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Processing of Modulated Sounds in the Zebra Finch Auditory Midbrain: Responses to Noise, Frequency Sweeps, and Sinusoidal Amplitude Modulations

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INTRODUCTION

Songbirds are particularly interesting model systems in which to study auditory processing because they use complex, learned vocalizations to communicate, and their brains contain a large number of auditory processing regions (see Brenowitz and Woolley 2004 for review). Behavioral experiments suggest that juvenile songbirds use genetic auditory preferences to guide song learning; conspecific songs are copied when songs are selectively responsive to particular portions of the noise, suggesting that, unlike forebrain neurons, many MLd neurons can encode specific acoustic components of highly modulated sounds such as noise. Selectivity for FM sweep direction was found in only 13% of cells that responded to sweeps. Those cells also showed asymmetric tuning curves, suggesting that asymmetric inhibition plays a role in FM direction selectivity. Responses to SAM showed that MLd neurons code temporal modulation rates using both spike rate and synchronization. Nearly all cells showed low-pass or band-pass filtering properties for SAM. Best modulation frequencies matched the temporal modulations in zebra finch song. Results suggest that auditory midbrain neurons are well suited for encoding a wide range of complex sounds with a high degree of temporal accuracy rather than selectively responding to only some sounds.

The anatomical connections to and from the songbird auditory midbrain region, the mesencephalicus lateralis dorsalis (MLd), suggest that it may be a site in which specialized auditory tuning mechanisms guide the brain’s acquisition of the appropriate acoustic material, conspecific song. In the frog TS, neurons are differentially responsive to AM rates that correspond to the amplitude modulations of conspecific calls (Diewald and Gerhardt 1992; Narins and Capranica 1980; Rose and Capranica 1983, 1985).

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vocalizations. While bat echolocation vocalizations have highly structured spectral and temporal properties, zebra finch songs are temporally complex sequences of broadband sounds and harmonic stacks of varying spectral modulation frequencies and durations. Therefore we might expect less frequency selectivity in the zebra finch MLd than is found in the bat IC. Given the complexity of zebra finch vocalizations, specialized tuning properties that correspond to conspecific vocalizations may be revealed only when complex stimuli are processed.

In an effort to describe how songbird auditory midbrain neurons process complex sounds and to identify specialized tuning characteristics, we examined the responses of single MLd neurons to four types of modulated sounds: white noise, band-limited noise, frequency modulated (FM) sweeps, and sinusoidally amplitude modulated (SAM) tones. To our knowledge, this is the first description of the response properties of songbird auditory midbrain neurons to these sounds.

METHODS

Animals

We used 22 adult male zebra finches that were purchased from a supplier (Magnolia Bird Farm, Anaheim, CA). Birds were housed in groups of 5–10 individuals and maintained on a 14:10-h light/dark cycle in a temperature controlled room. Food and water were available at all times. The University of Washington Animal Care Committee approved all animal procedures.

Surgical preparation

Birds were anesthetized with urethane (2.5 mg/g, given in 4 im injections delivered at 20-min intervals; Sigma, St Louis, MO). A bird was placed on a platform attached to a stereotaxic head holder. Body temperature was continuously monitored and adjusted to between 38 and 39°C using a custom designed heater with a thermistor placed in the cloaca and a heating blanket placed under the bird. Lidocaine was applied to the skin overlying the skull region covering the dorsal midline of the brain and the optic lobe. After lidocaine application, a small incision was made in the scalp along the midline of the cranium and a metal post was fixed to the surface of the skull using dental acrylic. Another incision was made in the skin over the skull covering the optic tectum. A small opening was made in the skin overlying the optic tectum, and the dura was resected from the surface of the brain.

Sound generation and stimuli

Sound stimuli were generated using custom software and a digital signal processor [DSP; Tucker Davis Technologies (TDT)]. The output of the DSP was routed through a digital to analog converter (TDT), through an anti-aliasing filter (TDT), and through digital attenuators (TDT) and finally through an analog attenuator and audio amplifier (Krohn-Hite 7500) to a free field loudspeaker (custom) that was placed 15 in. in front of the bird’s head. The output of the loudspeaker was calibrated at the beginning of each experiment with a 1/4-in microphone (Larson-Davis).

The noise stimuli were bursts of white noise and band-limited noise ranging between 10 and 1,000 ms in duration, presented at sound levels between 5 and 90 dB SPL, depending on the test. For any given set of trials, the noise bursts were identical or “frozen” from trial to trial. Band-limited noise stimuli varied from 100 to 3,000 Hz in spectral bandwidth, centered at the neuron’s characteristic frequency (CF). The CF was determined using pure tones. FM sweeps were centered at CF, with a defined frequency range, duration, and intensity, which depended on the cell. Sweeps ranged between 100 and 3,000 Hz above and below a neuron’s CF. Sweeps were 200 ms (equivalent to the maximum syllable duration in zebra finch song) or shorter in duration. In a test for directional selectivity, sweeps that were identical except for sweep direction (i.e., upward or downward) were presented in random order. SAM tones were varied between 10 and 500 Hz in modulation rate, in 10- to 20-Hz steps. Modulation depth was always set at 100%, and stimulus duration ranged between 100 and 500 ms. SAM intensities ranged between threshold and 90 dB SPL. Rise-fall times varied across stimuli but were generally 2–5 ms. Examples of the stimuli used are shown in Fig. 1. Presentation rates varied between one and three repetitions per second. Within a trial, stimuli were presented in a random sequence. Each stimulus was presented 15–25 times.

Recordings

All recordings were made inside a walk-in double-walled sound attenuation booth (Industrial Acoustics). The activity of single neurons was recorded extracellularly using glass micropipettes filled with 1 M NaCl. In some experiments, electrodes also contained 5% biotinylated dextran amine (BDA) to mark electrode locations by iontophoresic injection. Pipette tip diameters were typically <1.0 μm and impedances (at 1 kHz) ranged from 5 to 25 MΩ. Electrodes were aimed using visual landmarks and advanced in 1.0-μm steps using a Kopf hydraulic microdrive. Data were only collected from units that could be identified as cell bodies with reasonable certainty, in that they had a signal-to-noise ratio of ≥3:1 and biphasic action potential waveform. Recordings were amplified with a negative capacitance electrometer, filtered (300-Hz high-pass, 10-kHz low-pass), displayed on a Tektronix 5113 multi-channel oscilloscope, and monitored on an audio amplifier and loudspeaker. Spikes were discriminated with a TDT spike discriminator. Spike times were collected in 1-μs bins by TDT event timers and stored on a computer using custom software.

