Facilitatory Mechanisms Shape Selectivity for the Rate and Direction of FM Sweeps in the Inferior Colliculus of the Pallid Bat

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INTRODUCTION

FM sweeps are important and ubiquitous components of vocalizations. It is therefore not surprising that the neural mechanisms that process these spectrotemporally dynamic signals have received considerable attention for over four decades. In one of the earliest papers on this subject, Whitfield and Evans (1965) noted that some cortical neurons responded only to FM sweeps and that their responses could not be predicted by tones, anticipating the concepts of nonlinearity and spectrotemporal inseparability that are currently used (Andoni et al. 2007; Depireux et al. 2001; Linden et al. 2003) to describe the filters that shape such response selectivity. Whitfield and Evans further noted that some cortical neurons exhibited sweep direction selectivity for narrowband sweeps confined to their tonal response areas, suggesting that excitation is inherently coupled to the precise sequence of excitatory and inhibitory synaptic activation generated by spectral motion over a fraction of their receptive fields.

As also noted in the visual system (Barlow and Levick 1964; Livingstone 1998; Reid et al. 1991; Sillito 1977), the fundamental requirement for a preference for the direction of motion across a sensory surface is some form of asymmetry. This asymmetry has been modeled as the spatiotemporal integration of postsynaptic events (Gulledge et al. 2005; Rall 1959; Segev 1992; Softky 1994) as the result of interactions between networks of neurons (Gordon and O’Neill 1998; Suga 1965) and correlated with differential low- and high-frequency sideband inhibition (Brett and Starr 1976; Heil et al. 1992; Shannon-Hartman et al. 1992; Suga 1965; Zhang et al. 2003). The relative arrival times, bandwidths, and spectra of inhibitory and excitatory inputs can influence both sweep direction and rate selectivity (Andoni et al. 2007; Fishbach et al. 2003; Fuzessery et al. 2006; Gordon and O’Neill 1998). It has also been suggested that the precise timing of excitatory inputs and/or rebound from inhibition, occurring only during an FM sweep of given direction and rate, may coincide and summate to produce a response (Casseday et al. 1997; Covey and Casseday 2006; Gordon and O’Neill 1998). We have recently conducted a similar study in the auditory cortex of this species (Razak and Fuzessery 2008) and can therefore examine the transformation of this response selectivity at two levels of its auditory system. Most importantly, some of the mechanisms shaping selectivity for sweep direction and rate in the IC and auditory cortex have been documented (Fuzessery et al. 2006; Razak and Fuzessery 2006). In the IC, selectivity for the downward sweep direction of the echolocation pulse can be created by a low-frequency inhibitory input that arrives earlier than excitation during the course of an upward sweep. Sweep rate selectivity for downward sweeps is shaped by either an early on-best frequency inhibition that creates shortpass duration tuning (Fuzessery and Hall 1999) or a delayed high-frequency inhibitory input that allows responses to fast sweep rates but suppresses the response as sweep rate slows, thus creating a fastpass filter (Fuzessery et al. 2006). The above results were obtained using a two-tone inhibition.
(TTI) paradigm (Faure et al. 2003; Fuzessery et al. 2006; Gordon and O’Neill 1998), which is unfortunately useless when dealing with specialized neurons that do not respond to tones. Instead, a two-tone facilitation paradigm (TTF) (Finn et al. 2007; Jen et al. 2002; Nataraj and Wenstrup 2005; Razak and Fuzessery 2008) was used to simulate stimulus conditions present in FM sweeps. As reported in the auditory cortex (Razak and Fuzessery 2008), the excitatory spectrottemporal relationships of tone pairs predict selectivity for sweep direction and rate. Echoing the early results of Whitfield and Evans (1965), we report that these FM specialists retain their direction and rate selectivity even when presented with remarkably narrowband sweeps of 1–2 kHz, indicating that the asymmetrical spectrottemporal facilitation that shapes their selectivity is produced over a very narrow frequency range.

**METHODS**

Extracellular single-unit recordings were obtained from the central nucleus of the IC of adult pallid bats. Bats were 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 animal facility. The bats were fed mealworms raised on ground Purina rat chow. All surgical procedures, animal welfare, and experimental manipulations were approved by an 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). Briefly, bats were anesthetized with methoxyflurane, followed by an intraperitoneal bolus injection of pentobarbital sodium (30 mg/kg of body weight) and acepromazine (2 mg/kg of body weight). The level of anesthesia was light enough to allow the bats to respire on their own. On loss of reflexive responses to a toe pinch, animals were placed in a bite bar, and a midline incision was made in the scalp. Bats typically required at least one additional injection of anesthetic during a 6-h experiment, at one third of the presurgical dose. Pentobarbital is known to increase the relative strength of anesthetic during a 6-h experiment, at one third of the presurgical dose. Pentobarbital is known to increase the relative strength of anesthetic during a 6-h experiment, at one third of the presurgical dose.

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The muscles over the dorsal surface of the skull were carefully excised from the skull with a microscalpel. Exposed muscle was covered with petroleum jelly, and the brain was kept moist with periodic applications of physiological saline. After surgery, the animals were taken to the recording chamber and secured in a Plexiglas restraining device. An aluminum head pin was mounted to a cross bar and secured to the anterior skull with dental cement.

**Recording procedures**

Bats were isolated in a heated (85–90°F), soundproofed chamber lined with anechoic foam. Auditory stimuli were generated by digital hardware (Modular Instruments and Tucker Davis Technologies) controlled by custom software (Fuzessery et al. 1991). Waveforms were amplified with a stereo amplifier and presented as closed-field stimuli through Infinity emit-K ribbon tweeters with funnels for insertion into the pinnae close to the external meatus. Speaker output was calibrated with a Bruel and Kjaer 1/8-in microphone placed at the tip of funnel (±15 dB response from 20 to 70 kHz).

