Differential roles of GABAergic and glycinergic input on FM selectivity in the inferior colliculus of the pallid bat

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Submitted 21 June 2011; accepted in final form 20 July 2011

Williams AJ, Fuzessery ZM. Differential roles of GABAergic and glycinergic input on FM selectivity in the inferior colliculus of the pallid bat. J Neurophysiol 106: 2523–2535, 2011. First published July 20, 2011; doi:10.1152/jn.00569.2011.—Multiple mechanisms have been shown to shape frequency-modulated (FM) selectivity within the central nucleus of the inferior colliculus (IC) in the pallid bat. In this study we focus on the mechanisms associated with sideband inhibition. The relative arrival time of inhibition compared with excitation can be used to predict FM responses as measured with a two-tone inhibition paradigm. An early-arriving low-frequency inhibition (LFI) prevents responses to upward sweeps and thus shapes direction selectivity. A late-arriving high-frequency inhibition (HFI) suppresses FM sweeps and thus shapes rate selectivity for downward sweeps. Iontophoretic application of gabazine (GBZ) to block GABA_A receptors or strychnine (Strych) to block glycine receptors was used to assess the effects of removal of inhibition on each form of FM selectivity. GBZ and Strych had a similar effect on FM direction selectivity, reducing selectivity in up to 86% of neurons when both drugs were coapplied. FM rate selectivity was more resistant to drug application with less than 38% of neurons affected. In addition, only Strych could eliminate FM rate selectivity, whereas GBZ alone was ineffective. The loss of FM selectivity was directly correlated to a loss of the respective inhibitory sideband that shapes that form of selectivity. The elimination of LFI correlated to a loss of FM direction selectivity, whereas elimination of HFI correlated to a loss of FM rate selectivity. Results indicate that 1) although the majority of FM direction selectivity is created within the IC, the majority of rate selectivity is inherited from lower levels of the auditory system, 2) a loss of LFI corresponds to a loss of FM direction selectivity and is created through either GABAergic or glycinergic input, and 3) a loss of HFI corresponds to a loss of FM rate selectivity and is created mainly through glycinergic input.

glycine; inhibitory sidebands; iontophoresis

THE INFERIOR COLLCULUS (IC) is the midbrain integration center of the auditory system, responsible for assimilating both excitatory and inhibitory input from at least a dozen lower level nuclei (Casseday et al. 2002; Pollak and Park 1995). The complex pattern of parallel inhibitory projection pathways to the IC is somewhat unique among the sensory modalities and appears to be highly conserved in mammals (Winer et al. 1995). A key adaptive function of the auditory system is the creation of selectivity for complex sounds, much of which occurs within the IC (e.g., Casseday et al. 1997; Fuzessery and Hall 1996; Nataraj and Wenstrup 2006; Xie et al. 2005). In particular, frequency modulations (FM) are behaviorally relevant units of vocalization encoded by the auditory system with a high degree of selectivity across a variety of species (Casseday et al. 1997; Fuzessery 1994; Heil et al. 1992b; Mendelson and Cynader 1985; Poon et al. 1991; Shannon-Hartman et al. 1992; Suga 1968). Within the IC, two inhibitory pathways (GABAergic and glycinergic) are responsible for much of the de novo formation of this selectivity (Koch and Grothe 1998; LeBeau et al. 2001; Lu and Jen 2001; Vater et al. 1992), with multiple distinct mechanisms underlying selectivity for the different components of FM stimuli (Andoni et al. 2007; Fishbach et al. 2003; Fuzessery et al. 2011; Gordon and O’Neill 1998; Williams and Fuzessery 2010a). However, it is unclear whether these two inhibitory pathways play different roles in shaping FM sweep selectivity.

Multiple sources of ascending and descending input from the auditory system contribute to inhibitory responses in the IC. Prominent sources of GABAergic input to the IC include the dorsal nucleus of the lateral lemniscus (DNLL), commissural and intrinsic input, whereas both GABAergic and glycinergic inputs are derived from lower levels of the auditory system, including the superior olivary complex (Saldana et al. 2009) and intermediate (INLL) and ventral (VNLL) nuclei of the lateral lemniscus (Fubara et al. 1996; Merchan et al. 2005; Pollak and Park 1995; Winer et al. 1995). This divergent array of inhibitory input provides the framework for a complex network of sound processing within the IC. Most studies that have evaluated the effects of blocking GABAergic and glycinergic input on sound processing in the IC have not revealed major differences between the two pathways, including the encoding of sound duration (Casseday et al. 1994, 2000; Yin et al. 2008), binaural interactions (Klug et al. 1995), excitatory frequency tuning (LeBeau et al. 2001; Lu and Jen 2001), and FM stimuli (Koch and Grothe 1998). However, a few studies have found that these inputs play different roles in shaping response selectivity. In the IC of the horseshoe bat, excitatory tuning and lateral inhibition are shaped primarily by GABA (Vater et al. 1992), whereas facilitation of combination-sensitive neuronal responses in the mustached bat IC are created solely through glycinergic input (Sanchez et al. 2008).