FIG. 1. Four types of modulated sounds were used. A: white noise. B: band-limited noise. C: frequency modulated (FM) sweeps. D: sinusoidally amplitude modulated (SAM) tones.
Dot raster displays, spike rates, spike latencies, total spike number, peristimulus time histograms (PSTHs), and excitatory frequency response areas (FRAs) were seen on-line. Additional PSTHs and other graphic displays of the data were generated off-line. Using a combination of commercial and custom software packages, further statistical analyses such as interspike interval histograms, tuning curves, rate level functions (RLFs), and means and variability of spike latencies were performed.

**Search stimuli**

White noise was used as a search stimulus while approaching MLd. Once significant multunit activity was detected, a variety of search stimuli including pure tones, noise bands, white noise, FM sweeps, and zebra finch calls were used to isolate single units. A wide variety of stimuli were used to reduce the likelihood that neurons were isolated in a biased fashion based on the sounds presented. This variation in search stimuli is particularly important in the zebra finch MLd because we found that very few cells in this region show spontaneous firing under urethane anesthesia.

**Data analysis**

Four analyses were used to characterize responses to white noise and noise bands and to compare those with responses to tones. First, PSTHs were used to examine the temporal patterns of responses to white noise bursts. Neurons were classified as "onset," "primary-like," "primary-like with notch," or "sustained" based on the time varying spike rate across the duration of the stimulus (as in Woolley and Casseday 2004). Responses that included spiking beyond the onset of a stimulus were classified as "ongoing." This classification included the primary-like, primary-like with notch, and sustained temporal response patterns and excluded the onset response pattern. These patterns were classified on the basis of the cell’s responses to 100-ms noise bursts presented at varying intensities and compared with the tone-derived temporal response pattern for the same cell. A unit was assigned to a particular category only if a consistent response pattern was observed across a range of intensities. If a unit did not show consistent temporal response characteristics across intensities or if there were too few data to determine a pattern with reasonable certainty, it was not assigned a response pattern.

PSTHs were used to examine the selectivity and reliability of responses. For neurons that showed ongoing responses, significant changes in the time-varying mean spike rate that were not due to the response pattern inherent to that neuron (e.g., primary-like) suggested that a neuron was selectively responding to certain acoustic elements within the noise, such as amplitude or frequency transients, or frequency combinations that drove the neuron particularly well. Such responses were called "element selective." Under our experimental conditions, most zebra finch MLd neurons did not spontaneously fire. Therefore element selective responses to noise were defined by the following criteria: 1) at least one instance of a spike occurring within the same 3-ms time bin across 11 or more of the 15 trials, excluding the initial onset response, indicating that responses were reliable across trials; 2) ≥30% of the stimulus evoked no response; and 3) interspike intervals were not regular across the duration of the stimulus. This last criterion ensured that chopper-like responses inherent to the neuron were not mistaken for element selectivity across trials.

Therefore for a neural response to white noise to be considered element selective, the spike patterns had to be reliable across presentations of the same stimulus and temporally sparse in a way that suggested a relationship between specific acoustic elements within the noise and a response (i.e., firing that showed temporal patterns beyond the typical temporal response patterns).

To test whether element selective responses could have occurred by chance, we generated 15 random spike trains representing 15 responses from each of 60 model neurons (Matlab R12). Fifteen trials from each of 60 neurons were generated because that is the number of actual spike trains and neurons analyzed for element selectivity. These spike trains were matched in duration, sampling rate, and mean spike rate with the actual spike trains recorded in response to white noise. For each model neuron, the 15 spike trains were summed to get one model PSTH. Those 60 PSTHs were analyzed to determine how many of them met the criteria for element selectivity.

To examine the relationship between noise responses and frequency tuning, element selectivity in responses to noise was compared with tone-derived tuning curve shape by measuring bandwidths at 80 dB SPL, and Q₁₀ and Q₃₀. These measures quantify the sharpness of frequency tuning at a range of points along the tuning curve. Q₁₀ and Q₃₀ are equal to the CF divided by the linear bandwidth measured at 30 and 10 dB above threshold, respectively.

Last, intensity coding was examined. Mean spike rates averaged over the duration of the stimulus were calculated, and rate-level functions (RLFs) were plotted to examine thresholds and saturation intensities. RLFs were calculated from responses to 100-ms noise sweeps varying in intensity between 5 and 90 dB SPL. Noise RLFs were compared with RLFs obtained from responses to tones at CF. Threshold was defined as the lowest intensity to elicit a response on 10 of 15 trials. Threshold and saturation curves for responses to noise and to CF were plotted for each neuron and compared with the frequency tuning properties of the cell.

For analysis of band-limited noise, spike rates in response to noise bands of different widths were calculated and plotted as a function of bandwidth. Responses to noise bands were compared with the excitatory frequency tuning curves of individual cells to look for excitatory/inhibitory interactions among frequencies within a neuron’s tuning curve. Such interactions are not examined by presentation of single pure tones and may only become evident when those frequencies are presented simultaneously as in a noise band.

FM sweeps were used to test for directional selectivity of responses. Spike rates were calculated for responses to upward and downward sweeps that were matched in intensity, slope, and frequency range. Responses in terms of overall spike rate and time varying spike rate (temporal pattern) were compared with a neuron’s frequency tuning curve to determine whether differences in responses to sweeps of opposite directions could be explained by frequency tuning and/or the temporal response pattern inherent to the cell. For analysis of tuning to SAM stimuli, dot rasters were used to make phase histograms. Using those histograms, synchrony of responses to the stimulus modulations was measured using vector strength (VS), a measure of how well the occurrence of spikes synchronizes with the amplitude modulations of the stimulus. A VS of 0 indicates that action potentials were not synchronized to the stimulus cycle; a VS of 1 indicates that action potentials were perfectly synchronized to the stimulus (i.e., fell into a single phase histogram bin). Modulation transfer functions describing spike rate (rMTFs) or vector strength (vMTF) at different SAM rates were computed to determine the best modulation frequencies (BMFs) for driving MLd neurons. Based on the MTFs, cells were classified as low-pass, band-pass, high-pass, or all-pass in modulation bandwidth. Responses to noise bands were compared with RLFs obtained from responses to tones at CF. Threshold and saturation curves for responses to noise and to CF were plotted for each neuron and compared with the frequency tuning properties of the cell.

