Role of GABAergic Inhibition in the Coding of Interaural Time Differences of Low-Frequency Sounds in the Inferior Colliculus

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INTRODUCTION

The interaural time difference (ITD), created by the unequal path lengths that a sound must travel to reach each ear, is an important cue for localizing low-frequency sounds. For the rabbit, the ITD sensitivity of single neurons has been described for all major binaural nuclei: the nuclei of the superior olivary complex (SOC; Batra et al. 1997a,b), the dorsal nucleus of the lateral lemniscus (DNLL; Fitzpatrick and Kuwada 1996), the inferior colliculus (IC; Batra et al. 1993; Kuwada et al. 1987, 1989), the auditory thalamus (Stanford et al. 1992), and the primary auditory cortex (Fitzpatrick et al. 2000). From these studies, it is apparent that ITD tuning widths systematically sharpen at least to the level of the thalamus (Fitzpatrick et al. 1997).

Here, we examine the role of γ-aminobutyric acid (GABA) in the sharpening of ITD tuning curves in the IC. This role of GABA is supported in the barn owl where the GABA antagonist ejected onto neurons in the nucleus mesencephalicus lateralis dorsalis (MLD) could broaden ITD functions and shift their preferred ITD (Fujita and Konishi 1991). In the bat, GABAergic inhibition has been shown to be involved in shaping other IC responses, such as modulation tuning (Koch and Grothe 1998, 2000) and responses to interaural intensity differences (Park and Pollak 1993, 1994). We previously showed that the anesthetic sodium pentobarbital decreased the discharge rate and the width of ITD tuning curves and could shift the best ITD of IC neurons (Kuwada et al. 1989). Because it is known that barbiturates potentiate GABAergic inhibition (for review, see Ticku 1991), these experiments suggested that GABAergic inhibition played a major role in shaping ITD responses.

The IC is rich in sources of GABAergic inputs including those from the dorsal nucleus of the lateral lemniscus (DNLL) of both sides (Adams 1979; Adams and Mugnaini 1984; Beyerl 1978; Brunso-Bechtold et al. 1981; González-Hernández et al. 1996), sources intrinsic to the IC (Palombi and Caspary 1996; Yang et al. 2000), and descending sources (Winer et al. 1998). A role of these GABAergic inputs is to decrease the discharge rate of neurons in the IC (Fairgold et al. 1989, 1991; Fujita and Konishi 1991; Li and Kelly 1992). Recent experiments using iontophoresis have shown that GABAergic inputs are responsible for the complex frequency response areas in the IC (LeBeau et al. 2001) and make IC neurons less sensitive to directional changes in interaural phase modulations (McAlpine and Palmer 2002).

Previous studies investigating the effects of blocking GABA receptors have used anesthetized preparations where synaptic transmission and transmitter efficacy are potentially altered. Therefore in the present study we used an unanesthetized rabbit preparation to examine the role of GABAergic inhibition in shaping ITD sensitivity of cells in the IC. We blocked GABA receptors by iontophoresis of the GABA antagonists bicuculline methiodide (BIC) or SR-95531 (Gabazine) and in selected cases we iontophoresed the neurotransmitters GABA and glutamate. We suggest that GABAergic inhibitory inputs may also be involved in shaping ITD functions in the IC, including sharpening the function and optimizing the signal-to-noise ratio. Preliminary results were published previously in abstract form (Sterbing et al. 2002).

METHODS

Animals

Single unit recordings were made from 5 adult, female Dutch-belted rabbits (1.5–2.5 kg) with healthy external ears. All animal procedures were approved by the Committee for Animal Care and Use at the University of Connecticut Health Center and conformed to the guidelines for laboratory animal care and use published by the National Institutes for Health. Because surgical and experimental procedures were approved in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