Extracellular single-unit recordings were obtained with glass microelectrodes (1 M NaCl, 2–5 MΩ resistance) inserted orthogonal to the surface of the IC. Poststimulus time histograms (PSTHs) were used to record the number and temporal pattern of responses to the 30 successive stimulus presentations at intervals of 400 ms. Sounds were presented at a single intensity level 5–10 dB above threshold, with rise/fall times of 0.5 ms.

**Data acquisition protocol**

Data were recorded from the high-frequency region of the pallid bat IC [best frequencies (BF) = 30–60 kHz] using a recording protocol similar to that of Razak and Fuzessery (2008). First, the spectral width of the excitatory frequency domain was determined by the responses to individual tones (if present) or by using narrowband (1–6 kHz) FM sweeps for FM specialist neurons that responded maximally only to FM sweeps, and exhibited <20% of maximum response to tones (Fuzessery 1994). Broad FM sweeps (30–40 kHz bandwidths) were presented over a range of durations (0.5–100 ms) to establish the direction and rate selectivity of each cell. Pairs of pure tones delayed in time were used to establish spectral domains of facilitation and inhibition using the TTF and masking inhibition protocols described below.

**FM direction and rate selectivity**

To quantify FM direction selectivity, the maximal responses to upward and downward FM sweeps of the same bandwidth were compared with calculate a direction selectivity index (DSI): DSI = (D – U)/(D + U). D and U represent the maximum responses (number of spikes) to the upward and downward FM sweeps obtained at any sweep duration. Values of DSI ranged between −1 and +1, with positive values representing selectivity for downward sweeps. A cutoff DSI value of ≥0.3 was used to define the presence of direction selectivity; this represents a ≥50% response to one sweep direction over the other.

FM rate selectivity was quantified as the best rate and 50% cut-off rate of the FM sweep response (Fuzessery et al. 2006). Best rate was calculated as the median rate above 80% of the maximal response and could be calculated only for neurons with band-pass rate selectivity. The 50% cut-off rate was calculated as the slowest sweep evoking half the maximal response and could be calculated for both band-pass and fastpass rate-selective neurons. A neuron was tested for rate selectivity by first obtaining its response to a range of sweep rates, using three or more FM sweeps with similar bandwidths, but different sweep spectra. If the response magnitudes at a given sweep rate for all sweeps were within 20% of one another, the neurons was considered sweep rate selective.

**TTF**

Tone pairs (1 ms, 0.5 ms rise/fall time) near the center of the excitatory region (5–10 dB above threshold for response to FM sweeps) were used to characterize facilitatory responses that might occur during the course of an FM sweep. Facilitation was defined as a response to two tones that was ≥50% greater than the sum of responses to each individual tone. The time delay between presentation of the first and second tone was varied to characterize any preference for presentation of either the low- or high-frequency tone first and the optimal time delay between the tones.

The excitatory spectrottemporal combinations of tone pairs typically predicted neuronal selectivity for FM sweep parameters. To predict direction selectivity, responses were compared for each direction of tone presentation. Analogous to DSI calculation, an asymmetry index
(ASI) was calculated for each neuron: \( ASI = (H - L)/(H + L) \), where \( H \) and \( L \) represent the maximal responses (highest number of spikes) that occurred when the high- (HF) or low-frequency (LF) tone was presented first (Fig. 1) (Razak and Fuzessery 2008). The ASI is presented as the average value from multiple TTF pairs. The ASI ranged between \(-1\) and \(+1\), with positive values representing a preference for the HF tone presented first. As with DSI values, a cut-off ASI value of \( \pm 0.3 \) was used to define the presence of direction selectivity, which represents a 50% greater response to the tones presented in a particular direction.

The TTF data were used to predict the best sweep rates and 50% cut-off sweep rates and also responses over a range of sweep rates. Predicted best FM rates and 50% cut-off values were based on measurements of the best delay and 50% cut-off delay for each pair of tones (Razak and Fuzessery 2008). The best delay was the median delay value of a range of delay values that evoked 80% of maximum response. The 50% cut-off delay represented the longest delay between the tones that produced 50% of the maximal response; this was correlated to the slowest FM sweep rate that produced a 50% maximal response. These sweep rates were calculated from the TTF data as the spectral difference between tones (kHz) divided by the delay (ms) between tones.

To predict response to a range of sweep rates, each data point within the TTF curve was used to represent a specific FM rate. The predicted responses to a range of FM rates were calculated for nine neurons for which multiple TTF pairs were evaluated (3–5 pairs) and represent the average of 15–26 data points/neuron in 1 kHz/ms bins. The predicted range of preferred rates was only calculated for positive TTF delays in which the HF tone was presented first; they are compared with the actual responses to downward FM sweeps.

When possible, responses to multiple tone pairs varying in frequency difference were used to determine whether sweep rate selectivity could still be predicted. Finally, the duration of the HF tone was gradually increased (1–10 ms) to determine whether facilitation was occurring at stimulus onset and/or offset of the HF tone (Razak and Fuzessery 2008).

**Forward masking inhibition**

Forward masking inhibition experiments were conducted on FM specialists to determine whether tones that evoked facilitatory responses at appropriate delays were also able to suppress responses to excitatory sweeps, as suggested by a previous study (Fuzessery 1994). Narrowband downward FM sweeps (1–3 ms duration) were used to excite the neurons. Similar to the TTF paradigm, this excitatory probe stimulus was paired with an individual tone (1 ms) and the delay between sounds varied to determine the timing and duration of inhibition associated with the masker tone. Inhibition was defined as a >50% reduction in the number of spikes to the probe stimuli.

**Data presentation and analysis**

Response magnitudes (number of spikes) created by acoustic stimuli were reported as the number of action potentials in response to 30 stimulus repetitions presented every 400 ms and plotted for graphical interpretation. PSTHs were used to quantify the average timing of responses to each of the 30 stimulus repetitions. Pearson’s correlations were used to assess the relationship between calculated response parameters associated with the prediction of FM rate and direction selectivity. Group averages are presented as the mean \( \pm SD \). The differences in DSI values, with and without the contribution of the low-frequency region of an FM sweep, were evaluated by paired Student t-test. \( P < 0.05 \) was considered significant.