Histology

Electrode locations were histologically verified using iontophoretic injections of BDA (5 μA DC current pulsed 7 s on/7 s off). Usually, one to two recording sites within a pass were marked. After recording sessions, birds were overdosed with pentobarbital sodium (0.5 mg/g body weight) and transcardially perfused using formalin (10%). Brains were immediately dissected free of the head and postfixed for ≥2 days in formalin. After postfixation, brains were cryoprotected in...
Localization of auditory neurons

Recording sites were located according to Woolley and Casseday (2004). Briefly, we used Nissl-stained sections and BDA injections to locate the positions of electrodes and recording sites within the midbrain. The subregions and borders of MLd have yet to be thoroughly described in the zebra finch (see Mello et al. 1998), and Nissl staining does not define a clear lateral border between MLd and nucleus intercollicularis (ICo) through the rostro-caudal extent of the midbrain. Therefore it is possible that some of the cells described here were from ICo, although no consistent tuning differences were found between lateral and medial cells. The entire collection of BDA injections was used to define the borders of auditory responsive areas, and all single units within those borders were included in our analysis.

RESULTS

We recorded from 91 single cells in the auditory midbrains of 22 adult male zebra finches. The four stimulus types, white noise, band-limited noise, FM sweeps, and SAM tones, used are shown in Fig. 1. The major brain regions projecting to and from the avian auditory midbrain are shown in Fig. 2. The responses of zebra finch auditory midbrain neurons to pure tones have been described in detail elsewhere (Woolley and Casseday 2004). Here, responses to tones were examined only for comparison with responses to sounds with complex frequency content and temporal/spectral modulations.

Responses to white noise

Seventy-six cells were tested with white noise. Most (62) cells showed vigorous responses, and 14 cells were unresponsive. Of the 14 cells that did not respond to white noise, 8 showed onset only responses to tones at CF. The rest showed either sustained (3), primary-like (2), or primary-like with notch (1) response patterns to tones. There were no differences in either CF or thresholds at CF between cells that did and did not respond to white noise. However, the cells that did respond to noise had significantly ($P < 0.001$) wider frequency tuning bandwidths than those that did not respond. Excitatory tuning curve bandwidths at 80 dB SPL (BW80dB) determined using tones were $3,250 \pm 250$ (SE) Hz for cells that did respond to white noise and $1,340 \pm 340$ Hz for cells that did not respond to noise. Therefore it is likely that some cells did not respond to white noise because the power at the limited frequencies to which those cells were sensitive did not reach threshold or that responses to excitatory frequencies were suppressed by inhibitory sidebands at other frequencies.

Temporal response patterns to white noise

For each neuron, temporal response patterns to white noise were compared with the response patterns determined using tones. The same four response patterns, onset, primary-like, primary-like with notch, and sustained, were evident in responses to both white noise and tones (see Woolley and Casseday 2004). In 50% of cells (30 of 60), temporal response patterns to white noise were the same as those to tones, indicating that the response pattern inherent to a cell often persists despite the broadband spectral characteristics of white noise (Table 1). All but one of the cells that showed ongoing responses to tones also showed ongoing responses to noise. Eight cells (13%) responded only to the onset of noise, suggesting long-lasting inhibition to continuous sounds regardless of the frequency content (Fig. 3A). All but one of these cells showed onset responses to pure tones (Table 1). Eight cells showed primary-like with notch, and 10 showed primary-like response patterns to noise. Figure 3B shows a primary-like with notch pattern in response to white noise, suggesting that a transient, broad-band inhibition follows the onset response. Temporal response patterns to white noise were not the same as the responses to tones in 30 of the 60 neurons (Table 1). Onset units (defined using tones) showed the widest variety of response patterns to noise, most of which were selective for specific elements of the noise stimulus.

### TABLE 1. Temporal response patterns to noise and tones responses to tones

<table>
<thead>
<tr>
<th>Response Patterns</th>
<th>Onset</th>
<th>Primary-like w/notch</th>
<th>Sustained</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Responses to White Noise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Onset/ES sustained</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Primary-like</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Primary-like/ES</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Primary-like w/notch</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Primary-like w/notch/ES</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sustained</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Sustained/ES</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Weak</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>29</td>
<td>11</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

Response patterns for white noise are shown in column one. Response patterns for tones at CF are shown in row one. The counts show how many cells fell into each category. ES indicates “element selective”.

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Element selective responses to white noise

In addition to showing characteristic temporal response patterns, 39 of the 60 cells showed element selective responses to the stimulus such that responses to specific portions of the frozen noise stimulus were evident (see METHODS; Fig. 4, A and B). These cells all showed ongoing responses, with consistent spike patterns across trials of the same frozen noise stimulus. PSTHs with sharp response peaks characterized these cells; there were no responses to some portions of the noise and there were temporally reliable responses to other portions. Although a direct, casual relationship between the acoustic features of the white noise and the neural response could not be established, these spike patterns suggested that the neurons were responsive only to specific acoustic elements within the noise (e.g., particular frequencies or frequency transitions). First, responses to the same frozen white noise stimulus were reliable over time, and markedly different from the responses of the same neuron to a different white noise stimulus (cf. Fig. 4, A and B). It is therefore likely that the spiking patterns observed in responses to white noise were stimulus driven, rather than inherent to the neuron. It is important to note that, in some cases, a cell’s temporal response pattern shaped the responses (Table 1). Second, responses that appeared to be element selective did not occur by chance. PSTHs calculated using random spike trains that matched the actual responses to white noise in duration (100 ms), mean spike rate (53 spikes/s), and number of trials (15) did not meet the criteria for element selectivity (Fig. 4C). Of 60 random model neurons, none met the criteria for element selectivity. No PSTHs showed a spike occurring in the same 3-ms time bin in 11 of the 15 trials. Only four PSTHs showed an absence of spiking over ≥30% of the duration of the spike train. Temporally regular spiking was also absent.

Twenty-one of the 60 cells showed responses that did not have temporally consistent response patterns across trials and therefore did not appear to be time locked to any particular noise elements. PSTHs showing consistent spike rates across the duration of the stimulus characterized these responses. Element selectivity in response to noise was not predicted by the temporal responses to tones (Table 1). PSTHs to white noise and tones at CF for an element selective cell are shown in Fig. 5, A and C, and those for a nonelement selective cell are shown in Fig. 5, B and D. Both showed primary-like responses to tones.

