 52: 201–216

Faingold CL, Anderson CAB, Randall ME (1993) Stimulation or blockade of the dorsal nucleus of the lateral lemniscus alters binaural and tonic inhibition in contralateral inferior colliculus neurons. Hear Res 69: 98–106

Feng AS (1983) Morphology of neurons in the torus semicircularis of the northern leopard frog, *Rana pipiens pipiens*. J Morphol 175: 253–269

Feng AS (1986) Afferent and efferent innervation patterns of the superior olivary nucleus of the leopard frog. Brain Res 364: 167–171

Feng AS, Lin W (1991) Differential innervation patterns of three divisions of frog auditory midbrain (torus semicircularis). J Comp Neurol 306: 613–630

Ferster D (1986) Orientation selectivity of synaptic potentials in neurons of cat primary visual cortex. J Neurosci 6: 1284–1301

Fujita I, Konishi M (1991) The role of GABAergic inhibition in processing of interaural time difference in the owl's auditory system. J Neurosci 11: 722–739

Fuzessery ZM (1988) Frequency tuning in the anuran central auditory system. In: Fritzsch B, Ryan MJ, Wilczynski W, Hetherington TE, Walkowiak W (eds) The evolution of the amphibian auditory system. Wiley, New York, pp 253–273

Fuzessery ZM, Feng AS (1982) Frequency selectivity in the anuran auditory midbrain: single unit responses to single and multiple tone stimulation. J Comp Physiol A 146: 471–484

Fuzessery ZM, Feng AS (1983) Mating call selectivity in the thalamus and midbrain of the leopard frog (*Rana p. pipiens*): single and multiunit analyses. J Comp Physiol A 150: 333–344

Fuzessery ZM, Hall JC (1996) Role of GABA in shaping frequency tuning and creating FM sweep selectivity in the inferior colliculus. J Neurophysiol 76: 1059–1073

Gerhardt HC (1988) Acoustic properties used in call recognition by frogs and toads. In: Fritzsch B, Ryan MJ, Wilczynski W, Hetherington TE, Walkowiak W (eds) The evolution of the amphibian auditory system. Wiley, New York, pp 455–483

Gooler DM, Feng AS (1992) Temporal coding in the frog auditory midbrain: the influence of duration and rise-fall time on the processing of complex amplitude-modulated stimuli. J Neurophysiol 67: 1–22

Hall JC (1993) The effects of GABAergic inhibition on the response properties of neurons in the leopard frog's auditory midbrain (abstract). ARO Abstracts 16

Hall JC (1994) Central processing of communication sounds in the anuran auditory system. Am Zool 34: 670–684

Hall JC, Feng AS (1990) Classification of the temporal discharge patterns of single auditory neurons in the dorsal medullary nucleus of the northern leopard frog. J Neurophysiol 64: 1460–1473

Havey DC, Caspary DM (1980) A simple technique for constructing "piggy-back" multibarrel microelectrodes. Electroencephalogr Clin Neurophysiol 48: 249–251

Kaplan HM (1969) Anesthesia in amphibians and reptiles. Proc Fed Am Soc Exp Biol 28: 1541–1546

Katsuki Y, Sumi T, Uchiyama H, Watanabe T (1958) Electric responses of auditory neurons in cat to sound stimulation. J Neurophysiol 21: 569–588

Le Beau FEN, Rees A, Malmierca MS (1996) Contribution of GABA- and glycine-mediated inhibition to the monaural temporal response properties of neurons in the inferior colliculus. J Neurophysiol 75: 902–919

Muller CM, Scheich H (1987) GABAergic inhibition increases the neuronal selectivity to natural sounds in the avian auditory forebrain. Brain Res 414: 376–380

Muller CM, Scheich H (1988) Contribution of GABAergic inhibition to the response characteristics of auditory units in the avian forebrain. J Neurophysiol 59: 1673–1689

Narins PM, Zelick R (1988) The effects of noise on auditory processing and behavior in amphibians. In: Fritzsch B, Ryan MJ, Wilczynski W, Hetherington TE, Walkowiak W (eds) The evolution of the amphibian auditory system. Wiley, New York, pp 511–536