**RESULTS**

Single-unit recordings were obtained from 58 FM specialists within the IC to assess the contribution of facilitation to FM sweep selectivity. A neuron was classified as an FM specialist if the response to tones was <20% of the response to FM sweeps (Fuzessery 1994). The great majority of FM specialists tested (41/47, 87%) preferred downward over upward FM sweeps based on their DSI (DSI > 0.3). The remaining six neurons responded to both upward and downward sweeps (\(-0.3 < DSI < 0.3\)). None preferred upward FM sweeps (DSI < \(-0.3\)). This pronounced preference for the downward sweep direction of the bat’s echolocation pulse is in agreement with the results of earlier studies (Fuzessery 1994; Fuzessery et al. 2006) of the pallid bat IC.

Figure 1A shows a representative example of an FM specialist neuron. When presented with broadband sweeps of 25→55 and 55→25 kHz, this neuron responded only to the downward direction and consequently had a DSI = 1.0. This neuron was also selective for sweep rate. It was band-pass tuned, with a best sweep rate of \( \sim 3 \) kHz/ms. Of 33 neurons tested for sweep rate selectivity, 26 (78%) were either band-pass or fastpass rate selective.

In previous studies (Fuzessery et al. 2006; Razak and Fuzessery 2006), we used TTI to examine the inhibitory mechanisms that shape sweep direction and rate selectivity. TTI

![Fig. 1. Two-tone facilitation (TTF) in an FM specialist. A: an FM specialist selective for the sweep direction and rate of a 55→25 kHz downward sweep but unresponsive to an upward sweep of identical bandwidth. B: the TTF function of the neuron in A when presented with tones of 38 and 36 kHz delayed in time. Positive delays indicate the HF tone precedes the LF. Note the sharp response peak between 0.25 and 1.25 ms delay, with the high-frequency tone preceding the response to the individual tones.](/jn.physiology.org/abstract/282610801)
could not be used in this study because FM specialists respond weakly or not at all to tones. Instead, TTF was used, using pairs of tones delayed in time to determine whether the facilitatory frequency/delay combinations could be used to predict both sweep direction and rate selectivity (Razak and Fuzessery 2008). Figure 1B shows the TTF function of the neuron in Fig. 1A when tested with 1 ms duration tones of 36 and 38 kHz. The horizontal dashed line is the sum of responses to each tone alone. Note that the neuron’s response was greatly facilitated when the HF tone preceded the LF within a narrow range of delays and that it was suppressed when the tone sequence was reversed. This asymmetrical facilitation is what would be expected of a neuron that responded exclusively to downward sweeps, assuming that the tone pairs were able to simulate conditions in an FM sweep. As will be shown, there was a significant linear correlation between a neuron’s degree of asymmetrical facilitation and its FM direction and rate selectivity. Conversely, neurons that did not exhibit asymmetrical TTF, or any TTF at all, had less selectivity for FM sweep properties.

Figure 2 shows the variety of TTF responses observed in this study. Facilitation ranged from strongly asymmetrical (Fig. 2, A and C) to symmetrical (Fig. 2, B and D). Of 58 neurons tested, 21 (36%) exhibited TTF. The majority of these neurons tested (18/21, 86%) had asymmetrical TTF responses, with all preferring presentation of the HF followed by the LF. Symmetrical TTF responses were observed in the remaining three neurons (14%). None of the 21 neurons tested preferred the LF tone presented first. The remaining 37 neurons (64%) did not exhibit TTF; it is not clear whether they actually lacked this facilitatory mechanism or whether the use of two tones did not adequately simulate the facilitatory events that might have been evoked by an FM sweep.

In neurons that exhibited a single TTF response peak at or near 0 ms delay (e.g., Fig. 2D), this increase in response may have been the result of a transient increase in intensity level caused by amplitude modulations when two tones are combined (Hartman 1998) rather than a facilitatory spectrotemporal interaction. Three of the four neurons exhibiting this TTF profile did in fact respond to individual tones at higher intensities, suggesting that this may have been the case. Thirty-three percent (19/58) of all neurons tested did respond to tones at higher intensities. However, the majority remained FM specialists at all intensity levels tested.

Role of facilitation in shaping direction and rate selectivity

To what extent does TTF in FM specialists contribute to their selectivity for sweep direction and rate? This issue is addressed with three approaches. The first is to define their excitatory receptive fields with narrowband FM sweeps and show that selectivity persists when narrowband sweeps within these fields are used. The second is to determine whether frequencies outside of the excitatory receptive fields also contribute to FM sweep selectivity, as has been shown in neurons that respond to tones and sweeps (Fuzessery et al. 2006; Razak and Fuzessery 2006) or whether frequencies within the excitatory field are sufficient. Finally, can the spectrotemporal parameters that elicit maximal TTF be used effectively to predict selectivity for FM sweep direction and rate? This provides correlational evidence of its role.

FM tuning curves

We begin with examples of excitatory receptive fields and how they influence response selectivity (Fig. 3). If two tones closely spaced in frequency, as used in the TTF paradigm, can approximate the responses to broadband sweeps in different directions (Fig. 1), it is likely that only a small part of the sweep spectrum may be needed to produce this selectivity.
Because these neurons did not respond to single tones, narrowband FM sweeps were used to map excitatory receptive fields (Figs. 3–5). These are termed FM tuning curves. For example, the neuron in Fig. 3A preferred downward broadband sweeps (Fig. 3B, 45–15 kHz). The same was true for 4 kHz narrowband sweeps (Fig. 3A). A narrowband, downward 28–34 kHz sweep did not evoke a response, indicating the LF border was around 34 kHz. At higher frequencies, a downward 36–32 kHz sweep did not evoke a response, indicating the HF border was around 34 kHz. This defines the neuron’s FM tuning curve.