Element selectivity in response to noise was related to two properties of tuning to pure tones, frequency tuning bandwidth and CF thresholds (Figs. 5 and 6). First, the tuning curves obtained using tones were narrower for element selective cells than for cells that were not element selective (Figs. 5, E and F, and 6, A and B). The mean Q50 for cells that were classified as element selective, 3.05 ± 0.40, was significantly higher than for cells that were not element selective, 1.58 ± 0.21 (P < 0.002). Q10 and CF did not differ between selective and nonselective cells. Second, thresholds to CF were significantly higher (P < 0.05) in cells that responded selectively than in cells that did not (Figs. 5, E and F, and 6C). Mean CF thresholds were 37.3 ± 3.1 dB SPL for selective and 29.7 ± 2.03 dB SPL for nonselective cells. Therefore element selective responses to white noise were correlated with narrow frequency tuning and higher thresholds at CF.

Thresholds for responses to white noise and to tones at CF were positively correlated (r = 0.67; Fig. 7). As expected based on spectral density differences between white noise and pure tones, cells generally had higher thresholds to noise than to tones at CF. Threshold differences between white noise and tones ranged between −15 and 50 dB, with a mean of 20 dB higher to noise than to tones at CF. There did not appear to be any systematic differences between cells in which thresholds for tones and noise matched well and those in which they did not.
RLFs for white noise

We compared RLFs for responses to white noise and RLFs for responses to pure tones at CF. Figure 8 shows the white noise and CF-derived RLFs for four cells. The relationship between intensity and spike rates for responses to noise and tones was highly variable across cells. Some cells had similar RLFs to noise and tones (Fig. 8A). Others responded more robustly to tones at CF than to noise (Fig. 8, B and C). Cells that showed spike rate saturation within the tested intensity range for one stimulus type did not necessarily do the same for the other stimulus (Fig. 8B). RLF shapes could be very different between responses to the two stimuli (Fig. 8D). The average dynamic range (the range of sound intensities that produced changes in spike rates) of noise responses was not significantly different from that of tone responses because thresholds were higher in responses to noise.

Differences between the RLFs to noise and to CF tones were related to the sharpness of frequency tuning. Cells that were driven less well by noise than by CF tones had narrow tuning curves, with sharp tuning at CF near threshold and no responses to most of the frequencies in noise (Fig. 8, C and E). Cells that showed stronger responses to noise, with rapidly rising RLFs, were responsive to a wide range of frequencies (Fig. 8, D and F). That is, they were driven well by a broadband stimulus and were less selective for frequencies at and around CF.

Band-limited noise

Noise bands of varying widths were used in conjunction with frequency tuning curves to examine the possibility of excitatory/inhibitory interactions among frequencies presented simultaneously (noise bands) that were not evident when frequencies were presented independently (tuning curves). Sixty cells were presented with band-limited noise, ranging from 100 to 2,000 Hz in bandwidth. Noise bands were centered on a neuron’s CF and systematically increased in width to and

Fig. 5. Element selectivity was not predicted by a neuron’s temporal response pattern to tones. A: PSTH from 1 cell that showed element selectivity in response to white noise. B: PSTH from a cell that did not show element selectivity. C: PSTH showing response of the cell in A to a tone presented at characteristic frequency (CF). D: similar response recorded from the cell in B. Note the shorter time scale than in C. E: tuning curve for cell shown in A and C. This cell had a relatively high threshold and narrow tuning curve. F: tuning curve for cell shown in B and D. This cell had a relatively low threshold and wide tuning curve.

Fig. 6. Cells with element selective responses to noise had higher thresholds to tones at CF and narrower tuning curves than nonelement selective units. A: distribution of $Q_{30}$ values for element selective cells. B: distribution of $Q_{30}$ values for cells that were not element selective. C: average thresholds for element selective and nonselective cells. *$P < 0.05$. 
beyond the frequency limits of a neuron’s excitatory tuning curve. Of the 60 cells, 5 did not respond to noise bands of any width, and 8 responded only to noise onset. Of the 47 cells that gave ongoing responses to noise bands, 9 responded with the same spike rate to bands of varying widths, 14 responded best to mid-width bands (band-pass), 10 responded with systematically higher spike rates to narrowing bandwidths, and 14 responded with higher spike rates as bands widened. Figure 9, A–E, shows the relationship between spike rate and noise bandwidth for five cells. Of the cells with spike rate differences in responses to different bandwidths, most response differences could be explained by the excitatory frequency tuning curves. Figure 9, F–J shows the excitatory frequency tuning curves for the cells shown in Fig. 9, A–E. Gradual decreases in spike rate with increasing bandwidth occurred when bandwidths increased beyond the bounds of excitatory frequency tuning curves (Fig. 9, A and F). Similarly, rapid decreases in spike rate with increases in bandwidth occurred in cells that showed very narrow tuning curves (Fig. 9, B and G). In tuning curves that exhibited steep low frequency slopes, spike rates decreased to 0 or near 0 when bandwidths became wide enough to include frequencies outside the low frequency edge of the tuning curve (Fig. 9, C and H). In these cases, the excitation evoked by frequencies remaining within the bounds of the tuning curves may have been suppressed by inhibitory sidebands on the low frequency side.

Of the 14 cells showing higher spike rates to wider noise bands, five responded well to pure tones and/or to very narrow (e.g., 100 Hz) noise bands around CF, but did not respond to mid-width bands. Figure 9, D and E, shows examples of this response behavior. Even though the frequencies contained in the mid-width noise bands evoked responses when presented as separate pure tones, they appeared to suppress responses when presented together, within a noise band, suggesting excitatory and suppressive interactions among frequencies that fell entirely within the borders of the excitatory tuning curves. These suppressive bandwidths were not likely to be due to power differences because the intensity of the noise bands was well above the thresholds for those frequencies. These cells had more complex tuning curves than the other cells, suggesting that complex excitatory/suppressive interactions shape their frequency responses (Fig. 9, I and J). For example, the tuning curve in Fig. 9I is double peaked and the tuning curve in Fig. 9J is sharply peaked at mid- to low intensities, with a low frequency tail at higher intensities. In these cells with suppression at narrower bandwidths and excitation at wider bandwidths as well as complex tuning curves, nonlinear interactions among different frequencies may exist; some frequencies may be excitatory when presented alone but suppressive when presented in conjunction with other frequencies. Overall, however, the best noise bandwidth (evoking the highest spike rate) was highly correlated with tuning curve bandwidth at 80 dB SPL (r = 0.87, data not shown).