D’Angelo, W. R., S. J. Sterbing, E.-M. Ostapoff, and S. Kuwada. Role of GABAergic inhibition in the coding of interaural time differences of low-frequency sounds in the inferior colliculus. J Neurophysiol 93: 3390–3400, 2005. First published January 12, 2005; doi:10.1152/jn.00956.2004. A major cue for the localization of sound in space is the interaural time difference (ITD). We examined the role of inhibition in the shaping of ITD responses in the inferior colliculus (IC) by iontophoretically ejecting γ-aminobutyric acid (GABA) antagonists and GABA itself using a multibarrel pipette. The GABA antagonists block inhibition, whereas the applied GABA provides a constant level of inhibition. The effects on ITD responses were evaluated before, during and after the application of the drugs. If GABA-mediated inhibition is involved in shaping ITD tuning in IC neurons, then applying additional amounts of this inhibitory transmitter should alter ITD tuning. Indeed, for almost all neurons tested, applying GABA reduced the firing rate and consequently sharpened ITD tuning. Conversely, blocking GABA-mediated inhibition increased the activity of IC neurons, often reduced the signal-to-noise ratio and often broadened ITD tuning. Blocking GABA could also alter the shape of the ITD function and shift its peak suggesting that the role of inhibition is multifaceted. These effects indicate that GABAergic inhibition at the level of the IC is important for ITD coding.
procedures have been fully described elsewhere (e.g., Kuwada et al. 1987), they will be briefly recounted.

Surgical procedures

All surgery was performed under aseptic conditions. Under anesthesia [ketamine (44 mg/kg) and xylazine (6 mg/kg), administered intramuscularly (im)], a square, brass rod was anchored to the skull using screws and dental acrylic. About 1 wk later, the animal was again anesthetized to make custom-fitted ear molds. This was done by filling the external meatus and the concha with dental impression material (Reprosil, Dentsply International, Milford, DE). In a separate procedure, a craniotomy (about 6 × 4 mm) was performed under anesthesia to allow a dorsal approach to the IC. After each experimental session, the craniotomy was covered with sterilized medical elastopolymer (Sammons-Preston Rolyan, Germantown, IL).

Recording procedures and data collection

All recordings were conducted in a double-walled, sound-insulated chamber. The unanesthetized rabbit was placed in a spandex sleeve, seated in a padded cradle, and its head secured to the stereotaxic frame by clamping to the brass head bar. The elastopolymer was removed and the topical anesthetic Marcaine was applied to the exposed dura mater so that penetration by the electrode was painless. Our recording sessions were limited to about 2 h for practical and ethical reasons and could be terminated at any time if the rabbit showed signs of discomfort. Each rabbit participated in daily recording sessions over a period of 3–6 mo.

Recording and microiontophoresis

Initial experiments used a commercial carbon-fiber recording electrode surrounded by 6 glass barrels (Carbostar-7, Kation Scientific, Minneapolis, MN). In later experiments, we fabricated our own assembly using a glass-coated, tungsten microelectrode (tip diameter about 1 μm, about 10 μm exposed; Merrill and Ainsworth 1972) attached to a 5-barrel glass pipette, i.e., a piggyback configuration (Havey and Caspary 1980). Before assembly, the 5-barrel pulled pipette was broken to create a tip diameter of about 20 μm. The tungsten tip was placed about 15 μm past the tip of the barrels. Individual barrels were backfilled with BIC (10 mM in saline, pH 3.5), GABA (500 mM in distilled water, pH 3.5), glutamate (255 mM in distilled water, pH 8), or saline (165 mM, pH 5.5). For 3 neurons, we substituted Gabazine (10 mM in saline, pH 3.5) for BIC. Gabazine and BIC are both competitive antagonists for the GABA_A receptor. Typical impedance values for the filled barrels ranged from 20 to 60 MΩ. The current source (Dagan 6400, Minneapolis, MN) provided independent control of 6 iontophoresis channels and a balancing channel. Small retaining currents (about 5 nA) were set for all barrels. For GABA and glutamate, we typically used ejection currents of about 2–20 nA, and for the GABA antagonists, about 20–40 nA. Only neurons that showed at least a 20% change in firing rate were included in this study. For our analyses, we generally took the last recording session that showed at least a 20% increase in discharge rate during iontophoresis. Iontophoresis of saline as a control at current levels as high as 100 nA did not cause a noticeable change in neural response. CoolEdit (Syntrillium) was used to digitize the extracellular recordings.