Surprisingly, a sweep through only a fraction of an FM tuning curve, covering as little as 1 kHz, was, in the majority of cases, sufficient to produce both direction and rate selectivity. Not all neurons were tested with FM sweeps as narrowband as 1 kHz, but of those that were, 6/8% remained rate selective, and 13/18% remained direction selective.

Figure 3 shows the two general classes of FM specialists that were observed, based on responses to narrowband FM sweeps: neurons that responded exclusively to downward FM sweeps (Fig. 3A) and neurons that responded to both upward and downward narrowband FM sweeps (Fig. 3, C and E). Figure 3 also shows the responses of these neurons to broadband and narrowband sweeps in opposite directions (Fig. 3, right).

The response selectivity of the downward FM specialist shown in Fig. 3A was observed in 67% (12/18) of neurons tested. This neuron did not respond to narrowband upward FM sweeps, regardless of the absolute frequencies of the 4 kHz bandwidth sweeps used, showing a complete preference for the downward FM sweep direction throughout its FM tuning curve. It exhibited the same direction preference for 30 kHz broadband sweeps (Fig. 3B), although it is apparent that only a fraction of this broadband sweep was required to produce this selectivity. The neuron also exhibited sweep rate selectivity for both narrowband and broadband downward sweeps. Picking a narrowband sweep near the center of its excitatory receptive field (28–32 kHz) and varying the sweep duration showed a band-pass rate tuning preference between 0.5 and 1 kHz/ms (Fig. 3B). In response to broadband sweeps, the best rate rose to around 3 kHz/ms. In this and other neurons, rate selectivity sometimes changed with sweep bandwidth. It is possible that other mechanisms evoked by frequencies outside of the FM tuning curve (Fuzessery et al. 2006) may have also influenced its rate selectivity for downward sweeps. However, the salient

**FIG. 3.** The variety of FM tuning curves (left) and their responses to broadband and narrowband FM sweeps (right). A: a neuron that maintained its strong selectivity for narrowband downward sweeps throughout its FM tuning curve. C: a neuron that responded to both sweep directions and that had similar FM tuning curves in both sweep directions. E: a neuron with offset FM tuning curves for upward and downward sweeps. Right: these neurons differed in how they responded to broadband and narrowband sweeps and differed in whether they lost direction and rate selectivity when presented with narrowband sweeps.
point is that the sweep rate and direction selectivity of this neuron persisted for narrowband sweeps that remained within the FM tuning curve. It is likely that the strongly asymmetrical TTF (ASI = 1.0) exhibited by this neuron contributed to this selectivity.

The second class of neurons responded to both upward and downward narrowband FM sweeps and represented 33% (6/18) of the neurons tested. Their FM tuning curves could either overlap (Fig. 3C) or be shifted in frequency (Fig. 3E). The neuron in Fig. 3C responded similarly to upward and downward narrowband (DSI = 0.05) and broadband (Fig. 3D) sweeps. This neuron did not exhibit TTF. Although not direction selective, the neuron was band-pass rate selective for both narrowband and broadband sweeps (Fig. 3D), showing that the mechanism evoked by narrowband sweeps can shape rate selectivity independently of direction selectivity.

A shift in FM tuning curves with the direction of narrowband sweeps is seen in Fig. 3E. This neuron responded to broadband sweeps in either direction (DSI = 0.32; Fig. 3F), but response magnitude for downward sweeps was approximately twice that evoked by upward sweeps. In contrast to the previous neurons, this cell lost rate selectivity when presented
The assumption was that if neurons responded to upward sweeps, we eliminated lower frequencies by starting an upward sweep. Both frequency bands exert that action, whereas higher frequencies can shape rate selectivity for frequencies shaped direction selectivity. The neuron was strongly direction selective when presented with broadband sweeps but responded to upward sweeps at slow sweep rates when lower frequencies were eliminated (sweep 27-45 kHz), suggesting that lower frequencies shaped direction selectivity. However, the role of facilitation in shaping selectivity was tested by eliminating higher frequencies by starting a downward sweep at the high-frequency border of an FM tuning curve. Fifteen of these neurons were tested with upward sweeps starting within their FM tuning curves (Fig. 6A) to determine whether eliminating lower frequencies allowed the neuron to respond to upward sweeps and thus lose direction selectivity. Some cells completely or partially lost lower frequencies outside of the FM tuning curves played an important role. Conversely, if neurons still did not respond to upward sweeps, facilitation within the FM tuning curve was sufficient to shape direction selectivity.

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At the other extreme, the neuron in Fig. 5, although an FM specialist, did not exhibit TTF. It responded similarly to narrowband sweeps in either direction, and, like the neuron in Fig. 3E, had spectral offsets in the frequencies evoking maximum responses to upward and downward tuning curves. It did not respond to broadband upward sweeps (Fig. 5B; 15-45 kHz), but when the upward sweep began within the tuning curve (27-45 kHz), the neuron responded, indicating that lower frequencies shaped direction selectivity. The neuron was band-pass rate selective for broadband downward sweeps (45-15 kHz), but when the sweep started within the tuning curve (33-15 kHz), rate selectivity was lost, suggesting that higher frequencies created this selectivity. Both the absence of TTF and the finding that frequencies outside of the FM tuning curve are needed for selectivity to be expressed suggest that, for this neuron, facilitation played little or no role in shaping response selectivity.

