Differential Roles of GABAergic and Glycinergic Input on FM Selectivity in the Inferior Colliculus of the Pallid Bat

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Running Head: Inhibitory Sidebands and FM Sweep Selectivity

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Abstract:

Multiple mechanisms have been shown to shape FM selectivity within the central nucleus of the inferior colliculus (IC) in the pallid bat. Here we focus on the mechanisms associated with sideband inhibition. The relative arrival time of inhibition as compared to 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 slow FM sweeps and thus shapes rate selectivity for downward sweeps. Iontophoretic application of gabazine (GBZ) to block GABAa 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 co-applied. 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 while 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 while elimination of HFI correlated to a loss of FM rate selectivity. Results indicate that 1) while 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.
Introduction:

The inferior colliculus (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 (Pollak & Park 1995; Casseday et al. 2002). 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., Fuzessery & Hall 1996; Casseday et al. 1997; Xie et al. 2005; Nataraj & Wenstrup 2006). In particular, frequency modulations (FMs) are behaviorally relevant units of vocalization encoded by the auditory system with a high degree of selectivity across a variety of species (Suga 1968; Mendelson and Cynader 1985; Poon et al. 1991; Heil et al. 1992b; Shannon-Hartman et al. 1992; Fuzessery 1994; Casseday et al. 1997). Within the IC, two inhibitory pathways (GABAergic and glycinergeric) are responsible for much of the de novo formation of this selectivity (Vater et al. 1992; Koch and Grothe 1998; LeBeau et al. 2001; Lu & Jen 2001), with multiple distinct mechanisms underlying selectivity for the different components of FM stimuli (Gordon and O'Neill 1998; Fishbach et al. 2003; Andoni et al. 2007; Fuzessery et al. 2011; 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, while both GABAergic and glycinergic input is 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 (Pollak and Park 1995; Winer et al. 1995; Fubara et al. 1996; Merchan et al. 2005). 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) while 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 60-30 kHz (Brown 1976; Bell 1982; Fuzessery et al. 1993).
The mechanisms shaping FM selective responses in this system have also been well described (review, Fuzessery et al. 2011). Briefly, at least four mechanisms, alone or in combination, shape selectivity for FM sweep direction and rate. Here 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 fastpass rate filter for downward FM sweeps, suppressing responses when sweep rates drop below a given value (cutoff rate) (Suga 1968; Heil et al. 1992a; Shannon-Hartman et al. 1992; Gordon and O'Neill 1998; Zhang et al. 2003; Fuzessery et al. 2010). 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. Here we correlate the effects of blocking GABAa 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 glycineric 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 prior to 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, USP) followed by an intraperitoneal injection of pentobarbital sodium (30 mg/kg of body weight) and acepromazine (2 mg/kg of body weight). Upon loss of reflexive responses to a toe pinch, animals were placed in a bite bar and a midline incision made in the scalp. The superficial muscles over the dorsal surface of the skull were carefully separated and reflected by blunt dissection. The anterior region of the skull was gently scraped clean and a thin layer of glass microbeads was applied and secured with cyanoacrylate for placement of a head pin. A 1 mm² exposure was made over the left or right IC by carefully excising the skull with a microscalpel. Exposed muscle was covered with petroleum jelly (Vaseline®) and the skull was kept moist with periodic applications of
physiological saline throughout the course of the recording session. Following surgery, the animals were taken to the recording chamber (see below) and secured in a Plexiglas restraining device. A cylindrical aluminum head pin was mounted to a cross bar and secured to the anterior skull using dental cement to prevent movement of the head. A bat was used in four recording sessions, twice on each side of the IC, and then euthanized.

Recording and Data Acquisition Procedures

Bats were isolated in a heated (85-90° F), soundproof chamber lined with anechoic foam during the 6-8 h recording session. Auditory stimuli were generated by digital hardware (Modular Instruments and Tucker Davis Technologies) controlled by a custom-written software program (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 Brüel and Kjaer 1/8 inch microphone placed at the tip of the funnel (± 15dB response from 20-70 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 ‘piggy-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 1000-2000 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). Post-stimulus 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
approximately 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 (1M NaCl). A retaining current
(-15 nA) was used to retain the drugs during the search phase and pre-drug (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 pre-drug (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, while 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 the response. The tones were
delayed with respect to one another to determine the relative arrival time of inhibitory
input (Fig. 2B). Frequency and delay of the second tones were varied to achieve 100%
suppression of the response to the excitatory tone, or the maximum percent inhibition
present. The audiovisual mapping of inhibitory sidebands was greatly facilitated by the
fact that IC neurons in the pallid bat exhibited little or no spontaneous activity, and that
responses are typical phasic, onset responses consisting of 1-3 spikes/stimulus. The absence of a response is readily apparent.