In the present study, we compared the differential effects of blocking GABAergic and glycinergic inputs on two forms of FM selectivity (rate and direction) in the IC of the pallid bat (Antrozous p. pallidus). The ventral, high-frequency region of the pallid bat IC is a particularly useful model for studying FM selectivity due to the large percentage of neurons that respond preferentially or exclusively to the bat’s echolocation call, a short downward FM pulse sweeping from 60 to 30 kHz (Bell 1982; Brown 1976; Fuzessery et al. 1993).

The mechanisms shaping FM selective responses in this system also have been well described (see review, Fuzessery et al. 2011). Briefly, at least four mechanisms, alone or in com-
bination, shape selectivity for FM sweep direction and rate. In this study we focus on neurons that exhibited low- and/or high-frequency inhibitory sidebands, but not duration tuning, which shapes rate selectivity (Fuzessery et al. 2006), or asymmetrical facilitation, which shapes both rate and direction selectivity (Williams and Fuzessery 2010a). An early-arriving band of low-frequency inhibition (LFI) is associated with the inhibition of responses to an upward FM sweep, thus creating selectivity for downward FM sweeps. A late-arriving band of high-frequency inhibition (HFI) acts as a fast-pass rate filter for downward FM sweeps, suppressing responses when sweep rates drop below a given value (cutoff rate) (Fuzessery et al. 2011; Gordon and O’Neill 1998; Heil et al. 1992a; Shannon-Hartman et al. 1992; Suga 1968; Zhang et al. 2003). Predictions of cutoff rates can be made from the relative arrival time and spectral distance between an excitatory tone and that of a high-frequency inhibitory tone in both the IC (Fuzessery et al. 2006) and auditory cortex (Razak and Fuzessery 2006) of the pallid bat. It is already known that eliminating the frequencies in an inhibitory sideband from an FM sweep also eliminates response selectivity. In this study we correlate the effects of blocking GABA_A and glycine receptors on both FM direction and rate selectivity with the loss of the inhibitory sidebands associated with each form of FM selectivity. This approach also allows us to infer the extent to which FM rate or direction selectivity is created within the IC or inherited from a lower level, and whether these forms of selectivity are differentially shaped by GABAergic and/or glycinergic input.

MATERIALS AND METHODS

Extracellular single-unit recordings were obtained from the IC of 31 adult pallid bats. Bats were captured in New Mexico and housed in a free-flight environmental chamber (85–90°F) maintained on a reverse 12:12-h light-dark cycle at the University of Wyoming Biological Sciences animal facility. The bats were fed mealworms raised on ground Purina rat chow. All surgical procedures, animal welfare, and experimental manipulations were approved by the Institutional Animal Care and Use Committee based on guidelines required by the National Institutes of Health for animal research.

Surgical procedures. Each bat was isolated from the main colony room and allowed 2–3 days to acclimate to their home cage before surgery. All surgical procedures were performed as previously described (Fuzessery et al. 2006) in a designated surgical suite. In brief, bats were initially sedated with an inhalation anesthetic (isoflurane; Baxter Healthcare) followed by an intraperitoneal injection of pentobarbital sodium (Fuzessery et al. 1991). Modulated waveforms were amplified with a stereo amplifier and presented as monaural closed-field stimuli through Infinity emit-K ribbon tweeters with funnels attached for insertion into the pinnae. Speaker output was calibrated with a Bruel and Kjær 1/8-in. microphone placed at the tip of the funnel (±15 dB response from 20 to 70 kHz).

In vivo single-unit recordings of extracellular neuronal activity were obtained with glass microelectrodes (1 M NaCl, 2–5 MΩ resistance) mounted diagonally in a “pigg-y-back” configuration (Havey and Caspary 1980) to a five-barrel glass pipette (WPI) used for iontophoresis of inhibitory receptor antagonists (see below). All data were recorded from the high-frequency region of the pallid bat IC (best frequencies 30–60 kHz) at penetration depths of 1,000–2,000 μm from the surface of the brain using a similar recording protocol as described previously (Razak and Fuzessery 2009). Initially, the spectral width of the excitatory frequency band was determined by the response to individual tones. All subsequent recordings were performed at 5–10 dB above the minimum intensity threshold of the tonal response. Broad FM sweeps (30–40 kHz wide) were presented at increasing durations (0.5–100 ms) to establish direction and rate selectivity of each cell. Pairs of auditory stimuli (sequentially offset in time) were used to establish the extent of sideband inhibition using the two-tone inhibition protocol (see below). Poststimulus time histograms (PSTHs) with bin widths of 100 μs were used to record the temporal pattern of single-unit responses from the 30 successive stimulus presentations (400 ms apart) for each of the presented stimuli (e.g., tone or FM sweep). Single-unit output was identified using an oscilloscope and defined based on the consistency of action potential waveform and corresponding high signal-to-noise ratio of >10:1.