**FM direction selectivity**

Figure 6 and Table 1 summarize the role of facilitation in shaping selectivity for the downward sweep direction. There was a strong correlation in the ASI and DSI of 21 neurons that exhibited TTF ($r^2 = 0.542, P < 0.001$). Neurons with high, positive ASI values, indicating a strong preference for HF tones followed by LF tones, also showed a strong preference for downward sweeps. Fifteen of these neurons were tested with upward sweeps starting within their FM tuning curves (Fig. 6A) to determine whether eliminating lower frequencies allowed the neuron to respond to upward sweeps and thus lose direction selectivity. Some cells completely or partially lost direction selectivity, whereas the majority did not. These results suggest that, in some FM specialists, direction selectivity is shaped entirely by lower frequencies, whereas in others, facilitation is the dominant mechanism. In cases where there is only a partial loss of selectivity, both mechanisms may contribute to selectivity. Whether direction selectivity was lost seems to be related to the ASI of a neuron. On removal of lower frequencies, only 1 of 12 neurons with high ASI values ($>0.3$) lost direction selectivity, whereas the 2 neurons with low ASI values (symmetrical TTF functions) both lost directionality (Table 1).
The first approach is shown in Fig. 7. This neuron was band-pass rate selective (Fig. 7A), with a best rate of 1.1 kHz/ms and a 50% cut-off rate of 0.8 kHz/ms. Its TTF function (Fig. 7B) was generated with two tones separated by 1 kHz (30 and 31 kHz). Its best delay was 0.95 ms, which would simulate a sweep rate of 1.05 kHz/ms (1 kHz tone spectral difference/0.95 ms delay), which is close to the actual best sweep rate. Its predicted 50% cut-off rate is 0.85 kHz/ms, which is also close the actual value of 0.80 kHz/ms.

To test the range of frequencies and delays over which two tones would interact, and the accuracy with which they would predict rate selectivity, 9 of the 17 rate-selective neurons exhibiting TTF were tested with at least three different frequency combinations. The hypothesis was that, if TTF responses continued to predict rate selectivity, the best delay should increase with spectral difference. Neurons exhibited a range of responses that are represented by the three neurons in Fig. 7, C–H. When tone differences were increased over a narrow range, the neuron in Fig. 7C maintained peaked TTF functions of similar width, but with the expected shift in best delay. Consequently, the TTF functions continued to predict the best rate of the neuron (Fig. 7D). The second neuron (Fig. 7E) showed little shift in best delay, despite a larger change in spectral difference. However, as the spectral difference increased, the magnitude of the facilitatory response decreased, suggesting that, for this neuron, maximum facilitation occurred at a fixed delay around 1 ms. Consequently, the ability of the best delays to predict best rate also decreased with increasing spectral difference (Fig. 7F). Apparently, the interactions between the inputs generated by the two tones decreased with increasing spectral difference. The third neuron (Fig. 7G) exhibited yet another pattern. As spectral differences increased, the rise of the TTF functions from 0 ms delay remained relatively fixed, but the peaks broadened. The inputs generated by the two tones apparently interacted over a broader range of delays. The best delays calculated from the centers of these peaks accurately predicted the actual best rate of the neuron (Fig. 7H).

Figure 8 summarizes the predictions of best FM rate for 17 neurons tested. Predicted rate values are the averages across all tone combinations tested, as described above (Fig. 7). There was generally a strong correlation. Ten of these neurons were also tested with both downward broadband sweeps (filled

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**FM rate selectivity**

Of 33 FM specialists tested, 26 (78%) exhibited fastpass or band-pass sweep rate selectivity. Sixty-five percent (17/26) of these also exhibited TTF. The role of facilitation in shaping rate selectivity was tested in two ways: by predicting rate selectivity from TTF responses and by eliminating the influence of higher frequencies, which can shape rate selectivity.

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**TABLE 1. TTF and FM direction selectivity**

<table>
<thead>
<tr>
<th>TTF</th>
<th>Symmetry Index, ASI</th>
<th>FM Direction Selective, +LF</th>
<th>FM Direction Selective, −LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymmetrical</td>
<td>ASI &gt; 0.3</td>
<td>18/18 (100%)</td>
<td>11/12 (92%)</td>
</tr>
<tr>
<td>Symmetrical</td>
<td>−0.3 &lt; ASI &lt; 0.3</td>
<td>2/3 (67%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>Asymmetrical</td>
<td>ASI &lt; −0.3</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>No facilitation</td>
<td>—</td>
<td>18/23 (78%)</td>
<td>2/10 (20%)</td>
</tr>
</tbody>
</table>

Descriptive comparison of the number of FM specialists exhibiting TTF compared with the corresponding direction selectivity for FM sweeps. Neurons were classified according to the symmetry of TTF responses (ASI values). Positive ASI values represent a preference for HF to LF tone presentation, whereas negative ASI values represent a preference for LF to HF presentation. The number of FM direction-selective neurons are compared with (+) and without (−) the contribution of lower frequencies (LF) to direction selectivity by removing the low-frequency region from the upward FM sweep. All FM direction-selective neurons preferred a downward FM sweep. TTF, two-tone facilitation; ASI, asymmetry index; HF, high frequency; LF, low frequency.
circles connected to open circles) and downward sweeps that started within their FM tuning curves to determine whether the removal of higher frequencies changed or eliminated rate selectivity. Five neurons showed a change in rate selectivity, with their best rates shifting to slower rates, suggesting that higher frequencies contributed to selectivity for faster sweep rates. In the remaining neurons that did not show a change in rate selectivity, it is possible that facilitation contributed strongly to rate selectivity. The contribution of higher frequencies differed among rate-selective neurons, depending on whether they exhibited TTF (Table 2). Among neurons exhibiting TTF, 83% (10/12) retained rate selectivity when high

FIG. 7. Predicting sweep rate selectivity from multiple TTF functions. A: a rate function for a downward sweep, showing the best rate and 50% cut-off rate. B: the components of the TTF function used to predict the sweep rates in A. C, E, and G: the range of changes in TTF responses of neurons when the spectral difference was varied. The neuron in C showed a systematic increase in best delay (arrows) as spectral difference increased and predicted best rate accurately (D). The neuron in E showed little shift in best delay but rather a decrease in response as spectral difference increased and did not accurately predict best rate (F). The neuron in G showed a broadening of best delay peaks and a shift in best delay and accurately predicted best rate (H).
or offset of tones. Five IC FM specialists were similarly tested by increasing the duration of the preceding HF tone while LF tone duration was held constant (Fig. 11). Four of the five neurons exhibited the interaction shown in Fig. 11A. As the duration of the HF tone was increased by 1 ms, the best delay peak shifted accordingly, indicating that facilitation was occurring at the offset of the HF tone. The remaining neuron exhibited interactions at both sound onset and offset (Fig. 11B). At a duration of 1 ms, there was one sharp response peak at \(-1\) ms delay. At a 3 ms delay, the response peak broadened and began to break into two components. At 5 and 10 ms delays, the onset and offset response peaks are now separate and are approximately one half the amplitude of those evoked by the shorter HF tone durations. This change in peak amplitude suggests that, for shorter tone durations, the onset and offset components overlap and summate. As the tone duration increases, these events become temporally separate.