Acoustic stimulation

Stimuli were generated using a digital stimulation system (Rhode 1976) delivered to the 2 ears through Beyer DT-48 earphones coupled to the ear molds to form a sealed system. The ear molds were fitted with a sound-delivery tube that extended to within about 2.5 cm of the tympanum. The system was calibrated for amplitude and phase from 60 Hz to 40 kHz in 20-Hz steps by means of a probe tube that extended about 1 mm from the end of the sound-delivery tube. Sensitivity to ITDs was assessed by recording the response to a binaural beat stimulus. The binaural beat stimulus was created by delivering 5.1-s tones to each ear that differed by 1 Hz. This stimulus results in a complete cycle of interaural phase change every second. The beat was typically tested in one direction, although some neurons were tested with both directions. The frequency range tested was usually from 200 to 1500 Hz. For some neurons, only one frequency was tested because of time limitations. The intensity of the tones was typically 70 dB SPL. In some cases, we added the same sinusoidal modulation frequency to each ear (about 25 Hz) to optimally evoke ITD-sensitive responses (D’Angelo et al. 2003; Sterberg et al. 2003). When time permitted, a neuron’s best frequency (BF) was measured, using tones (250 Hz to 16 kHz in 0.5-octave steps) at or below 50 dB SPL delivered to the contralateral ear.

Data analysis

Analyses of neural responses to binaural beat stimuli have been described previously (Stanford et al. 1992; Yin and Kuwada 1983). We divided our sample into peak-type and trough-type neurons based on their characteristic phase. Peak-type neurons had characteristic phases between 0.00 and 0.25 cycles or between 0.75 and 1.00 cycles, whereas trough-type neurons had characteristic phases between 0.26 and 0.74 cycles. For a portion of our neurons, time constraints allowed us to test the effect of the antagonists at only one frequency. Here, we estimated the “peak ITD” by using the peak of a parabola fitted to the peak of the ITD function. For neurons that were tested at multiple frequencies, composite ITD curves were obtained by averaging the ITD functions across the responsive frequency range for each neuron using the limitation that the frequency range was matched for the control and iontophoresis conditions. Here, the “best ITD” was estimated from a parabolic fit of the peak of the composite ITD function. The peak width was measured at 50% down from the maximum firing rate. Signal-to-noise ratio was calculated by dividing the firing rate at the peak of the ITD function by the firing rate at the trough of the ITD function. Changes in rate, width, and signal-to-noise ratio were calculated by subtracting the value in the control condition from the value in the test condition and dividing the result by the value in the control condition.

Localization of recording sites

During the last several recording sessions, electrolytic lesions (10 μA, 10 s) were made at selected sites where ITD sensitivity was present. Under deep anesthesia [ketamine (44 mg/kg) and xylazine (6 mg/kg), im; Nembutal (37.5 mg/kg), intravenous], the animals were perfused with a 2.5% solution of formol saline. The brains were sectioned and stained with cresyl violet. Based on the histologically assessed locations of the lesions, all of our recordings were located in the IC.

RESULTS

We examined responses of IC neurons to ITDs in low-frequency tones or modulated tones before and after the iontophoretic application of neurotransmitters (GABA and glutamate) and GABA antagonists (BIC or Gabazine). Only neurons that showed at least a 20% increase in discharge rate were included in our sample. This included 60 neurons tested with GABA antagonists. We were able to record full recovery from the effects of BIC in about half the neurons (33/60). Additionally, 9 neurons were tested while ejecting the inhibitory transmitter GABA, and 9 neurons with the excitatory transmitter glutamate. We excluded from our sample the responses from 25 single neurons that showed little (<20%), if any, increase in...
discharge rate under BIC. This could be because BIC was released and had no effect, or not released at all. Thus we feel that it is inappropriate to attribute meaning to the number of cells that did not change their response.

**GABA antagonists increased the rate and altered the shape of ITD functions**

Blocking GABA receptors increased the firing rate, could broaden the width of the ITD functions, and could shift the best ITD. The effects were similar for both peak-type (42/60) and trough-type (18/60) neurons so we pooled these groups. To illustrate these effects, we provide the raw recording traces from 3 neurons to a binaural beat stimulus under control, and while iontophoresing the GABA receptor antagonist BIC (Figs. 1–3). Also, in each case we present the ITD functions derived from these recordings as spike rate and as normalized functions. The rate plots show the changes in spike rate under BIC, and the normalized functions allow a visualization of any changes in the shape or width, and of any shifts of the ITD functions.