Microiontophoresis. Microiontophoretic applications of inhibitory receptor blockers were delivered using a previously described protocol (Razak and Fuzessery 2009). Immediately before recording, individual iontophoresis barrels were loaded with gabazine (GBZ; 10 mM, pH 4.0; Sigma) or strychnine (Strych; 3 mM, pH 4.0; Sigma) dissolved in physiological saline. The center barrel was used as a balance electrode (1 M NaCl). A retaining current (–15 nA) was used to retain the drugs during the search phase and predrug (control) recording phase. Escalating iontophoretic ejection currents of +10 to +60 nA were used to apply drug. Three types of tests were performed following drug application to confirm the effectiveness of inhibitory receptor blockade, as previously described (Razak and Fuzessery 2009): 1) recovery data (22/54 neurons) were obtained at 5-min intervals after the ejection current was turned off to monitor the return to predrug (control) response levels, 2) current was passed through the balance barrel (19/54 neurons) to verify that the ejection current did not affect the response, and 3) ejection currents were gradually increased from +10 to +60 nA, with responses monitored at each interval, to avoid possible response saturation. Typical results indicated an increase in response following application of GBZ or Strych with a return to baseline levels 10–30 min after iontophoresis was stopped and no effect on the response during current ejection through the balance electrode (Fig. 1).

Two-tone inhibition. The spectral width and relative arrival time of inhibitory sidebands were determined using a two-tone inhibition (TTI) over time protocol as previously described (Fuzessery et al. 2006). In brief, the inhibitory sidebands were mapped audiovisually using an excitatory tone paired with a second tone at frequencies higher or lower than the excitatory tuning curve. The inhibitory tone was 20 ms in duration, whereas the excitatory tone was 5 ms in duration. A longer duration inhibitory tone was used so that the excitatory tone was completely overlapped by the inhibitory tone, providing the opportunity for inhibitory input to completely suppress
determine the relative arrival time of inhibitory input (Fig. 1). The tones were delayed with respect to one another to determine when the response was suppressed.

**Method**

We focused on how the disruption of the bandwidth and arrival times of inhibitory input affected selectivity for FM sweep rate and direction. Single-unit responses were obtained from 53 neurons that displayed strong selectivity for the rate and/or direction of a downward FM sweep (Fig. 2A). They all had narrow excitatory frequency tuning curves bounded by sideband inhibition (Fig. 2B), similar to those described in previous studies of the pallid bat IC (Fuzessery and Hall 1996; Fuzessery et al. 2006). The arrival times of inhibition, relative to those of excitatory input, were calculated from TTI responses (Fig. 2C), in which an excitatory and inhibitory tone were delayed with respect to one another to determine when the response was suppressed.

Thirty-five neurons tested for direction selectivity all preferred downward FM sweeps and had DSI values ranging from 0.3 to 1.0 (0.82 ± 0.04). This direction selectivity was associated with a broad band of early-arriving LFI that inhibited the rate function. This represents the slowest FM sweep rate exhibiting half the maximal response (see Fig. 2A). The 50% cutoff rate was used instead of best rate because fast-pass rate-selective neurons did not have best rates, whereas both band-pass and fast-pass rate-selective neurons had a 50% cutoff rate.

**Data presentation and analysis.** Response magnitudes are represented as the number of action potentials in response to 30 stimulus repetitions presented every 400 ms. Responses to FM stimuli were presented at multiple sweep rates for both upward and downward sweeps to evaluate maximal response levels. Changes in maximal spike counts across treatment paradigms were evaluated by analysis of variance (ANOVA) followed by a Dunnett’s post hoc analysis for comparison with baseline values. The differences in DSI values and 50% cutoff rates between baseline and postdrug application were evaluated using paired Student’s t-tests. All data are means ± SE. P values <0.05 were considered significant.

**RESULTS**

This study examined the effects of removing sideband inhibition on FM sweep selectivity by using iontophoretic application of GBZ to block GABA_A receptors and/or Strych to block glycine receptors. Neurons that also exhibited other mechanisms known to shape FM selectivity, i.e., duration tuning for tones (Fuzessery et al. 2006) or asymmetrical two-tone facilitation (Williams and Fuzessery 2010a), were not included in this study to ensure that only the inhibitory sidebands contributed to response selectivity.

Once the sidebands were delineated, single inhibitory tones from the low- and high-frequency regions (if both present) were paired with an excitatory tone. The delay between an excitatory and inhibitory tone was then varied to create a TTI function, which was used to accurately determine the relative arrival time of inhibition for each sideband region (Fig. 2C). Specifically, the delay between the onset of the two tones was varied to determine the delay-frequency combination resulting in at least a 50% reduction in the response. If the inhibitory tone had to be presented before the excitatory tone for suppression to occur (i.e., positive delay), then inhibitory input was assumed to arrive before excitation.