Their extreme selectivity for narrowband downward FM sweeps suggests that the FM specialists may be very sensitive to the spectral sequence and exhibit what is commonly referred to as spectrotemporal inseparability. If so, if a tone that is part of an excitatory narrowband sweep is presented out of sequence, it may prevent a response to the sweep. To test this hypothesis, we used a variation of the TTI paradigm, in which an excitationary narrowband sweep was pitted against a tone from the center of the sweep. All four neurons exhibited the type of inhibitory interaction shown in Fig. 12. In this example, a 4 ms duration, 38±34 kHz sweep was delayed with respect to a 1 ms duration, 36 kHz tone from the center of this sweep. As the FM sweep was delayed with respect to the tone, the response was suppressed but gradually returned as the delay was further increased. These results suggest that a frequency that may contribute to excitation in the appropriate spectrotemporal context is also capable of disrupting or suppressing the facilitatory interaction when that frequency is presented out of the correct sequence.

**DISCUSSION**

We have previously described three mechanisms that shape selectivity for the direction and rate of downward FM sweeps in the pallid bat IC (Fuzessery et al. 2006). Sweep rate selectivity in roughly one half of the neurons examined is shaped by a delayed HF inhibition that allows a response to fast downward sweeps traversing this inhibitory sideband. However, as sweep rate slows, the arrival of inhibitory inputs eventually coincides or precedes excitatory inputs and suppresses the response. In the remainder of the rate-selective neurons, their selectivity is hypothesized to be shaped by an early on-BF inhibition (Fuzessery and Hall 1999) that makes a

**TABLE 2. TTF and FM rate selectivity**

<table>
<thead>
<tr>
<th>TTF</th>
<th>FM Rate Selectivity, +HF</th>
<th>FM Rate Selectivity, −HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>17/19 (89%)</td>
<td>10/12 (83%)</td>
</tr>
<tr>
<td>No</td>
<td>9/16 (56%)</td>
<td>1/5 (20%)</td>
</tr>
</tbody>
</table>

Descriptive comparison of the number of FM specialists exhibiting TTF compared with the rate selectivity for downward FM sweeps. Preference for FM rate selectivity is compared with (+) and without (−) the contribution of higher frequencies (HF) by removing the high-frequency region from the FM sweep. See Table 1 for abbreviations.
neuron shortpass duration tuned for tones, as well as fastpass rate tuned for FM sweeps. If a tone is too long, or an FM sweep too slow, the early on-BF inhibition persists, overlaps with a delayed excitatory input, and suppresses the response. Selectivity for a downward sweep direction is created by an early LF inhibition that suppresses responses to upward sweeps when they traverse this inhibitory sideband. Thus inhibitory inputs triggered by frequencies inside and outside of the excitatory tuning curve shape FM sweep selectivity.

Here we described a fourth mechanism, facilitation, that can shape both sweep rate and direction selectivity. All neurons studied were downward FM specialists that did not respond to tones, and therefore their excitatory tuning curves could not be delineated. Their excitatory domains were instead defined with narrowband, downward FM sweeps, and termed FM tuning curves. The majority of neurons exhibited both FM sweep rate and direction selectivity for narrowband sweeps that covered only part of their FM tuning curves; the mechanisms that underlie this selectivity can be evoked within frequency bands as narrow as 1 kHz. These mechanisms may be both excitatory and inhibitory. The TTF method showed that responses are greatly facilitated when tone pairs are presented in the correct spectrotemporal sequence (high frequency followed by low frequency). When reversed, the response was less than the sum of the responses to each tone alone (e.g., Fig. 1), suggesting that an inhibitory mechanism contributes to this asymmetrical facilitation. The contribution of inhibition is also suggested by the finding that a frequency that is excitatory within a narrowband downward FM sweep will inhibit the response to that sweep if a tone of that frequency is presented at the same time (Fig. 12). Thus both excitation and inhibition may contribute to the precise spectrotemporal resolution displayed by these facilitated downward FM specialists.

Several findings suggest that facilitation within FM tuning curves is sufficient to create sweep rate and direction selectivity, independent of the other three mechanisms. First, narrowband sweeps that remain within the FM tuning curves are sufficient to evoke selectivity, indicating that frequencies outside of the tuning curves are not required. Second, the frequencies and timing of the two tones evoking maximum facilitation, in most cases, can reasonably predict rate selectivity for actual FM sweeps, which suggests that facilitation is the dominant mechanism. Third, we eliminated the contribution of frequencies higher and lower than the FM tuning curves by removing these frequencies from broadband FM sweeps and comparing the response selectivity evoked by FM sweeps that included these frequency domains. As seen in Fig. 6, neurons exhibiting TTF were less likely to lose selectivity than those that did not, suggesting that facilitation was sufficient for creating selectivity. We assumed that the elimination of frequencies outside of the FM tuning curves was most likely removing inhibitory sidebands that are known to shape the rate and direction selectivity of other FM selective neurons in the pallid bat IC (Fuzessery et al. 2006). However, because these FM specialists...
showed no response to tones, a property that is needed to test for sideband inhibition through the TTI paradigm, the presence of these sidebands could not be verified in this study.

On the other hand, facilitation may work in concert with the other three mechanisms to shape selectivity. Some FM specialists exhibiting facilitation showed a change in sweep rate selectivity when frequencies outside of FM tuning curves were eliminated from broadband sweeps (Fig. 8), suggesting more than one mechanism contributed to shaping rate selectivity.