**FIG. 7.** Intensity thresholds for white noise and for tones at CF were positively correlated. Thresholds to tones at CF were generally lower than white noise thresholds.

**FIG. 8.** Most responses to white noise were monotonic. Differences between noise and CF rate level functions (RLFs) within a cell were usually predictable based on a cell’s tuning curve. A: RLFs showing similar intensity coding in response to noise and tones. B: RLFs for a cell that fired at rapidly rising and saturating spike rates to CF but was less responsive to noise. C: RLFs from a cell that was less excited by white noise than by tones at CF. D: RLFs from a cell that showed a slightly nonmonotonic response to noise and was less responsive to tones at CF. E: narrow tuning curve for the cell shown in C indicates that this cell was not excited by frequencies other than CF and would not be likely to respond well to noise. F: wide tuning curve for cell shown in D indicates that this cell was excited by a wide range of frequencies and was therefore likely to respond well to noise.
neurons. The examples in Fig. 10, response patterns to tones (Fig. 10, FM. Figure 10 compares spike rates in response to FM sweeps activity, in terms of mean spike rate, for upward or downward slopes. Therefore the vast majority of cells showed no selection, and slope. This lack of sensitivity to sweep direction was downward FM with the same frequency range, power, duration, that showed ongoing responses to FM sweeps, 22 showed no a frequency within the excitatory tuning curve. These cells also responded only to the onsets of sweeps, provided that the sweep began at CF, did not respond to wider bands, and resumed responding to even wider bands. E: another example of the same band-pass firing. F: tuning curve for cell shown in A. G: tuning curve for cell shown in B. H: tuning curve for cell shown in C. I: tuning curve for cell shown in D. J: tuning curve for cell shown in E. Open squares in F–J indicate center frequency (always at CF) and intensity of noise bands.

**FM sweeps**

Forty-five cells were tested with FM sweeps. Of those cells, five did not respond to FM sweeps. These cells showed only sparse onset responses to pure tones. Thirteen cells responded only to the onsets of sweeps, provided that the sweep began at a frequency within the excitatory tuning curve. These cells also showed purely onset responses to pure tones. Of the 27 cells that showed ongoing responses to FM sweeps, 22 showed no significant differences in spike rates between upward and downward FM with the same frequency range, power, duration, and slope. This lack of sensitivity to sweep direction was consistent across different frequency ranges, durations, and slopes. Therefore the vast majority of cells showed no selectivity, in terms of mean spike rate, for upward or downward FM. Figure 10 compares spike rates in response to FM sweeps (Fig. 10, A and B) with the tuning curves (Fig. 10, C and D) and response patterns to tones (Fig. 10, E and F) from the same neurons. The examples in Fig. 10, A and B, show spike rates for two representative cells that were not selective for direction. Those cells also had symmetric, V-shaped tuning curves (Fig. 10, C and D). The range of sweeps that evoked responses was predictable based on tuning curve width and the tone-derived temporal response pattern of a cell (Fig. 10, E and F). For example, the cell shown in Fig. 10A had a wider tuning curve and a more sustained response pattern than the cell in Fig. 10B. Consequently, all sweeps presented to the cell in Fig. 10A evoked responses, but only the sweeps that began at a frequency that was near CF evoked responses from the cell shown in Fig. 10B.

Five cells showed selectivity for sweep direction, in terms of significant differences in spike rate. Four cells were selective for upward FM and one was selective for downward FM. In each of these cells, spike rate differences between upward and downward sweeps appeared to be related to the shape of the neuron’s tuning curve, the first spike latency, and the cell’s temporal response pattern to tones. Figure 11 shows the responses and tuning characteristics of a neuron that responded best to sweeps that progress from low to high frequencies. This selectivity may be explained by three tuning properties observed in responses to tones: 1) the asymmetric tuning curve; 2) the long first spike latency; and 3) the sustained temporal response pattern that builds in spike rate as the stimulus progresses in time (Fig. 11, B and C). For this and similar cells, it is possible that these three properties combine to generate directional selectivity (see DISCUSSION).

The temporal patterns of responses to sweeps were often different between upward and downward directions and were related to a neuron’s tuning curve, latency, and response pattern (e.g., onset or primary-like). Time-dependent differences in spike distribution that distinguished the responses to upward and downward sweeps were more common than differences in the mean spike rates calculated across the entire stimulus duration. Figure 12A shows the responses of one unit to upward sweeps and downward sweeps that were identical except for direction, ranging in bandwidth from 0 to 3,000 Hz. The tuning curve for that cell is shown in Fig. 12B. In this case, the cell’s response appeared to be related to two tuning characteristics: 1) the temporal response pattern inherent to the cell (primary-like with notch) and 2) the order of the frequencies presented. For pure tones or sweeps that begin within the bounds of the tuning curve (all downward sweeps and 1 upward sweep), the response pattern was primary-like with notch. In responses to upward sweeps that began outside of the tuning curve, however, the initial spike burst and a portion of
the sustained response were absent. Thus the sequence of excitation and inhibition is dependent on the cell’s inherent temporal response pattern and how the sweep progresses through the tuning curve. These response characteristics were observed in 21 of the 40 cells with ongoing responses, such that FM sweeps of opposite directions were coded differently in terms of time-varying spike rates.

**SAM tuning**

Fifty-eight cells were tested with SAM tones presented at CF, at varying intensities, and between 10 and 200 Hz modulation frequencies. Cells that showed synchronization or interesting responses at 200 Hz were tested at 500 Hz in modulation frequency. A surprisingly large number of cells (21%) gave no response to SAM tones at any of the modulation rates tested. All of these cells showed purely onset responses to tones. SAM stimuli elicited strictly onset responses from 34% of the cells. These cells showed onset, primary-like, and primary-like with notch, but not sustained response patterns to tones. The remainder of the cells responded robustly to SAM stimuli, showing variations in spike rate and synchronization, depending on the modulation rate (Fig. 13). None of these cells showed purely onset responses to tones. Figure 13A shows the responses of a cell that did not change spike rate across the sustained response were absent. Thus the sequence of excitation and inhibition is dependent on the cell’s inherent temporal response pattern and how the sweep progresses through the tuning curve. These response characteristics were observed in 21 of the 40 cells with ongoing responses, such that FM sweeps of opposite directions were coded differently in terms of time-varying spike rates.