**FM direction and rate selectivity.** For evaluation of FM direction selectivity, the maximal responses to an upward and downward FM sweep of the same bandwidth were compared to calculate a direction selectivity index (DSI): DSI = (D - U)/(D + U) (Fuzessery et al. 2006), where D and U represent the maximum responses (number of spikes) to the upward and downward FM sweeps, respectively. Values of DSI range between -1 and +1, with positive values representing selectivity for downward sweeps. A DSI value larger than ±0.3 was used to define the presence of direction selectivity. This represents a 50% greater response to one sweep direction over the other. A neuron was classified as FM rate selective if it exhibited a fast-pass or band-pass rate-selective response to a downward FM sweep and if the responses to multiple FM sweep bandwidths were within 10% of each other on the decreasing rate slope of the FM rate function (Fuzessery et al. 2006). In particular, we focused on mechanisms shaping fast-pass FM rate selectivity associated with the presence of HFI. Evaluation of fast-pass FM rate selectivity was based on calculation of the 50% cutoff rate of the decreasing rate slope of the rate function. This represents the slowest FM sweep rate exhibiting half the maximal response. The 50% cutoff rate was used instead of best rate because fast-pass rate-selective neurons did not have best rates, whereas both band-pass and fast-pass rate-selective neurons had a 50% cutoff rate.

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responses to upward FM sweeps; arrival times of the LFI ranged from 0.36 ± 0.41 ms.

Twenty-nine neurons were tested that exhibited fast-pass or band-pass rate selectivity, with baseline FM cutoff rates ranging from 0.4 to 5.0 kHz/ms (1.89 ± 0.20 kHz/ms). FM rate-selective neurons were associated with a narrow band of late-arriving HFI (e.g., Fig. 2B) that acted as a fast-pass filter of FM rate selectivity; arrival times ranged from −0.5 to 5 ms (2.01 ± 0.44 ms).

We addressed three questions related to the formation of both FM direction and rate selectivity using iontophoretic application of inhibitory receptor blockers: 1) Is FM selectivity created in the IC by local inhibition or inherited from lower levels of the auditory system? 2) Are GABAergic or glycinergic inputs similar in their effects on shaping FM selectivity? and 3) Does the loss of response selectivity correspond to a loss of the sideband inhibition associated with that form of selectivity?

**Effect of GABAergic and glycinergic input on FM direction selectivity.** The effect of blocking inhibition on FM direction selectivity was tested in 20 neurons treated with GBZ and 23 neurons tested with Strych. In the majority of neurons tested, the selectivity for downward FM sweeps was eliminated or reduced following application of either GBZ (Fig. 3A) or Strych (Fig. 3D) as indicated by the significant reduction in DSI values (Fig. 3, B and E, P < 0.05 for both GBZ and Strych, paired t-test). The majority of neurons exhibited a reduction in DSI value >50% (Table 1). GBZ and Strych exhibited similar physiological effects on FM direction selectivity, indicating that both GABAergic and glycinergic inputs equally participate in the formation of FM direction selectivity within the IC. In seven neurons, the simultaneous application of both drugs was more effective at reducing FM direction selectivity than application of the individual drugs alone (Table 1), indicating a possible synergistic effect between the two inhibitory pathways.

Given that both GBZ and Strych affected FM direction selectivity, an additional set of experiments was used to directly compare the effects of each drug on individual neuronal responses (n = 7). In all neurons tested, DSI values were reduced by >50% following application of either GBZ or Strych (Fig. 4). In one neuron, only the application of GBZ reduced FM direction selectivity (Fig. 4, A–C). Conversely, in two neurons, only the application of Strych could reduce FM selectivity (Fig. 4, D). Fig. 2. Sideband inhibition and FM selectivity. A: neuron exhibiting strong FM direction and rate selectivity. This neuron only responded to the downward FM sweep [direction selectivity index (DSI) = 1.0] and within a narrow range of FM sweep rates. The arrow indicates the 50% cutoff rate used to evaluate the fast-pass filter effects of high-frequency inhibition (HFI). B: neuron bounded by a broad band of low-frequency inhibition (LFI) arriving at the same time as excitation and a narrow band of HFI arriving 4 ms later than excitation. These differential arrival times are typical of the pallid bat IC. C: two-tone inhibition (TTI) functions of the neuron in B, pairing an excitatory tone (40 kHz) with a tone from the low (38 kHz)- or high-frequency (43 kHz) inhibitory sidebands. The horizontal bars show the response to the 3 tones presented singly. The arrival time of LFI and HFI was measured as the relative delay between the excitatory and inhibitory tones. A delay of 0 ms indicates that the tones were presented simultaneously. A positive delay indicates that the inhibitory tone was presented first. A negative delay indicates that the excitatory tone was presented first. For this neuron, the response was completely suppressed when the low-frequency tone was presented at the same time as the excitatory tone, but maximum inhibition by the high-frequency tone occurred only when the excitatory tone was delayed 3.5–4 ms.
direction selectivity (Fig. 4, D–F). In the remaining four neurons, both GBZ and Strych were effective (Fig. 4, G–I).

Together, these results indicate that the majority of FM direction selectivity is created at the level of the IC, through either GABAergic or glycinergic input to the recorded neuron. However, it is important to note that even when both GABAergic and glycinergic inputs were blocked, 14% of the neurons tested retained direction selectivity (Table 1), suggesting that some neurons inherit at least part of their direction selectivity.