We described facilitation only in FM specialist neurons because it is easiest to show facilitation through the TTF method in neurons that respond weakly, or not at all, to tones. We are not suggesting that the facilitation mechanism is present only in these highly specialized neurons. Indeed, previous studies that used similar TTF paradigms reported facilitation in IC and cortical neurons that did not respond exclusively to FM sweeps (Brosch and Schreiner 2000; Brimijoin and O’Neill 2005; Gordon and O’Neill 1998). In this study, a few of the neurons that exhibited TTF also responded to tones at higher sound intensities, suggesting that facilitation is not limited to FM specialists but is also present in less specialized neurons that respond to tones as well as FM sweeps.

A striking feature of the asymmetrical facilitation described here is the high degree of spectrotemporal resolution that seems to underlie it. Temporally, facilitated neurons were sensitive to delays between tones on the order of hundreds of microseconds. Spectrally, neurons displayed direction selectivity for FM sweeps with spectral bandwidths as narrow as 1 kHz, which constituted only a fraction of their FM tuning curves. The majority of neurons maintained this direction selectivity throughout their FM tuning curves, as first described in a study of cat auditory cortex by Whitfield and Evans (1965).

Also striking is that two tones separated in frequency by as little as 1 kHz were able to produce the asymmetrical facilitation. This bandwidth is narrower than the excitatory tuning curves of the great majority of IC neurons in the pallid bat (Fuzessery 1994) and likely narrower than the tuning curves of inputs to these neurons. Modeling the origin of this spectrotemporal resolution is therefore problematic. However, a recent whole cell recording study of the IC of another bat species (Xie et al. 2007) showed that the timing, duration, and magnitude of excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) could change significantly in response to tones presented in 1 kHz increments. How these subthreshold events interact in response to delayed tone pairs or narrowband FM sweeps in opposite directions may underlie the observed asymmetrical facilitation that shapes rate and direction selectivity. Indeed, intracellular recording of IC neurons responding to FM

![Fig. 10. Summary of the actual and predicted sweep rate ranges of 9 neurons, based on the method shown in Fig. 9.](image)

![Fig. 11. An examination of whether TTF occurs at stimulus onset or offset. A: 2 TTF functions obtained from the same neuron when the low-frequency tone duration was held constant at 1 ms and the HF tone duration set at 1 and 2 ms. Note the 1 ms delayed shift in the response peak, indicating that facilitation occurred at the offset of the HF tone. B: a neuron in which, as the HF tone duration was increased from 1 to 10 ms, 2 response peaks became apparent, suggesting that facilitation occurred to both the onset and offset of the HF tone.](image)
Mechanisms of facilitation

A general mechanism thought to account for the facilitation of subthreshold events in sensory systems is some form of asymmetrical organization of delay lines. In its simplest form, facilitation can occur in response to a stimulus traversing a sensory epithelium that sequentially excites inputs with longer to shorter latencies, resulting in an overlap of EPSPs. A stimulus in the opposite direction may be nonexcitatory or, as suggested above, inhibitory. In the rodent vibrissal system, facilitation can occur after stimulation across multiple whiskers, resulting in a specific set of spatiotemporal pattern (Egoshengel et al. 2005; Kida et al. 2005). Offsets in response latency have also been described in the visual system as slanted “space-time plots” recorded from motion-selective cells of the visual cortex (Livingstone 1998). For example, Priebe and Ferster (2005) have shown that, in direction-selective neurons of the IC (Andoni et al. 2007; Brimijoin and O’Neill 2010) and auditory cortex (deCharms et al. 1998; Depireux et al. 2001; Linden et al. 2003), particularly with models that take into account inherent nonlinearities (Ahrens et al. 2008). An- doni et al. (2007) found a strong correlation between an auditory neuron’s directional selectivity and its spectrotempo- ral inseparability index, derived from the spectrotemporal tilt of the STRF.

These results support the notion that both excitatory and inhibitory mechanisms contribute to the spectrotemporal inseparability observed within the FM tuning curves described here. The TTF paradigm showed that tone pairs delayed in time can result in either facilitation or suppression, depending on their temporal relationship. Moreover, frequencies that contribute to excitation when presented within an FM sweep can suppress responses to sweeps if presented simultaneously as tones, suggesting that frequencies must occur in the correct temporal sequence to contribute to excitation.

Similar mechanisms are thought to underlie selectivity for other types of complex sounds. Temporal combination sensi- tivity has been described in songbird forebrain neurons that may fail to respond to a single syllable within a song but are driven by the combination of two syllables present in the correct temporal order (Doupe 1997; Lewicki and Konishi 1995; Margoliash 1983). In the mustached bat IC, combina- tion-sensitive neurons serving echolocation or communication show facilitated responses to tone combinations presented in specific temporal relationships (Nataraj and Wenstrup 2005), much like the TTF reported in this study.

Possible synaptic mechanisms for facilitation

All neurons in this study that were tested for whether TTF occurred at the onset or offset of the first tone showed facilitation at offset or at both onset and offset. If TTF occurs first in the IC (which is not yet known), a possible explanation for facilitation at offset is that an excitatory rebound from inhibi- tion is part of the mechanism. A neuron may receive inhibitory input that lasts the duration of the tone and respond through postinhibitory rebound at the end of the tone. This rebound may overlap with an excitatory subthreshold event evoked by the second tone. The required coincidence of subthreshold events may arise, in part, through an excitatory rebound from inhibition overlapping with an EPSP produced by excitatory input (Cassey and Casseday 1997; Covey and Casseday 1999; Mar- goliash 1983). Alternatively, facilitation can also be produced by a coincidence of postinhibitory rebound from multiple inhibitory inputs (Peterson et al. 2009; Sanchez et al. 2008). Compelling support that this second mechanism is present in the IC is the blockade of glycine receptors and the subsequent loss of facilitation (Nataraj and Wenstrup 2005), suggesting input decreased in opposition to sweep direction, with the resulting overlap in EPSPs producing facilitation.