**FIG. 10.** Responses to FM sweeps could be explained by tuning curve widths and temporal response patterns. A: example of spike rates for 1 cell’s responses to FM sweeps. Sweeps that began with frequencies that were further away from CF evoked fewer spikes. B: some cells showed no response to FM sweeps that began outside of a cell’s tuning curve. In this example, sweeps of 1-kHz bandwidth or higher evoked no response. C: tuning curve for cell shown in A. A linear scale is used for graphic clarity. D: tuning curve for cell shown in B. Sweeps of 1-kHz bandwidth or higher began outside of the cell’s tuning curve. E: tone-derived response pattern for cell shown in A and C. Sweeps that began near the edge of or outside the cell’s tuning curve evoked less firing because the initial, strong onset response was absent in responses to those sweeps. F: tone-derived response pattern for the cell shown in B and D. This cell did not respond to sweeps of bandwidths wider than 1 kHz because it gave only onset responses, and those sweep onsets fell outside of the tuning curve.

**FIG. 11.** Cells that exhibited selectivity for FM sweep direction also exhibited asymmetric tuning curves. A: histogram showing that, in 1 cell, higher spike rates were elicited by upward sweeps than by otherwise identical downward sweeps. B: asymmetric tuning curve for the same cell. Gray bar shows frequency range of the FM sweeps used. *CF that was the center point for all sweeps. A linear scale is used for graphic clarity. Vertical black lines show frequency ranges of sweeps with successively wider ranges. For example, bars closest to * show frequency range of sweeps with 0.5-kHz bandwidth. C: PSTH showing tone-derived response pattern for the same cell. Directional selectivity can be explained by the combination of a steep tuning curve slope on the low-frequency side and an increase in spike rate as stimulus duration increases.
pure tones, which is the same pure tone response type as the mid-frequencies. This neuron showed sustained responses to poor to both low and high frequencies, but was good in the spike rate and synchronization. Response synchronization was apparently suppressed at higher modulation rates. The re-
have been expected based on responses to pure tones was not notch patterns. The ongoing portion of the response that may appeared at modulation frequencies above 60 Hz. The re-
modulation frequencies. For this cell, periodic responses dis-
ated signals and therefore do not elicit periodic responses. The
response pattern to tones; neurons with narrower tuning curves
ward vs. downward sweeps). Those that did show directional
response to tones; neurons with narrower tuning curves
threshold at CF, but not to its temporal
neurons appeared to respond selectively to specific acoustic
observed in the responses of the same cells to pure tones. Many
tuning properties such as tem-
observed in responses to white noise was related to a neuron's
3
patterns differed between two different samples of noise; and
a random model analysis indicated that such responses were
not generated by chance. The degree of element selectivity
observed in responses to white noise was related to a neuron’s
tuning curve and threshold at CF, but not to its temporal
response pattern to tones; neurons with narrower tuning curves
higher thresholds were more often element selective and
could exhibit any of the four response types (see Table 1).
Most cells showed no significant differences in spike rates to
FM sweeps that were identical except for direction (i.e., upward
rightward vs. downward sweeps). Those that did show directional
selectivity based on spike rate (13% of responsive cells) also
showed asymmetric tuning curves, suggesting that sideband
inhibition shaped both frequency tuning and directional selec-


differences between responses to upward and downward sweeps were not
side of CF and their inherent temporal response patterns. For these cells,
overall spike rate differences between upward and downward sweeps were not significant. A: raster plot showing 1 cell’s responses to FM sweeps. B: that
cell’s tuning curve. *CF that was the center point for all sweeps. Vertical black
d lines show frequency ranges of sweeps with successively wider ranges.
Differences between responses to upward and downward FM most likely occur
because 2- and 3-kHz width upward sweeps begin outside of the tuning curve and 2- and 3-kHz downward sweeps end outside of the tuning curve.

modulation frequencies but did synchronize better to lower
frequencies; this cell showed low-pass synchronization characteristics, with significantly higher vector strengths in re-
sponses to lower modulation frequencies. Its responses to pure
tones were sustained. The stimuli presented at higher modulation
frequencies elicited responses that were similar to pure
tone responses. It is possible that, in this type of unit, higher
frequency SAM stimuli are not distinguished from unmodu-
lated signals and therefore do not elicit periodic responses. The
responses in Fig. 13B exhibited low-pass tuning, with both higher spike rates and vector strengths in responses to low modulation frequencies. For this cell, periodic responses dis-
appeared at modulation frequencies above 60 Hz. The re-
sponses of this cell to pure tones showed primary-like with
notch patterns. The ongoing portion of the response that may have been expected based on responses to pure tones was
apparently suppressed at higher modulation rates. The re-
sponses in Fig. 13C showed band-pass tuning characteristics in
spike rate and synchronization. Response synchronization was poor to both low and high frequencies, but was good in the
mid-frequencies. This neuron showed sustained responses to
pure tones, which is the same pure tone response type as the

cell in Fig. 13A. However, the SAM tuning is very different
between the two cells. Unlike the neuron in Fig. 13A, the
synchronization of the neuron in Fig. 13C is specifically tuned
to modulation rates centered at 80 Hz.

Modulation transfer functions were used to examine the relationship between modulation frequency and spike rate (rMTF) and synchronization measured with vector strength (vsMTF). In most of the cells that gave ongoing responses to SAM tones, spike rates varied in responses to different modulation frequencies. Twenty-four of those cells synchronized their responses to the stimulus modulation rates, with vector strengths ≤0.94. Figure 14 shows examples of rMTFs and vsMTFs for four cells. Cells were classified as having low-pass, band-pass, high-pass, or all-pass filtering properties for both rMTFs and vsMTFs. There was no consistent relationship between filter classification for rMTF and classification for vsMTF. BMFs for spike rate (rBMFs), the frequencies that elicited the highest spike rates, were calculated from the rMTFs. Best temporal modulation frequencies (tBMFs) were calculated from the vsMTFs. The distributions of rBMFs and tBMFs are shown in Fig. 15. rBMFs were spread across the full range of modulation frequencies tested, with a slight population preference for mid-frequencies. rBMFs ranged from 20 to 200 Hz across cells, with a mode of 80 Hz (Fig. 15A). In contrast, most units (70%) that synchronized to SAM stimuli showed low-pass synchronization; vector strengths were higher to lower modulation frequencies (Fig. 15B). The modulations to which neurons synchronized best (tBMF) ranged widely (between 20 and 140 Hz), but most fell below 100 Hz. A cell’s synchronization to modulation rates was related to frequency tuning. A weak correlation (0.3) existed between tuning curve bandwidth at 80 dB SPL and tBMF, the modulation frequency to which a cell synchronized best; cells with wider frequency tuning at 80dB SPL synchronized best to higher modulation frequencies.