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 two-tone inhibition function, which was used to accurately determine the relative arrival time of inhibition for each sideband region (Fig. 2C). Specifically, the delay between the onsets 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 excitatory input was assumed to arrive before inhibition. If the inhibitory tone suppressed the excitatory tone even when presented after the excitatory tone (i.e. negative delay), then inhibitory input was assumed to arrive before excitation.

**FM Direction and Rate Selectivity**

For evaluation of FM direction selectivity, the maximal response to an upward and downward FM sweep of the same bandwidth was compared to calculate a direction selectivity index (DSI): $DSI = (D-U) / (D+U)$ (Fuzessery et al. 2006). $D$ 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 of $>+/-.3$ 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 fastpass or bandpass 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 fastpass 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 (see Fig. 2A). The 50% cutoff rate was used instead of best rate because fastpass rate-selective neurons did not have best rates, while both bandpass and fastpass 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 an 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 post-drug application were evaluated by paired Student t-tests. All data are presented as the mean ± S.E.M. p values < 0.05 were considered significant.
**Results:**

This study examined the effects of removing sideband inhibition on FM sweep selectivity using iontophoretic application of GBZ to block GABA\textsubscript{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 assure that only the inhibitory sidebands contributed to response selectivity.

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 0.3-1.0 (0.82 ± 0.04). This direction selectivity was associated with a broad band of early-arriving LFI that inhibited responses to upward FM sweeps; arrival times of the LFI ranged from -5 to 2 ms (-0.36 ± 0.41).
Twenty-nine neurons were tested that exhibited fastpass or bandpass rate selectivity, with baseline FM cutoff rates ranging from 0.4-5.0 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 fastpass filter of FM rate selectivity; arrival times ranged from -0.5 to 5 ms (2.01 ± 0.44).

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. 3B,E, p < 0.05 for both GBZ and STRYCH, paired t-test). The majority of neurons exhibiting a reduction in DSI value of > 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 7 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. 4A-C). Conversely, in two 
neurons, only the application of STRYCH could reduce FM direction selectivity (e.g., 
Fig. 4D-F). In the remaining four neurons, both GBZ and STRYCH were effective (e.g., 
Fig. 4G-I).

Taken together, these results indicate that the majority of FM direction selectivity 
is created at the level of the IC, through either GABA- or glycinergic input to the 
recorded neuron. However, it is important to note that even when both GABA- 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) in order 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 (e.g. Fig. 6A). In all 14 neurons in which LFI was
eliminated, FM direction selectivity was also eliminated (e.g. 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 3.5 ms shift in arrival time of inhibition from a baseline value of -2.5 ms 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 4 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 Figure 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, fastpass 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, in the majority of neurons tested (e.g., Fig. 7D) with STRYCH (66%), FM rate selectivity was largely unaffected (Table 1) with only a slight reduction in the 50% cutoff rate (Fig. 7E, p < 0.05, paired t-test).

Only STRYCH or the combination of GBZ and STRYCH proved effective at eliminating FM rate selectivity (Table 1). In 33% of neurons tested with STRYCH, fastpass FM rate selectivity was eliminated (Table 1), as illustrated by the neuron in Figure 7D. In addition, 8 neurons were tested with the co-application of GBZ and STRYCH to assess possible synergistic effects on FM rate selectivity. However, similar to the effects of STRYCH alone, only 38% of neurons co-treated with both drugs lost FM rate selectivity (Table 1). It thus appears that the FM rate selectivity created within the IC is largely due to glycinergic input. Given that only STRYCH could eliminate FM rate
Loss of FM rate selectivity is associated with a loss of HFI

Fastpass 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) in order 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. 9A,B). However, in 50% (4/8) of neurons tested with STRYCH, HFI was eliminated (Fig. 9C), corresponding with a loss of fastpass FM rate selectivity (Fig. 9D). 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. First, based on the effects of blockade of inhibitory receptors, FM direction selectivity is created or refined within the IC in the majority of neurons. In contrast, FM rate selectivity was disrupted in only a 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 GABAa or glycine receptors, while 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, while 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 a third of the neurons tested, even when both GABAa 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. 2009), and the transformation of binaural response properties in the auditory brainstem (Pollak et al. 2002).

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 GABAa 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, 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 ionotophoresis 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 GABAa 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 (38% vs 33%) that lost rate selectivity, 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 erred in data interpretation, it is to assume that too great a percentage of neurons inherit response selectivity.