Palombi PS, Caspary DM (1996) GABA inputs control discharge rate primarily within frequency receptive fields of inferior colliculus neurons. J Neurophysiol 75: 2211–2219

Park TJ, Pollak GD (1993) GABA shapes a topographic organization of response latency in the mustache bat's inferior colliculus. J Neurosci 13: 5172–5187

Park TJ, Pollak GD (1994) Azimuthal receptive fields are shaped by GABAergic inhibition in the inferior colliculus of the mustache bat. J Neurophysiol 72: 1080–1102

Pollak GD, Park TJ (1993) The effects of GABAergic inhibition on monaural response properties of neurons in the mustache bat's inferior colliculus. Hear Res 65: 99–117

Pollak GD, Park TJ, Larue DT, Winer JA (1992) The role inhibitory circuits play in shaping spatial receptive fields of neurons in the mustache bat's inferior colliculus. In: Singh RN (ed) Principles of design and function in nervous systems. Wiley Eastern, New Delhi, pp 271–290

Rand AS (1988) An overview of anuran acoustic communication. In: Fritzsch B, Ryan MJ, Wilczynski W, Hetherington TE, Walkowiak W (eds) The evolution of the amphibian auditory system. Wiley, New York, pp 415–431

Rose G, Wilczynski W (1984) The anuran superficial reticular nucleus: evidence for homology with nuclei of the lateral lemniscus. Brain Res 304: 170–172

Shumway CA, Maler L (1989) GABAergic inhibition shapes temporal and spatial response properties of pyramidal cells in the electrosensory lateral line lobe of gymnotiform fish. J Comp Physiol A 164: 391–407

Sillito AM (1975) The contribution of inhibitory mechanisms to the receptive field properties of neurons in the striate cortex of the cat. J Physiol (Lond) 250: 305–329

Sillito AM (1977) Inhibitory processes underlying the directional specificity of simple, complex and hypercomplex cells in cat's visual cortex. J Physiol (Lond) 271: 699–720

Smith DV, Li CS (1998) Tonic GABAergic inhibition of taste-responsive neurons in the nucleus of the solitary tract. Chem Senses 23: 159–169

Suga N (1995) Sharpening of frequency tuning by inhibition in the central auditory system: Tribute to Yusuji Katsuki. Neurosci Res 21: 287–299

Suga N, Manabe T (1982) Neural basis of amplitude-spectrum representation in the auditory cortex of the mustached bat. J Neurophysiol 47: 225–255

Suga N, Tsuzuki K (1985) Inhibition and level-tolerant frequency tuning in the auditory cortex of the mustached bat. J Neurophysiol 53: 1109–1145

Suga N, O'Neill WE, Manabe T (1979) Harmonic-sensitive neurons in the auditory cortex of the mustached bat. Science 203: 207–274

Suga N, Zhang Y, Yan J (1997) Sharpening of frequency tuning by inhibition in the thalamic auditory nucleus of the mustached bat. J Neurophysiol 77: 2098–2114

Vater M, Habicht H, Kossl M, Grothe B (1992) The functional role of GABA and glycine in monaural and binaural processing in the inferior colliculus of horseshoe bats. J Comp Physiol A 171: 541–553

Walsh EJ, McGee J, Fitzakerley JL (1990) GABA actions within the caudal cochlear nucleus of developing kittens. J Neurophysiol 64: 961–977

Watanabe T, Simada Z (1973) Pharmacological properties of cat's collicular auditory neurons. Jpn J Physiol 23: 291–308

Wilczynski W (1988) Brainstem auditory pathways in ranid frogs. In: Fritzsch B, Ryan MJ, Wilczynski W, Hetherington TE, Walkowiak W (eds) The evolution of the amphibian auditory system. Wiley, New York, pp 209–231

Yang L, Pollak GD (1994) GABA and glycine have different effects on monaural response properties in the dorsal nucleus of the lateral lemniscus of the mustache bat. J Neurophysiol 71: 2014–2024

Yang L, Pollak GD, Resler C (1992) GABAergic circuits sharpen tuning curves and modify response properties in the mustache bat inferior colliculus. J Neurophysiol 68: 1760–1774