DISCUSSION

The responses of MLd units to complex (modulated) sounds were most often explained by tuning properties such as tem-
poral response patterns, tuning curves, thresholds, and RLFs observed in the responses of the same cells to pure tones. Many
neurons appeared to respond selectively to specific acoustic elements within the frozen white noise stimuli. We called these
responses element selective for three reasons: 1) they were temporally precise and reliable across trials for a given noise
sample; 2) within the same neuron, the specific response patterns differed between two different samples of noise; and
3) a random model analysis indicated that such responses were not generated by chance. The degree of element selectivity
observed in responses to white noise was related to a neuron’s
tuning curve and threshold at CF, but not to its temporal
response pattern to tones; neurons with narrower tuning curves
and higher thresholds were more often element selective and
could exhibit any of the four response types (see Table 1).
Most cells showed no significant differences in spike rates to
FM sweeps that were identical except for direction (i.e., upward
rightward vs. downward sweeps). Those that did show directional
selectivity based on spike rate (13% of responsive cells) also
showed asymmetric tuning curves, suggesting that sideband
inhibition shaped both frequency tuning and directional selec-

FIG. 12. Some cells responded with different temporal patterns to upward and downward sweeps, reflecting differences in their tuning curves on either side of CF and their inherent temporal response patterns. For these cells, overall spike rate differences between upward and downward sweeps were not significant. A: raster plot showing 1 cell’s responses to FM sweeps. B: that cell’s tuning curve. *CF that was the center point for all sweeps. Vertical black lines show frequency ranges of sweeps with successively wider ranges. Differences between responses to upward and downward FM most likely occur because 2- and 3-kHz width upward sweeps begin outside of the tuning curve and 2- and 3-kHz downward sweeps end outside of the tuning curve.
tivity. Roughly one-half of neurons either did not respond to SAM signals or responded only to the onsets of SAM stimuli, regardless of rate. The rest of the cells distinguished among different SAM rates with differences in spike rate and synchronization. The range of best temporal modulation frequencies determined by the reliability of synchronization to the modulation phase matched the range of temporal modulations found in zebra finch song (see Singh and Theunissen 2003).

FIG. 14. For each cell, rate modulation transfer functions (rMTFs) and vector strength (vs) MTFs were used to measure the response differences across modulation frequencies. A wide variety of functions were observed. A: example of similar, band-pass functions for changes in rate and synchronization across modulation frequencies. This cell showed the same best modulation frequency (BMF) for both measures. B: example of more gradual increases and decreases in spike rate and vector strength across frequencies. C: some cells showed very different functions for the 2 measures. Spike rate gradually increased with increased frequency but synchronization decreased. D: example of unstable spike rates but stable synchronization across frequencies.

FIG. 13. Examples of responses to SAM tones. A: raster plot showing response of 1 cell to SAM tones that ranged between 20 and 200 Hz in modulation rate. The cell synchronized better to modulation frequencies <100 Hz. B: raster plot showing response of another cell to SAM tones ranging between 20 and 200 Hz in modulation rate. This cell synchronized firing to low modulation frequencies and gave only onset responses to frequencies >60 Hz. C: a third cell shows band-pass synchronization, following 80 Hz most accurately and giving less synchronized and/or more sparse responses to frequencies >120 and <80 Hz.
Responses to noise—what acoustic information is encoded?

Nearly all units responded robustly to white noise and noise bands that contained energy within a neuron’s excitatory tuning curve. Scheich et al. (1977) found that many guinea fowl MLd units were highly responsive to broadband sounds but did not test responses to white noise. Fifty-five percent of the cells in this study appeared to respond selectively to particular acoustic elements within noise stimuli. These acoustic elements were likely power transients at or near a neuron’s CF but may also have been short-duration frequency combinations that drive neurons particularly well. Keller and Takahashi (2000) showed that owl MLd (IC) neurons respond to broadband noise with specific patterns that are driven by the acoustic properties of the stimulus. They also showed that some cells were tightly frequency tuned and temporally precise in responses to noise. The selectivity that we observed was not dependent on inherent onset characteristics of a neuron’s response; nearly 50% of neurons with element selectivity gave ongoing responses to tones. Intensity may play an important role in element selectivity. Because the power in a noise stimulus is distributed across a wide range of frequencies, the power at any one frequency is much lower than the overall dB SPL intensity of the stimulus. Because most zebra finch MLd units have V-shaped tuning curves (Woolley and Casseday 2004), the distribution of power in a noise stimulus creates the effect of testing a neuron at a narrower point in the tuning curve than the tuning curve width at the overall stimulus intensity. Therefore a neuron that is widely tuned to tones at behavioral sound levels may show a significant degree of frequency selectivity in responses to white noise presented at behavioral levels.

Both the consistently robust responses to noise and the reliable responses to specific acoustic elements in the noise are unlike the responses of auditory forebrain neurons in zebra finches (Grace et al. 2003) and other birds (Bonke et al. 1979; Leppelsack 1978; Muller and Leppelsack 1985); many forebrain neurons do not respond well to white noise and very few respond to particular elements of the noise. This difference between midbrain and forebrain neurons suggests that midbrain neurons may encode the time-varying acoustic elements of rapidly modulated sounds whereas many forebrain neurons are either suppressed by such sounds or respond with general excitation that is not reliable over trials. MLd neurons are not particularly selective for sounds that contain specific phase relationships in spectrum or in time. Instead, an individual neuron may respond well to a range of stimuli with highly varied statistics such as pure tones, noise, FM sweeps and, to some extent, SAM signals. Thus selectivity for specific acoustic patterns like that found in bat auditory midbrain neurons (see Casseday and Covey 1996) appears to be less common in the zebra finch midbrain. Additionally, MLd neurons show no evidence of response selectivity for one single complex stimulus, as do neurons in the song system nuclei; neurons in the adult song system respond selectively to the bird’s own song (Doupe and Konishi 1991; Margoliash 1983; Theunissen and Doupe 1998).