Differential Effects of Blocking GABAa and Glycine

In the present study, blocking GABAa 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 fastpass filter for sweep rate
appears to be provided only by glycinergic input. None of the 16 rate-selective neurons
tested lost selectivity when GABA\textsubscript{a} receptors were blocked. This leads to the second
unexpected conclusion that these inhibitory inputs are tuned to different frequencies.
While both GABA- and glycinergic inputs are tuned to frequencies in low-frequency
inhibitory sidebands, only glycinergic 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. But while there is overlap in
the frequency tuning of excitatory and inhibitory inputs, present results suggest that glycinergic (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 glycinergic. 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 glycinergic 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 glycinergic (Vater et al. 1997), and contains neurons that have unusually broad frequency tuning curves (Covey and Casseday 1991). If a similar situation exists in the pallid bat, this could account for the broader glycinergic tuning observed in its IC. Glycinergic cells are present in the VNLL of other species as well (Winer et al. 1995; Fubara et al. 1996). However, there are known to be considerable species differences (e.g., Peterson et al. 2009) 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 GABAA 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).

While 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 biosonar's higher harmonics delayed with respect to the first harmonic. The first harmonic input is inhibitory and solely glycinergic, while both glycinergic and GABAergic inputs are both 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 brainstem. 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, when it is essential for the offset response typical of neurons in this nucleus (Kulesza et al. 2007), while GABA has a more subtle role in shaping response magnitude. An excitatory role for glycinergic input has also 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 GABAa receptors resulted only in disinhibition, producing an increase in response magnitude and duration. This excitatory effect of glycinergic input has also 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 glycinergic effect played a role in shaping rate selectivity in the present study.

In summary, the differences in the roles of GABA- and glycinergic 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 glycinergic 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 GABAa and glycine receptors in the IC. In bats, GABAa and glycine receptors are reported to have either opposing gradients of distribution, in which GABAa receptors are densest dorsal-medial, and glycine ventral-lateral, or an even distribution of GABAa receptors, with glycine receptors confined mainly to the ventral-lateral region (Vater et al. 1990; Winer et al. 1995; Fubara et al.
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 (Lu and Jen 2001, Koch and Grothe 1998). 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:

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 GABA- or glycinergic 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 glycinergic HFI. The multiple effects of blocking inhibition further suggest that a form of selectivity may range from being refined to being created de novo at the IC level. In some cases, inhibitory sidebands were completely eliminated, while 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.

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Table 1. Comparison of GBZ and STRYCH application on FM selectivity. All neurons tested were selective for either the direction or rate of a downward 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 to the co-application of both drugs together.

<table>
<thead>
<tr>
<th>Drug Application</th>
<th>FM Direction Selectivity</th>
<th>FM Rate Selectivity</th>
</tr>
</thead>
<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>
</tr>
<tr>
<td>GBZ+STRYCH</td>
<td>6/7 (86%)</td>
<td>3/8 (38%)</td>
</tr>
</tbody>
</table>
Figures:

**Figure 1: Microiontophoresis.** A: Response of an IC neuron to microiontophoretic application of the inhibitory receptor blockers GBZ and STRYCH. Both drugs increased the spike response across FM sweep rate with recovery to baseline values between drug applications and no effect of a +60 nA current delivered through the balance electrode. B: Comparison of maximal FM spike responses across the entire population of neurons tested (n=54). * p < 0.05 (ANOVA followed by Dunnett’s post-hoc comparison to baseline values).

**Figure 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 (DSI = 1.0) and within a narrow range of FM sweep rates. The black arrow indicates the 50% cutoff rate used to evaluate the fast-pass filter effects of HFI. B: This neuron was bounded by a broad band of 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: The two-tone inhibition 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.

**Figure 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 as (B)n, 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). Closed circles and vertical bars represent mean ± S.E.M. F: Same as (E), shown in normalized format.

**Figure 4: IC Neurons Use GABAergic Input, Glycinergic Input, or Both to Create FM Direction Selectivity.** The responses of 3 neurons (columns) to GBZ and STRYCH, with each drug applied independently. A-C: Direction selectivity was eliminated with GBZ, but not STRYCH. D-F: Direction selectivity was eliminated by STRYCH, but not GBZ. G-I: Direction selectivity was eliminated by either drug.

**Figure 5: LFI is Associated with the Formation of FM Direction Selectivity.** The contribution of LFI on FM direction selectivity can be tested by removing the low-frequency region from an upward FM sweep. A: This neuron responded equally well to upward and downward sweeps when frequencies below 55 kHz were removed from the upward sweep. B: The excitatory and inhibitory frequency bands of the neuron in (A),
showing that starting the upward sweep at 55 kHz removed the low-frequency inhibitory sideband.