Loss of FM direction selectivity is associated with a loss of LFI. Selectivity for downward FM sweeps can be eliminated by starting an upward sweep at a frequency higher than the low-frequency inhibitory sideband (Fig. 5) (Fuzessery et al. 2006). This manipulation demonstrates the importance of LFI but does not determine whether the inhibitory sideband is inherited or created in the IC. In a subset of the neurons tested above, LFI and FM direction selectivity were measured both before and after application of GBZ (n = 12) or Strych (n = 10) to correlate the loss of LFI to the loss of FM direction selectivity.

LFI was eliminated in 50% (6/12) of neurons treated with GBZ and 80% (8/10) of neurons treated with Strych (Fig. 6A). In all 14 neurons in which LFI was eliminated, FM direction selectivity was also eliminated (Fig. 6B). Overall, DSI values dropped to <0.33 after drug application, with an average reduction in DSI of 90 ± 3%. In other neurons, LFI was not eliminated, but arrival times were delayed, as shown in the

Table 1. Comparison of GBZ and Strych application on FM selectivity

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<thead>
<tr>
<th>Drug Application</th>
<th>FM Direction Selectivity</th>
<th>FM Rate Selectivity</th>
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<tbody>
<tr>
<td>GBZ only</td>
<td>12/20 (60%)</td>
<td>0/16 (0%)</td>
</tr>
<tr>
<td>Strych only</td>
<td>13/23 (57%)</td>
<td>7/21 (33%)</td>
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<tr>
<td>GBZ + Strych</td>
<td>6/7 (86%)</td>
<td>3/8 (38%)</td>
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All neurons tested were selective for either the direction or rate of a downward frequency-modulated (FM) sweep. Values represent the number of neurons affected (loss of selectivity following drug application) over the total number of neurons tested. Individual drug responses are compared with the coapplication of both drugs together. GBZ, gabazine; Strych, strychnine.

Fig. 3. Both GABAergic and glycinergic inhibition shape FM direction selectivity. A: the role of GBZ in eliminating FM direction selectivity. B: across the population, a significant reduction in DSI was seen in the majority of neurons following GBZ application (n = 20, *P < 0.05). C: same data as in B, shown in normalized format. D: the role of Strych in eliminating FM direction selectivity. E: a significant reduction in DSI was seen in the majority of neurons following Strych application (n = 23, *P < 0.05). F: same data as in E, shown in normalized format. In B and E, closed circles and vertical bars represent means ± SE.
3.5-ms shift in arrival time of inhibition from a baseline value of −2.5 to +1.0 ms after drug application (Fig. 6C). This delay in arrival of inhibition correlated with a reduction in FM direction selectivity, with the DSI dropping from 0.75 to 0.03 (Fig. 6D). The arrival time of LFI was delayed in this fashion in 25% (3/12) of neurons treated with GBZ and 10% (1/10) of neurons treated with Strych. In all four neurons, DSI values dropped to 0.39, with an average reduction in DSI of 77.10%. This latter effect is important because it demonstrates that even though the strength of the inhibitory input is not affected, the change in its arrival time is sufficient to disrupt direction selectivity.

In contrast, LFI was relatively unaffected in other neurons, as illustrated by the minimal change in arrival time and strength of LFI seen in the neuron in Fig. 6E. As expected, there was little effect on direction selectivity (Fig. 6F). Overall, LFI was unaffected in 25% (3/12) of neurons treated with GBZ and 10% (1/10) of neurons treated with Strych. In these four neurons, the average delay in the arrival time of LFI was minimal (0.6 ± 0.2 ms). These four neurons also retained their FM direction selectivity with an average reduction in DSI of only 15 ± 9%. These results indicate that there was a strong correlation between the loss of LFI and the loss of FM direction, implicating LFI input in the creation of FM direction selectivity. In addition, direction selectivity could be reduced or eliminated if LFI remained intact, but the arrival time of LFI was sufficiently delayed relative to the excitatory input.

Effect of GABAergic and glycinergic input on FM rate selectivity. The effect of blocking inhibition on FM rate selectivity was tested in 16 neurons treated with GBZ and 21 neurons tested with Strych. In contrast to the effect on FM direction selectivity, blocking local inhibitory input was largely ineffective at eliminating FM rate selectivity (Table 1), indicating that this form of FM selectivity may be largely inherited from lower levels of the auditory system. In all 16 neurons tested with GBZ, fast-pass FM rate selectivity remained intact (Table 1, Fig. 7A), with only a slight reduction in the 50% cutoff rate (Fig. 7B, *P < 0.05, paired t-test). Similarly,
Loss of FM rate selectivity is associated with a loss of HFI. Fast-pass FM rate selectivity can be eliminated by removing the high-frequency region from a downward FM sweep (Fig. 8), suggesting that HFI shapes FM rate selectivity (Fuzessery et al. 2006). In a subset of the neurons tested above, HFI and FM rate selectivity were measured both before and after application of GBZ ($n = 6$) or Strych ($n = 8$) to correlate the loss of HFI to the loss of FM rate selectivity. Again, GBZ was ineffective at eliminating FM rate selectivity, and in all neurons tested ($6/6$), both HFI and FM rate selectivity remained intact (Fig. 9, $A$ and $B$). However, in $50\%$ ($4/8$) of neurons tested with Strych, HFI was eliminated (Fig. 9$C$), corresponding to a loss of fast-pass FM rate selectivity (Fig. 9$D$). In the remaining neurons tested with Strych ($50\%$, $4/8$), both HFI and FM rate selectivity remained intact. Thus a strong correlation between the loss of HFI and the loss of FM rate selectivity was observed, implicating local, glycinergic HFI input in shaping sweep rate selectivity, at least in a minority of the IC neurons tested.