Inhibitory components have been added to this excitatory model. Neural models based on “rotatable” excitatory and inhibitory spectrotemporal fields (STRFs) have also been suc- cessful in predicting responses to FM stimuli in auditory neurons based on the manipulation of a small number of variables (Fishbach et al. 2003). Direct measurement of rotated or tilted STRFs, termed spectrotemporal inseparability, has been associated with selectivity for complex sounds in auditory neurons of the IC (Andoni et al. 2007; Brimijoin and O’Neill 2010) and auditory cortex (deCharms et al. 1998; Depireux et al. 2001; Linden et al. 2003), particularly with models that take into account inherent nonlinearities (Ahrens et al. 2008). An- doni et al. (2007) found a strong correlation between an auditory neuron’s directional selectivity and its spectrotempo- ral inseparability index, derived from the spectrotemporal tilt of the STRF.
that coincident inhibitory rebound is sufficient for creation of combination sensitivity. Functionally, it has been suggested (Person and Perkel 2005; Sanchez et al. 2008) that excitation through inhibitory rebound may provide greater temporal resolution than an EPSP generated through excitatory input. This is consistent with the submillisecond temporal resolution displayed by the neurons described in this study.

Speculation about the role of inhibitory inputs in exciting postsynaptic targets is of interest, but, in this study, whether this asymmetrical facilitation that shapes FM sweep rate and direction selectivity appears first in the IC cannot be resolved. FM sweep direction selectivity is not present in the auditory nerve of the cat (Sinex and Geisler 1981), but it has been suggested that cochlear mechanisms such as two-tone suppression (Gans et al. 2009; Kiang and Moxon 1974), and intermodulation distortions (Abel and Kossl 2009; Portfors et al. 2009) may contribute to the processing of complex signals. However, these studies all involved interactions between more widely spaced tone frequencies than those used in this study; it remains unclear whether cochlear mechanisms contribute to the selectivity observed.

Selectivity for FM sweep parameters has been reported as low in the central pathway as the cochlear nucleus (Britt and Starr 1976; Erulkar et al. 1968). In the pallid bat IC, the blockade of GABAA receptors has been shown to degrade or eliminate the direction selectivity of the FM specialists examined in this study (Fuzessery and Hall 1996), suggesting that, in some neurons, selectivity is created or sharpened within the IC. However, this disinhibition did not, in all cases, cause a loss of response selectivity, raising the possibility that, in some neurons, selectivity for FM sweeps is inherited by the IC from lower levels of the system. Thus these high-resolution spectrotemporal filters may arise through a combination of inherited selectivity and integration of input at the collicular level. Future studies using the iontophoretic application of receptor blockers may resolve these issues.

Comparison with auditory cortex

These results are much like those of a previous study of the pallid bat auditory cortex (Razak and Fuzessery 2008). Similarities are that TTF asymmetry was able to predict FM sweep rate and direction selectivity, that multiple mechanisms can contribute to shaping selectivity, and that neurons exhibited the same high degree of spectrotemporal resolution. Differences are that ~30% of cortical neurons showed TTF only at the onset of the first tone of the pair. This was not observed in the IC. The second difference is in the TTF functions. In cortex, the TTF response peaks broadened rather than shifted when the frequency difference between the two tones was increased. A shift in the response peak, without broadening, is what would be expected of neurons that were more sensitive to the spectrotemporal features of FM sweeps. Some broadening of response peaks was also observed in the IC, but in the majority of IC neurons, the peaks shifted systematically with spectral difference. IC neurons thus seem to expression greater temporal precision. It is likely that auditory cortex inherits this extreme spectrotemporal acuity from lower levels of the system.

Conclusions

The pallid auditory system has an unusually large percentage of neurons that are highly selective for the downward FM sweep of its echolocation pulse. This bat is a gleaner that, when hunting, must simultaneously use echolocation for obstacle avoidance, while listening passively for prey-generated sounds, i.e., it performs two acoustically mediated behaviors at the same time. We suggested that the adaptive response was to evolve an auditory system that is segregated into two pathways dedicated to echolocation and passive hearing. A functional component of this segregation is the presence of highly selective spectrotemporal filters that, in the case of the FM specialists described here, may respond exclusively to biosonar echoes. From this perspective, it is perhaps not unexpected that the system uses at least four mechanisms to shape response selectivity.

It is, however, somewhat unexpected that different mechanisms would be used to produce identical forms of selectivity. These mechanisms can operate alone or in concert with others to shape these spectrotemporal filters. A delayed high-frequency inhibition, a shortpass duration tuning, and facilitation are three mechanisms that can shape sweep rate selectivity. An early low-frequency inhibition and asymmetrical facilitation are two mechanisms that can create a selectivity for downward FM sweeps. The receptive fields of the neurons, and the circuitry that creates them, must be quite complex. The question must be asked regarding whether these mechanisms are actually discrete or different expressions of a common mechanism. This question can be partially answered by separating the frequency domains that evoke these mechanisms. Cells responding to tones are generally thought to receive both excitatory and inhibitory inputs within their excitatory tuning curves (Caspari et al. 1994; Fuzessery and Hall 1996; Xie et al. 2007) and, as noted above, so do the FM tuning curves described in this study. The shortpass duration tuning that shapes sweep rate selectivity is thought to be created by an early, on-BF inhibition that also acts within excitatory tuning curves. Separating the effect of shortpass duration tuning from asymmetrical facilitation was not possible in this study because quantifying duration tuning requires a response to tones, which was absent in the recorded population. These two mechanisms may therefore overlap. Sideband inhibition occurs in spectrally discrete domains outside of the excitatory frequency domains. Eliminating the frequency domains of these inhibitory sidebands from FM sweeps can also eliminate sweep rate or direction selectivity (Fuzessery et al. 2006), so these would appear to be discrete mechanisms. A future pharmacological dissection of these receptive fields should shed light on whether these mechanisms are indeed discrete and how and at what level of the auditory system they are created.

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Disclosures

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