**Figure 6: Elimination of LFI by Blocking Inhibitory Input is Associated with a Loss of FM Direction Selectivity.** The left column shows the TTI functions of 3 neurons, created using an excitatory tone and a tone from the low-frequency inhibitory sideband. The right column shows 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 was 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 change in DSI (same neuron as in (E)).

**Figure 7: FM Rate Selectivity was Largely Resistant to Blocking Inhibition and only Affected by Removal of Glycinergic Input.** A: Lack of effect of GBZ on fastpass FM rate selectivity. Vertical black arrows and horizontal dashed lines indicate 50% cutoff rates. Response shown as percentage of maximum. Actual maximum spike counts are
34 (baseline) and 51 (GBZ). B: Effect of GBZ on the 50% cutoff (CO) rate across the
recorded population (n = 16, p < 0.05). C: Same as (C) in normalized format. D: Only
STRYCH was effective at eliminating fastpass FM rate selectivity in a minority of
neurons tested (33%), as indicated here by the response to slow FM sweep rates
following drug application. Actual maximum spike counts are 45 (baseline) and 35
(STRYCH). E: Effect of STRYCH on 50% cutoff rates across the recorded population (n
= 21, p < 0.05). Horizontal black arrow indicates the loss of FM rate selectivity (50%
cutoff rate = 0) observed in 7 neurons following STRYCH application. Closed circles
and vertical bars in B and D represent mean ± S.E.M. F: Same as (E) in normalized
format.

Figure 8: HFI is Associated with the Formation of FM Rate Selectivity. The
contribution of HFI on fast-pass FM rate selectivity can be tested by removing the high-
frequency region from a downward FM sweep. A: This neuron lost its fastpass rate
selectivity and responded to slow FM sweeps when frequencies above 41 kHz were
removed from the downward sweep. B: The excitatory and inhibitory frequency bands of
the neuron in (A), showing that starting the downward sweep at 41 kHz removed HFI.

Figure 9: Elimination of HFI by Blocking Inhibitory Input is Associated with a Loss
of FM Rate Selectivity. The TTI functions (left column) and sweep rate functions (right
column) of two neurons. A: GBZ application only slightly altered HFI in this neuron as
indicated by the slight shift in arrival time (black arrows). B: Similar to the lack of effect
on HFI, fastpass FM rate selectivity was also unaffected in this neuron (same neuron as in
(A), as indicated by the relatively small change in the 50% cutoff rate. C: STRYCH application eliminated HFI in this neuron as indicated by the inability of a high-frequency tone to inhibit a response. D: Similar to the elimination of HFI, fastpass FM rate selectivity was also eliminated (same neuron as in (C)), as indicated by the response to slow FM sweeps following drug application.

**Figure 10: Overlapping but Offset Inhibitory Receptive Fields Shape FM Selective Responses in the IC of the Pallid Bat.** The IC integrates excitatory and inhibitory inputs from multiple sources of the lower auditory system. Based on the data presented here, pallid bat IC neurons appear to consist of overlapping but offset GABAergic and glycinergetic receptive fields critical to the formation of FM selectivity. The low-frequency region appears to be composed of both GABAergic and glycinergetic input while the high-frequency region is composed exclusively of glycinergetic input.
References:


Gittelman JX, Pollak GD. It's about time: how input timing is used and not used to create emergent properties in the auditory system. J Neurosci 31: 2576-2583, 2011.


Heil P, Rajan R, Irvine DR. Sensitivity of neurons in cat primary auditory cortex to
tones and frequency-modulated stimuli. I: Effects of variation of stimulus

Huffman RF, Argeles PC, Covey E. Processing of sinusoidally frequency modulated
signals in the nuclei of the lateral lemniscus of the big brown bat, *Eptesicus

Klug A, Park TJ, Pollak GD. Glycine and GABA influence binaural processing in the

Koch U, Grothe B. GABAergic and glycinergic inhibition sharpens tuning for frequency

Kulesza RJ, Kadner A, Berrebi AS. Distinct roles for glycine and GABA in shaping
the response properties of neurons in the superior parolivary nucleus of the rat. *J

Kutscher A, Covey E. Functional role of GABAergic and glycinergic inhibition in the
intermediate nucleus of the lateral lemniscus of the big brown bat. *J Neurophysiol*

LeBeau FE, Malmierca MS, Rees A. Iontophoresis in vivo demonstrates a key role for
GABA(A) and glycinergic inhibition in shaping frequency response areas in the

Lu Y, Jen PH. GABAergic and glycinergic neural inhibition in excitatory frequency

Mendelson JR, Cynader MS. Sensitivity of cat primary auditory cortex (AI) neurons to

Merchan M, Aguilar LA, Lopez-Poveda EA, Malmierca MS. The inferior colliculus
of the rat: quantitative immunocytochemical study of GABA and glycine.