**DISCUSSION**

There are three main findings of the present study. First, based on the effects of blockade of inhibitory receptors, FM direction selectivity was created or refined within the IC in the majority of neurons. In contrast, FM rate selectivity was disrupted in only one-third of neurons tested and therefore appears to be largely inherited from lower levels of the auditory system. It should be noted that this degree of inheritance applies only to selectivity shaped by inhibitory sidebands. The extent to which duration tuning and asymmetrical facilitation, which also shape FM sweep selectivity (Fuzessery et al. 2006; Williams and Fuzessery 2010a), are inherited remains to be determined. Second, FM direction selectivity was disrupted by blockade of either GABA$_A$ or glycine receptors, whereas the FM rate selectivity was disrupted only by blockade of glycinergic input. Third, the loss of FM selectivity correlated with a loss of the inhibitory sidebands associated with each form of selectivity. That is, a loss of direction selectivity correlated with a loss of LFI, whereas a loss of rate selectivity correlated with a loss of HFI.

**Origins of FM sweep selectivity.** Sweep direction selectivity was disrupted by receptor blockade in the majority of neurons tested, but not in all, suggesting that direction selectivity is created largely within the IC. In contrast, rate selectivity was disrupted in only about one-third of the neurons tested, even when both GABA$_A$ and glycine receptors were blocked (Table 1), suggesting that this form of selectivity is largely inherited from lower levels of the system. Nothing is known of sweep selectivity at lower levels of the pallid bat auditory system, but in other species, direction and/or rate selectivity has been reported in the cochlear nucleus (Britt and Starr 1976; Godfrey et al. 1975) and nuclei of the lateral lemniscus (Huffman et al. 1998), as well as an extralemniscal region of the IC (Gordon and O’Neill 2000). The modeling of synaptic inputs to IC neurons further suggests that the inputs themselves may be selective for sweep direction and rate (Gittelman et al. 2009). Finally, blocking inhibition in the big brown bat IC eliminated direction selectivity for sinusoidally frequency-modulated (SFM) signals in only a minority of neurons, suggesting considerable inheritance of response selectivity (Koch and...
Grothe 1998). This is consistent with the finding that, in this bat species, the VNLL, which projects to the IC, contains neurons that are already selective for SFM direction (Huffman et al. 1998).

These results indicate, first, that similar forms of response selectivity may be created at different levels across species, and second, that within a species, selectivity for different features of relevant sounds may be created at different levels. Other examples of the latter are the creation of delay tuning in the mustached bat IC, which is shaped by a combination of inherited selectivity and selectivity shaped at the level of the IC (Peterson et al. 2008) and the transformation of binaural response properties in the auditory brain stem (Pollak et al. 2002).

Fig. 6. Elimination of LFI by blocking inhibitory input is associated with a loss of FM direction selectivity. A, C, and E show the TTI functions of 3 neurons, created using an excitatory tone and a tone from the low-frequency inhibitory sideband. B, D, and F show the effect on the neuron’s direction selectivity. A: LFI was eliminated in this neuron following GBZ application, as indicated by the lack of effect of a low-frequency tone to inhibit the excitatory tone. B: the loss of LFI was associated with the elimination of FM direction selectivity, as indicated by the drop in DSI (same neuron as in A). C: the arrival time of LFI was delayed in this neuron following Strych application, as indicated by the rightward shift in the arrival time of inhibition, but the inhibitory effect was not eliminated. D: the delay of LFI was associated with a loss of FM direction selectivity, as indicated by the drop in DSI (same neuron as in C). E: the strength and arrival time of LFI were relatively unaffected in this neuron following GBZ application, as indicated by only a slight shift in arrival time. F: the relative lack of effect on LFI was associated with a minimal change in FM direction selectivity, as indicated by the minimal lack in DSI (same neuron as in E).
One caveat to the above arguments is that the presence of a form of response selectivity at a lower level does not necessarily preclude its refinement or de novo creation at a higher level. In the pallid bat, sweep rate and direction selectivity in the auditory cortex can be eliminated by GABA$_A$ receptor blockade (Razak and Fuzessery 2009), indicating that intracortical GABAergic inhibition creates this response selectivity, even though the expressions of selectivity are very similar to those seen in the IC. The rat auditory system is similar in that intracortical inhibition contributes to sweep direction selectivity (Zhang et al. 2003), even though this response selectivity is present in the IC (Poon et al. 1992; Poon and Yu 2000). One possible explanation for this seemingly redundant operation is that response selectivity may be degraded or lost through the synaptic convergence of selective and nonselective inputs as the information ascends to auditory cortex, requiring that selectivity be recreated.