The neuron in Fig. 1 is an example of a broadening of the ITD function under BIC. The extracellular recordings to 5.1 s of a 1-Hz binaural beat stimulus (contra: 1500 Hz, ipsi: 1501 Hz) before and about 1 min after the application of BIC (20 nA) are shown in Fig. 1, A and B, respectively. In both conditions, there are 5 bursts of action potentials, each locked to the 1-Hz beat frequency. However, these bursts under BIC cover a greater portion of the 1-Hz cycle and the number of spikes is more than twice that in the control condition. From the binaural beat responses we derived the ITD function (Stanford et al. 1992). These functions in control and under BIC are depicted in Fig. 1C and normalized in Fig. 1D. This neuron was a trough-type neuron and under BIC the rate increased most at the peaks and the peak ITDs remained relatively unchanged. In contrast, the peak widths measured 50% down from the peak response increased by 118% (dashed line) compared with the control (solid line).

The second example is a neuron that under BIC displayed dramatic changes in its ITD function (Fig. 2). The organization is similar to Fig. 1 with the exception that the ITD functions are illustrated for 2 frequencies (Fig. 2, C and D: 800/801 Hz and Fig. 2, E and F: 700/701 Hz). The extracellular traces in the control show, as in the previous examples, bursts of spikes synchronized to the 1-Hz binaural beat (Fig. 2A: 800/801 Hz). Under BIC (Fig. 2B), the spike rate increases, as does the duration of the burst. Noteworthy is that the highest discharge rates within a burst occur in almost antiphase with the control bursts. This is clearly seen in the rate (Fig. 2C) and normalized rate (Fig. 2D) functions where the peak shifted by almost 180° (459 ms). A similar shift in peak ITD occurred at 700/701 Hz, but in addition a prominent secondary peak emerged (Fig. 2, E and F). This created a noticeable change in ITD shape and the overall width of ITD tuning.

This example also illustrates the limitations of our measurements of peak position and width. Under BIC, at 800/801 Hz, the width actually decreased by 39% and at 700/701 Hz, the primary peak width remained unchanged (630 ms). However, these metrics hardly reflect the complex changes in these ITD functions and indicate that for some neurons, the effect of blocking GABA is multifaceted.

The third example is a neuron that under BIC altered its peak position, width, and shape. It is also an example where blocking GABA receptors could cause action potentials to abort. As in Figs. 1 and 2, in Fig. 3, A and E we show the neuron’s

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**FIG. 1.** Extracellular recording of the response of a neuron to 5.1 s of a 1-Hz binaural beat before (A) and approximately 1 min after (B) the start of iontophoretic application of the γ-aminobutyric acid (GABA) antagonist bicuculline methiodide (BIC, 20 nA), and corresponding interaural time difference (ITD) tuning curves plotted as spike rate (C) and as normalized spike rate (D). The binaural beat stimulus was created by delivering a 1500 and 1501 Hz to the contralateral and ipsilateral ear, respectively, both at 70 dB SPL. Solid and dashed lines depict the ITD function under control and BIC, respectively. In D the functions shown in C were normalized to the maximum firing rate for each condition.
response to 5.1 s of a 1-Hz binaural beat stimulus before and about 5 min after the application of BIC (25 nA). The tones to each ear (1100/1101 Hz) were both amplitude modulated at 23 Hz. The neuron clearly follows the 1-Hz beat frequency (Fig. 3, A and E) and when only one cycle of the beat frequency is viewed, the following to the modulation frequency is clearly evident at favorable ITDs and absent at unfavorable ITDs (Fig. 3, B and F). Furthermore, the height of action potentials systematically decreases and recovers as the ITD approaches and leaves the region of maximal discharge. When the time-scale is further magnified around the ITDs that produced the maximum firing rate under BIC and within a modulation cycle, the height of the action potential systematically decreases and leads to a complete spike abortion (Fig. 3, C and G). In this neuron, even in control conditions the height of the action potential is reduced where the discharge rate is maximal. The sample recording in Fig. 3 demonstrates that blocking GABA can cause the height of the action potentials to systematically shorten, completely abort, and then gradually recover around the ITDs that evoked the highest discharge rate. This pattern primarily occurs because excessive depolarization causes a reduction of the driving force of sodium through its channels. The ITD functions derived from the illustrated binaural beat responses are shown in Fig. 3, D and H. The function under BIC displays a notch in the middle of the ITD function (Fig. 3H) that is attributed to the aborted spikes seen in Fig. 3G. In this example, in addition to increasing the discharge rate, blocking GABA receptors created a peak shift of −25 μs and a width increase of 21% compared with the control ITD function (Fig. 3D).