Nataraj K, Wenstrup JJ. Roles of inhibition in creating complex auditory responses in
the inferior colliculus: facilitated combination-sensitive neurons. *J Neurophysiol*

Nataraj K, Wenstrup JJ. Roles of inhibition in complex auditory responses in the
inferior colliculus: inhibited combination-sensitive neurons. *J Neurophysiol* 95:


Figure 1

A

![Line graph showing spikes against FM Rate (kHz/ms). The graph compares different conditions: baseline, GBZ, STRYCH, recovery, and balance.](image)

B

![Bar chart showing spikes across different conditions: baseline, GBZ, STRYCH, recovery, and balance. The STRYCH condition has a significant increase indicated by an asterisk.](image)
Figure 2

A. FM Sweep Response

- Spikes vs. FM Rate (kHz/ms)
- FM 55-25

B. Sideband Inhibition

- Arrival Time of Inhibition (ms) vs. Frequency (kHz)
- LFI, excitatory, HFI

C. Two-Tone Inhibition

- Spikes vs. Delay Between Tones (ms)
- LFI, HFI, 40 kHz, 43 kHz, 38 kHz
Figure 3

A

B

DSI (normalized)

0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4

baseline GBZ

FM Rate (kHz/ms)

0.1 1 10 100

Spikes

FM 60-30 (baseline)

DSI = 1.00 (baseline)

FM 30-60 (baseline)

DSI = 0.10 (GBZ)

FM 60-30 (GBZ)

DSI = 0.14 (STRYCH)

FM 30-60 (GBZ)

DSI = 0.79 (baseline)

FM 45-15 (baseline)

DSI = 0.14 (STRYCH)

FM 15-45 (baseline)

FM 45-15 (STRYCH)

FM 15-45 (STRYCH)

FM Rate (kHz/ms)

0.1 1 10 100

Spikes

B

DSI

0.0
0.2
0.4
0.6
0.8
1.0

baseline

GBZ

DSI (normalized)

0.0
0.2
0.4
0.6
0.8
1.0

baseline

GBZ

DSI (normalized)

0.0
0.2
0.4
0.6
0.8
1.0

baseline

GBZ

DSI = 1.00 (baseline)

DSI = 0.10 (GBZ)

DSI = 0.79 (baseline)

DSI = 0.14 (STRYCH)

n=4

n=7

A

B

C

D

E

F
Figure 4

A. Neuron #1

B. Neuron #2

C. Neuron #3

D. Neuron #4

E. Neuron #5

F. Neuron #6

G. Neuron #7

H. Neuron #8

I. Neuron #9

FM Rate (kHz/ms)
Figure 5

A

![Graph showing spikes vs. FM rate (kHz/ms)]

- FM 65-45
- FM 45-65
- FM 55-65

B

![Graph showing arrival time of inhibition vs. frequency (kHz)]

- (+)
- (-)

- FM 55-65
- FM 45-65
- FM 65-45
Figure 6

(A) Low-Frequency Inhibition

(B) FM Response

(C) Low-Frequency Inhibition

(D) FM Response

(E) Low-Frequency Inhibition

(F) FM Response
Figure 7

A. GABA

B. 50% CO Rate (kHz/ms)

C. 50% CO Rate (normalized)

D. glycine

E. 50% CO Rate (kHz/ms)

F. 50% CO Rate (normalized)

FM 65-35

FM 70-50

no rate selectivity

n=6

baseline GBZ

baseline STRYCH

FM Rate (kHz/ms)

Response (normalized)

response (normalized)

FM Rate (kHz/ms)

Response (normalized)

FM Rate (kHz/ms)

Response (normalized)
Figure 9

A. High-Frequency Inhibition

B. FM Response

C. Delay of Excitatory Tone (ms)

D. FM Rate (kHz/ms)

% Response (normalized)

50% tone response

Delay of Excitatory Tone (ms)

% Response (normalized)

50% tone response

Delay of Excitatory Tone (ms)

% Response (normalized)

50% tone response

Delay of Excitatory Tone (ms)

FM Rate (kHz/ms)
Figure 10

GABAergic Input  
(-) 

Glycinergic Input  
(+), (-) 

LFI  
(GABA/glycine) 

HFI  
(glycine) 

Intensity  

Excitatory Input  

Frequency