A second caveat regarding the differential effects of drug iontophoresis on sweep rate and direction selectivity is that...
the lesser effect on rate selectivity could have been due to an inability to block enough receptors serving the creation of rate selectivity. However, the drug applications typically elevated response magnitude and/or eliminated direction selectivity, so drug perfusion was at least sufficient to affect other response properties. Moreover, when both GABA_A and glycine receptors were blocked, a greater percentage of neurons lost selectivity for sweep direction (86% vs. 60%). In contrast, there was little increase in the percentage of neurons that lost rate selectivity (38% vs. 33%), suggesting that rate selectivity was not disrupted due to a need to block both receptor types. However, it is worth emphasizing that if not enough receptors are blocked, and a form of response selectivity remains intact, this will be erroneously interpreted as an inheritance of response selectivity. Therefore, if we have errored in data interpretation, it is to assume that too great a percentage of neurons inherit response selectivity.

**Differential effects of blocking GABA_A and glycine.** In the present study, blocking GABA_A or glycine receptors had the same effects on sweep direction selectivity in a similar percentage of neurons. Moreover, when direction selectivity was eliminated or reduced, LFI was also eliminated or its arrival time delayed relative to excitation. In order for an inhibitory sideband to suppress a response to a given sweep direction, the inhibitory input it triggers must arrive early enough to suppress a neuron before excitation arrives (Fuzessery et al. 2011). If the arrival of LFI is delayed, the response to an upward sweep increases (e.g., Fig. 6).

An unexpected finding is that the HFI that creates a fast-pass filter for sweep rate appears to be provided only by glycineric input. None of the 16 rate-selective neurons tested lost selectivity when GABA_A receptors were blocked. This leads to the second unexpected conclusion, that these inhibitory inputs are tuned to different frequencies. Although both GABAergic and glycineric inputs are tuned to frequencies in low-frequency inhibitory sidebands, only glycineric inputs are tuned to the frequencies in the high-frequency inhibitory sidebands (Fig. 10). In general, the frequency tuning of inhibitory inputs are thought to roughly match their excitatory tuning (Palombi and Caspary 1996), since the frequencies of the inhibitory sidebands vary with a neuron’s best excitatory frequency, although the spectral bandwidth of inhibitory inputs can be considerably wider than the excitatory inputs (Voytenko and Galazyuk 2007; Xie et al. 2007).

There is considerable evidence from the pallid bat IC that the tuning of excitatory and inhibitory inputs overlap (Fuzessery 1994; Williams and Fuzessery 2010a). For example, a tone burst tuned to a neuron’s best frequency can suppress a response to an excitatory FM sweep if presented at the same time as the sweep. However, if the same tone is presented in the correct sequence and delayed relative to a second tone, it can contribute to an asymmetrical two-tone facilitation. Thus it appears that a given frequency can activate both excitatory and inhibitory inputs. However, although there is overlap in the frequency tuning of excitatory and inhibitory inputs, present results suggest that glycineric (but not GABAergic) inputs are tuned to frequencies that are slightly higher than the excitatory tuning curve. This assertion is based on the argument that because only the blockade of glycine receptors eliminates rate selectivity, and because HFI creates rate selectivity, HFI must therefore be glycineric. However, given the unprecedented nature of this finding, the axiom that “absence of proof is not proof of absence” should be observed. Additional studies might have revealed that GABAergic input also contributes to HFI. Present results do, however, suggest that glycineric input dominates this higher frequency band.

The functional and anatomical substrates for this difference in the tuning of inhibitory inputs are unknown. In the big brown bat, the columnar region of the VNLL, which projects to the IC, is largely glycineric (Vater et al. 1997) and contains neurons that have unusually broad frequency tuning curves (Covey and Caseday 1991). If a similar situation exists in the pallid bat, this could account for the broader glycineric tuning observed in its IC. Glycineric cells are present in the VNLL of other species, as well (Fubara et al. 1996; Winer et al. 1995). However, there are known to be considerable species differences (e.g., Peterson et al. 2008) in the functional neuroanatomy of the lateral lemniscal nuclei, making such speculation problematic.
We are not aware of similar findings in other systems regarding the differences in the roles of these inhibitory circuits in creating low- and high-frequency inhibitory sidebands. In the big brown bat IC, both inhibitory inputs are reported to have similar roles in shaping inhibitory sidebands (Lu and Jen 2001). In contrast, in the greater horseshoe bat IC, inhibitory sidebands shaping frequency tuning curves are created primarily by GABAergic inputs (Vater et al. 1992); blocking glycine receptors had comparatively little effect. In the big brown bat IC, FM direction selectivity in response to SFM was similarly disrupted by blocking either GABA_A or glycine receptors (Koch and Grothe 1998). Both inhibitory inputs also have a similar influence on response magnitude and shaping frequency tuning curves in the guinea pig IC (LeBeau et al. 2001).