The bulk of our analysis was not performed on the ITD responses to a single frequency, but instead on the average ITD response across many frequencies (i.e., the composite curve). In Fig. 3, I and J, we show how the composite curve is derived for the neuron in Fig. 3, A–H. Figure 3I depicts 5 ITD functions derived from the binaural beat at 700/701 Hz to 1500/1501 Hz and tested at 200-Hz intervals (light lines) under control conditions. These curves are then averaged to create the composite curve (bold line). The same format is used in Fig. 3J to illustrate the individual and composite ITD functions recorded under BIC. In Fig. 3K, the composite curves under control and BIC are each normalized to their maximum response to allow a visual comparison. For this neuron, under BIC, the best ITD shifted by −55 μs and its width increased by 29%. This is a greater change than seen at 1,100/1,101 Hz (peak shift = −25 μs, width increase = 21%).

In Fig. 4, we present 5 examples of the different effects of GABA antagonists on the firing rate, best ITD, and ITD tuning width as seen in the composite curves of our neurons. In all cases, the peak discharge rate substantially increased (e.g.,
96% in Fig. 4C to 270% in Fig. 4E). The changes in best ITD were often modest (e.g., $-5\mu s$ in Fig. 4E and $-43\mu s$ in Fig. 4B). The ITD tuning width could increase (e.g., Fig. 4, A–C), remain relatively unchanged (e.g., Fig. 4D), or even decrease (e.g., Fig. 4E). Blocking GABA receptors could selectively increase the peak portion of the ITD function (e.g., Fig. 4D) or elevate the whole function (e.g., Fig. 4C). Just increasing the peak rate increases the signal-to-noise ratio (peak-to-trough ratio), whereas increasing the whole function does not alter this ratio. An increase in the trough rate relative to the peak rate would decrease the signal-to-noise ratio. Blocking GABA receptors could produce all 3 effects, but the most prevalent effect was to decrease signal-to-noise ratio. A majority of the neurons in our population (35/60) decreased their signal-to-
higher frequency was delivered to the ipsilateral ear, the different forms of broadening are likely not the result of directional effects of the binaural beat. Moreover, we tested a subset of our neurons \((n = 13)\) to both directions of the 1-Hz binaural beat stimulus. This was done by reversing the frequencies to the 2 ears. No consistent relationship could be detected between the direction of the binaural beat and the type of broadening.

Figure 5 displays the distributions of the changes in discharge rate, best ITD, and tuning width. These distributions were for the most part \((52/60)\) neurons performed on their composite ITD functions. In 8 neurons, only a single frequency was tested and these are also included in the distributions of Fig. 5. The mean increase in discharge rate was 210% and the bulk of the neurons increased their discharge rate well beyond our inclusion criteria of 20% \((Fig. 5A)\). The shift in best ITD could occur almost equally in either direction and most of them \((65\%)\) had shifts that fell between \(\pm 50\, \mu s\) \((Fig. 5B)\). In general, GABA antagonists increased the ITD tuning width

**FIG. 4.** Five examples of composite ITD functions taken before (solid line) and during (dotted line) the iontophoretic application of the GABA antagonist BIC. Spike rate and normalized firing rates are shown in the left and right columns, respectively. Percentage increase in firing rate and the shift in the best ITD are given in the left column; percentage increase in tuning width is given in the right column. Ejection currents and time after the start of iontophoresis were \((A)\) 20 nA, about 3 min; \((B)\) 30 nA, about 1 min; \((C)\) 20 nA, about 4 min; \((D)\) 30 nA, about 5 min; and \((E)\) 30 nA, about 3 min.

**FIG. 5.** Distributions of the changes in peak firing rate \((A)\), shifts in best ITD \((B)\), and changes in ITD tuning width \((C)\) attributed to the application of GABA antagonists \((n = 60)\). Metrics are based on the composite ITD functions of 52 neurons and on single-frequency ITD functions of 8 neurons where only a single frequency was tested.
Most of the neurons (52%) showed tuning widths that increased by \( \geq 20\% \). In contrast, only about 7% of the neurons decreased their tuning widths by \( \geq 20\% \). Overall, the mean change in width was 21%.

Finally, there was little, if any, relationship between the magnitude of the rate change and either the shift in best ITD or the change in ITD tuning width. The BIC effect was graded with time. Figure 6 shows a neuron’s composite ITD function in control and at about 1, 3, and 5 min after the start of BIC ejection. The functions are plotted in the left column as spike rate and in the right column as normalized rate. During the continuous iontophoresis of BIC, the firing rate increased by 52% after about 1 min and by 563% after about 5 min; the best ITD shifted to larger ipsilateral delays by 15 and 30 ms; and the ITD tuning width increased by 9 and 24%.

Effects of blocking GABA receptors for neurons with different best frequencies

The effects of blocking GABA receptors did not appear to be related to the frequency tuning of the neuron. Sensitivity to ITDs was observed over a wide range of best frequencies (BFs). We measured the BF for 19 of 60 neurons using tones delivered to the contralateral ear, at or below 50 dB SPL. Of these neurons, 74% (14/19) had BFs between 350 and 2000 Hz (mean = 1,247 Hz, SD = 562 Hz), which covers the range of neural ITD sensitivity seen in the rabbit. About 26% had BFs between 2 and 4 kHz (mean: 3297 Hz, SD = 642 Hz) so in these neurons it is possible that ITD sensitivity arises from stimulating the low-frequency tails. We found no relationship between BF and increase in firing rate or changes in ITD tuning width with GABA antagonists.

Effects of blocking GABA receptors on tuning width across frequency

Our lab has shown that some IC units show relatively constant ITD tuning width across frequency (Fitzpatrick and Kuwada 2001). This suggests that a potential sharpening mechanism is more potent at lower frequencies. We tested this possibility by examining the tuning widths across frequency. Figure 7 shows that the mean percentage change in width was in the direction of broadening and remained relatively constant across frequency. This change was significant at all frequencies (one-tailed \( t \)-test, \( P < 0.05 \)) and the average change across frequency of 22% agrees well with the changes measured neuron by neuron (Fig. 5C: 21%). These results suggest that GABAergic inhibition cannot explain constant tuning widths in the IC.

Effects of blocking GABA receptors on the frequency range of ITD sensitivity

Application of GABA antagonists has been shown to broaden the frequency response area of IC neurons (LeBeau et al. 2001). Consequently, for a portion of our sample (12/60), we examined the effect of GABA antagonists on the frequency range of ITD sensitivity. The frequency range of ITD sensitivity for 8 of these neurons increased, usually at the high (5/8) or both ends (2/8) of the frequency range. Only one neuron compressed its frequency range under GABA-receptor block. This occurred at the low-frequency end primarily because the spike rate increased so that the signal-to-noise ratio became substantially reduced and no longer met our statistical criterion for ITD sensitivity (\( P < 0.001 \)). The remaining 3 neurons did not change their ITD frequency range.

Effects of the neurotransmitters GABA and glutamate

We have demonstrated that blocking GABA receptors invariably increased the response rate and could alter the shape of the ITD function, including shifts and broadening. Therefore applying GABA should have the opposite effects. We tested this idea in a subset of our neurons (\( n = 9 \)). Examples of the effect of iontophoresing GABA on single-frequency ITD functions are shown for 3 neurons in Fig. 8. In all 3 examples, GABA reduced the discharge rate and this reduction could be graded with the iontophoretic current (Fig. 8C). Furthermore, GABA decreased the ITD tuning width (Fig. 8, A and B) and

![FIG. 7. Mean and SD of the change in ITD tuning width as a function of stimulation frequency. Each point reflects the mean and SD of the responses at that frequency and those within ±100 Hz (e.g., 300 ± 100 or 200 to 400 Hz).](http://jn.physiology.org/)
this decrease could also be graded with the iontophoretic current (Fig. 8C). In most cases, the activity of a neuron could be completely suppressed with higher levels of iontophoretic current. Unlike the effect of the GABA antagonists, the shape and peak of the ITD function remained stable.