Although other examples of differential tuning of inhibitory sidebands are lacking, there is evidence of differences in the frequency tuning of inhibitory pathways tuned to widely separated frequency bands. In the mustached bat, delay-tuned neurons in the IC are created in part by inhibitory inputs tuned near different harmonics (Portfors and Wenstrup 1999). Delay tuning emerges through a combination sensitivity for the bio-sonar’s higher harmonics delayed with respect to the first harmonic. The first harmonic input is inhibitory and solely glycinergic, whereas both glycinergic and GABAergic inputs are tuned to the higher harmonics near the neurons’ best excitatory frequency (Nataraj and Wenstrup 2005; Sanchez et al. 2008).

The above studies of delay-tuned neurons highlight another significant difference in the roles of glycinergic and GABAergic inhibitory inputs in the mammalian auditory brain stem. Evidence to date indicates that only glycinergic inputs are capable of providing excitatory drive through a rebound from inhibition (Wenstrup and Leroy 2001). Glycine has a similar excitatory role in the superior paraolivary nucleus, where it is essential for the offset response of neurons in this nucleus (Kulesza et al. 2007), whereas GABA has a more subtle role in shaping response magnitude. An excitatory role for glycinergic input also has been reported in a minority of neurons in the INLL; the majority of these neurons showed a more typical disinhibition of response (Kutscher and Covey 2009), highlighting the fact that glycinergic input may have multiple effects on a target nucleus. In contrast, blocking GABA_A receptors resulted only
in disinhibition, producing an increase in response magnitude and duration. This excitatory effect of glycineric input also has been observed in the pallid bat IC (Williams and Fuzessery 2010b). It underlies the asymmetrical facilitation that creates both sweep direction and rate selectivity (Williams and Fuzessery 2010a), and blockade of glycine receptors completely eliminates the response. It is, however, unlikely that this unusual glycineric effect played a role in shaping rate selectivity in the present study.

In summary, the differences in the roles of GABA- and glycineric inputs in shaping response selectivity appear to vary across species and auditory nuclei. In the pallid bat IC, rate selectivity through high-frequency inhibitory sidebands appears to be shaped only by glycineric inputs. This is somewhat peculiar because sweep rate selectivity is also recreated in the auditory cortex of the pallid bat, but in this case through intracortical GABAergic inhibition (Razak and Fuzessery 2009), suggesting that one inhibitory transmitter system is not inherently more effective in shaping this form of response selectivity.

One methodological consideration that could potentially influence present conclusions is the distribution of GABA<sub>A</sub> and glycine receptors in the IC. In bats, GABA<sub>A</sub> and glycine receptors are reported to have either opposing gradients of distribution, in which GABA<sub>A</sub> receptors are densest dorsomedial, and glycine ventral-lateral, or an even distribution of GABA<sub>A</sub> receptors, with glycine receptors confined mainly to the ventral-lateral region (Fubara et al. 1996; Vater et al. 1990; Winer et al. 1995). A similar glycine receptor distribution is seen in the gerbil (Sanes et al. 1987). A physiological corollary is that the effects of blocking glycine are seen only in deeper regions of the IC (Koch and Grothe 1998; Lu and Jen 2001). Receptor distribution in the pallid bat IC is not known, but our recordings were all made in the ventral and lateral aspects of the IC, where both receptor types are likely to be present at similar densities. Present physiological results also suggest that both receptor types are present.

Conclusions. Our present results add to a growing body of evidence that the spectrotemporal receptive fields of IC neurons are often constructed at multiple levels of the auditory system, through the interactions of multiple transmitter systems. In the pallid bat, neuronal selectivity for the direction and rate of the downward FM sweep of its echolocation pulse is shaped by different mechanisms. Direction selectivity is created largely within the IC, with GABAergic or glycineric input creating the LFI that prevents responses to sweeps in the upward direction. In contrast, rate selectivity is largely inherited, and that which is created within the IC is shaped by glycineric HFI. The multiple effects of blocking inhibition further suggest that a form of selectivity may range from being created de novo at the IC level. In some cases, inhibitory sidebands were completely eliminated, whereas in others, the strength of the inhibitory input was unchanged but the arrival time of the inhibitory input was altered, suggesting a fine-tuning of the timing of synaptic inputs at the IC level. Gittelman and Pollak (2011), based on the modeling of in vivo patch-clamp recording in the IC, have suggested that the modulation of the strength of inhibitory conductances may provide a more robust way to control the relative timing of inhibitory and excitatory inputs. To an extent, synaptic strength and timing may be interchangeable. Such a mechanism could account for the changes in the relative arrival time of inhibition observed in the present study. Perhaps the most general and conservative conclusion to be drawn from present study is that it is likely that there is no single mechanism responsible for shaping neuronal selectivity for FM sweeps in the IC.

ACKNOWLEDGMENTS

We thank Terese Zumsteg for comments on this manuscript.

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