We also tested the effects of the excitatory transmitter glutamate in 9 neurons. We applied glutamate to simulate the increased excitability seen with BIC but without the effect being dependent on the stimulus. Iontophoretic application of glutamate invariably increased the neuron’s firing rate (e.g., Fig. 9, left column), but had little, if any, effect on the shape and peak of the ITD function (Fig. 9, right column). In fact, across our sample, glutamate slightly decreased the ITD tuning width (−11 ± 15 μs). Therefore applying the transmitters themselves (glutamate and GABA) produces predictable effects consistent with a simple gain control mechanism, whereas blocking the transmitter (GABA) can produce complex effects.

**DISCUSSION**

The role of GABAergic inhibition in shaping ITD sensitivity in the IC appears to be multifaceted. Blocking GABA_A receptors increased the discharge rate, could alter the shape of the ITD function, could decrease the signal-to-noise ratio (peak-to-trough ratio), could create shifts in best ITD, and could broaden the width of ITD tuning. In contrast, application of the transmitters GABA or glutamate predictably changed the discharge rate but did not alter the shape or create shifts in the ITD function. The difference in these 2 experiments is that the antagonists block the effects of GABAergic inputs that are activated by sound, whereas application of the transmitters simply serves to elevate or depress the neurons overall response to sound. The GABA antagonists Gabazine and BIC affect the receptor in different ways. Although both agents block inhibitory postsynaptic currents, BIC also blocks a tonic inhibition (Mody 2001). This broader-based inhibition may contribute to the multitude of the effects we observed under BIC.

**GABAergic inputs shape ITD functions**

About half of the neurons had ITD functions that broadened by >20% when GABA_A receptors were blocked. Of these, about 25% broadened on both sides of the peak (e.g., Figs. 1C and 4A). A possible explanation is that the GABAergic inputs allow only a portion of the peak to be visible. When the
GABAergic inputs are inactivated a greater proportion of the peak region becomes revealed, much like an iceberg rising above the water. In such a scheme, the peak portion of the ITD function would broaden symmetrically. Applying the transmitter GABA had a similar, but opposite effect in that the peak portion was symmetrically narrowed, as if an iceberg was being further submerged in the water. Such symmetrical changes in peak width could also arise from monaural inhibitory inputs or from inhibitory ITD inputs with an ITD function similar to that of the excitatory ITD inputs to the IC neuron. Inhibitory ITD inputs with such features could come from the DNLL on the same side because both the IC and DNLL on the same side receive similar ITD inputs from the medial superior olive (MSO) and lateral superior olive (LSO) (Brunso-Bechtold et al. 1981; Glendenning and Masterton 1983).

The most common broadening (about 75%) occurred on one side of the peak or the other (e.g., Fig. 4, B and C). Such asymmetrical effects could be a result of interactions of inhibitory and excitatory ITD-sensitive inputs with different best ITDs. Inactivating the inhibitory input would serve to broaden the ITD function on the side of its best ITD relative to the best ITD of the excitatory input. A logical candidate for this inhibitory input is the GABAergic ITD inputs from the contralateral DNLL. The peak-type neurons in the contralateral DNLL have their best ITDs tuned to the opposite hemispheric space than those in the IC. However, the medial slopes of their ITD function can overlap and in this way the contralateral DNLL input could serve to suppress the medial slope of peak-type neurons in the IC (see Fig. 9 in Kuwada et al. 1997). Inactivating this GABAergic input would then broaden the medial slope of the ITD function. Spitzer and Semple (1998) proposed a similar mechanism of converging ITD-sensitive inputs to explain sensitivity to sound motion cues in the IC, but this idea was not supported by the findings of McAlpine and Palmer (2002). It is also possible that symmetrical or asymmetrical sharpening in the IC is attributable to intrinsic projections within the IC itself. Most, if not all, IC neurons display axon collaterals and many neurons in the IC stain positively for GABA (Oliver et al. 1991).

Because the best ITD was estimated from the center of a parabola fitted to the peak region, any broadening on one side of the peak or the other will cause a shift in the best ITD. Thus it is not surprising that many neurons showed a shift in best ITD when GABA_A receptors were blocked. However, several neurons showed large shifts in their best ITDs that cannot simply be attributed to changes in peak width (e.g., Fig. 2).
There is compelling evidence that individual IC neurons receive converging inputs with different best ITDs or even from both peak- and trough-type neurons (McAlpine et al. 1998; Stanford et al. 1992). If a role of inhibitory inputs is to maintain a balance between the converging binaural and monaural inputs to the IC, then inactivating the GABAergic inputs could upset this balance and create large shifts in best ITD and in the shape of the ITD function.

Another effect of blocking GABA_A receptors was to reduce the peak-to-trough ratio for most IC neurons. This reduction primarily occurred because of a disproportionate increase in the discharge rate at unfavorable ITDs compared with that at favorable ITDs. If the effects were proportional (i.e., multiplicative), then this ratio would not change. A reduction in the peak-to-trough ratio was also seen with glutamate application where a constant increase in discharge rate (i.e., nonproportional) was seen at favorable as well as unfavorable ITDs. One explanation for the reduced peak-to-trough ratio is the finding that BIC blocks both driven inhibition and tonic inhibitory currents for neurons in the hippocampus (Mody 2001). If this holds for IC neurons, this would lead to increased excitability similar to that seen by applying glutamate.

The width of ITD tuning decreases at higher levels along the auditory pathway, both in rabbits and owls (Fitzpatrick et al. 1997; Fujita and Konishi 1991; Stanford et al. 1992). In the rabbit, the ITD functions become progressively narrower from the SOC to the auditory thalamus. The ITD functions of single neurons in the SOC are broader by 33% compared with those of the IC (S. Kuwada, unpublished observation). The present study found the broadening effect of blocking GABA to be 21%. This discrepancy suggests that GABAergic mechanisms are not wholly responsible for the sharpening between the SOC and the IC, that we did not inactivate all of the GABAergic inputs to our IC neurons, or that additional mechanisms are involved in sharpening. For example, glycineergic inhibitory inputs from trough-type neurons in the LSO could sharpen the ITD functions of IC neurons. This is a feasible scenario because inputs from the MSO and LSO have been found to converge in the low-frequency region of the IC (Loftus et al. 2004).

GABA controls the discharge rate of IC neurons

The most salient effect of blocking GABA receptors was to substantially increase a neuron’s discharge rate. We rarely tested the limits of this effect because, in many cases, even after a short exposure, action potentials began to systematically decrease in height and finally abort as if the neuron were going into depolarization block. In the brain slice, all IC neurons display depolarization block to lemniscal shock when GABA_A receptors are blocked and a similar pattern is observed in the responses of IC neurons in the unanesthetized rabbit to tone bursts under iontophoresis of GABA_A antagonists (Sivaramakrishnan et al. 2004). A similar observation was made by McAlpine and Palmer (2002). Thus a major role of GABAergic inputs may be to regulate the postsynaptic membrane potential to prevent excitotoxic effects and to optimize neural coding of sounds.

For about half of our neurons ITD peak widths changed by <20% (e.g., Fig. 4, C–E, Fig. 5C), despite the fact that their discharge rate could increase severalfold under BIC. Presumably, such neurons receive little, if any, excitatory drive at unfavorable ITDs. Consequently, blocking inhibition is ineffective where the excitatory response is negligible. In contrast, at favorable ITDs, where excitation is robust, blocking GABAergic inhibition allows this excitation to further reveal itself. In this way, the activity in the peak region is enhanced, whereas the trough region remains unaltered. In such a scheme, the GABAergic inputs are not ITD sensitive and simply serve to attenuate the ITD function in a multiplicative way (i.e., they act like a gain control). Such inhibition could arise from GABAergic projections from monaural neurons in the ventral and/or dorsal nucleus of the lateral lemniscus. This result is similar to the finding that V-type units of the IC did not show a broadening of the frequency response area when BIC was applied, even though the rate increased severalfold (LeBeau et al. 2001; Palombi and Caspary 1996).

In summary, GABAergic inputs may serve to keep a neuron at its optimal activity level, improve its signal-to-noise ratio, and sharpen and/or shape ITD functions. Given an array of ITD-sensitive neurons tuned to different ITDs, an increased signal-to-noise ratio would improve ITD coding, thus allowing better discrimination performance. This idea is similar to that expressed by Fujita and Konishi (1991) on the role of GABA in the owl’s midbrain. Functionally, sharper ITD tuning curves may improve the coding accuracy of ITDs, provide a more energy-efficient code, and require fewer neurons to accomplish this task (Fitzpatrick et al. 1997; Zhang and Sejnowski 1999). However, sharper ITD functions at the level of the IC cannot contain more information than initially derived by the superior olivary complex (Pouget et al. 1999).

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REFERENCES