We found two main differences between MLd cells that did and did not appear to encode the specific acoustic elements of noise stimuli. Cells that were classified as element selective had both higher thresholds at CF and narrower tuning curves than cells that were not. Threshold differences have been suggested to account for the element selectivity of responses to noise-like stimuli in the mammalian IC (Escabi et al. 2003). The correlation between these two factors and element selectivity suggests that responses to complex sounds can be at least partially predicted by a neuron’s basic tuning characteristics. In the processing of complex sounds such as vocalizations, cells with narrower tuning curves and higher thresholds may be useful in detecting particular spectro-temporal patterns that identify sounds produced by particular species or even individuals. Evidence for the use of narrow tuning curves as a potential strategy for detection of vocal signals has been shown in the bat IC, where cells with very narrow tuning curves match echolocation frequencies (Casseday and Covey 1992; Fuzessery and Hall 1996; Haplea et al. 1994; Pollak and Bodenhamer 1981).

FM sweeps—directional selectivity

We found that 89% of MLd units were responsive to FM sweeps. This percentage of responsive cells in the MLd agrees with numerous studies showing that most neurons in the mammalian ICC respond well to FM sweeps of varying frequency range, duration, speed, and direction (Felsheim and Ostwald 1996; Fuzessery 1994; Lee et al. 2002; Pollak et al. 1978; Suga 1969). Zebra finch songs contain more downward than upward FM sweeps (Singh and Theunissen 2003). This suggests that MLd neurons might be directionally selective for FM downward sweeps. However, the vast majority of cells that we studied showed no differences in spike rate based on sweep direction. And, four of the five cells that did prefer one direction to the other preferred upward sweeps. A general lack of directional selectivity has also been observed in MLd neurons using spectro-temporal receptive fields during the processing of song (Woolley and Casseday 2004). Therefore sensitivity to sweep direction such as that found in neurons of the bat IC (Fuzessery 1994; Pollak et al. 1978) does not appear to be widespread in the zebra finch MLd. This relative lack of tuning for sweep direction suggests that it is not an acoustic feature that contributes to tuning mechanisms that distinguish zebra finch songs from other stimuli. Scheich et al. (1977) found that some guinea fowl MLd neurons responded particularly strongly to FM sweeps, suggesting that MLd neurons in other birds may respond well to FM stimuli. However, the tuning curves of those cells were unknown, and selectivity for upward versus downward sweeps was not directly tested. Therefore it is difficult to interpret how their results relate those reported here. The prevalence of neurons that show selectivity...
for FM sweep direction varies considerably across species and studies. FM directionally selective neurons have been reported as rare in the rat IC (Felsheim and Ostwald 1996; Poon et al. 1991, 1992). However, Lee et al. (2002) found that roughly 30% of rat ICC neurons show directional selectivity, and Hage and Ehret (2003) found that 56% of the neurons they studied in the mouse ICC were directionally selective. These differences across studies suggest that the proportion of units that are found to be directionally sensitive is highly dependent on the acoustic features of the sweeps used to test neurons and the subregions of the auditory midbrain that are sampled. Both factors may have played a role in the relative rarity of directionally selective cells found in the zebra finch midbrain.

Although sensitivity to FM sweep direction was uncommon, 13% of the cells that responded to FM sweeps showed spike rate differences in response to sweeps of opposite direction. In all cases, FM directionality was related to three parameters of a neuron’s tuning properties that are revealed using tones: 1) the frequency tuning curve; 2) the temporal response pattern; and 3) the spike latency to CF. For example, cells that showed directional selectivity had asymmetric tuning curves, in which one side of the tuning curve had a steep slope and the other side had the shallower slope that is typical of most MLd neurons (Woolley and Casseday 2004). Cells without directional preferences did not have asymmetric tuning curves. The co-occurrence of tuning curve asymmetry and selectivity for FM sweep direction suggests that inhibitory sidebands shape asymmetric tuning curves and could provide the mechanism for FM directional selectivity. Although we were not able to directly measure inhibition because nearly all MLd units showed no spontaneous firing, the possibility that inhibition causes directional selectivity in MLd cells is supported by the role that inhibition plays in shaping the FM tuning properties of mammalian IC cells. Fuzessery and Hall (1996) showed that selectivity for FM sweep direction in the bat IC was due to inhibition. In the cells we studied, it is possible that as a downward sweep progresses through the excitatory frequencies (inside the tuning curve), the long first spike latency delays the response to those frequencies. The delayed response to these excitatory frequencies may then be suppressed by a less delayed inhibition at frequencies just outside the steep, low frequency border of the tuning curve (i.e., an inhibitory sideband). By this mechanism, a low spike rate would be elicited by downward FM. Conversely, when a sweep progresses from low to high, the response builds without suppression because the latter half of the sweep contains frequencies to which the neuron is sensitive (inside the tuning curve). An excitatory response representing rebound from suppression may also contribute to the strong response to upward FM.

**SAM—encoding temporal modulations**

The Mld appears to process SAM sounds much like the mammalian IC. Seventy-nine percent of MLd cells responded to SAM stimuli. Those cells that did not respond to SAM showed strictly onset responses to tones. This relationship between temporal response pattern determined using tones and the response to SAM has also been found in IC neurons in gerbils (Krishna and Semple 2000), rats (Shaddock Palombi et al. 2001), and bats (Condon et al. 1996). Periodic responses to SAM stimuli were elicited from only 52% of cells; 36% of cells gave onset only responses to SAM. Condon et al. (1996) found that only bat IC units with chopper or tonic temporal response patterns responded in a time-locked way to the periods of SAM stimuli and that onset units did not respond to periods after the first one. This is very similar to our results. The fact that a large percentage of MLd and IC cells do not follow periodic tones suggests that neurons with onset characteristics are suppressed after initial onset responses for durations that exceed the modulation frequency of the stimulus. It is also possible that, for these cells, the sinusoidal amplitude rise time at low modulation frequencies was too shallow to reset the neuron for another onset response. It can be argued from an ethological standpoint that the SAM stimuli used in this study, amplitude modulated tones presented at CF, are highly unnatural sounds for the songbird to process. Zebra finch vocalizations contain complex spectral and temporal modulations. Thus it may not be surprising that SAM tones fail to drive many neurons. This is in contrast to frogs, who communicate using highly periodic calls and in which midbrain neurons turn SAM rates into tonic responses with graded spike rates that depend on the period (Rose and Capranica 1985).

In cells that did respond periodically to SAM stimuli, temporal BMFs measured using vector strength were generally at or under 100 Hz. This corresponds well with the modulation frequencies to whichowl MLd neurons synchronize best in response to SAM noise (Keller and Takahashi 2000). However, these modulation frequencies are much lower than those that are encoded by the eighth nerve, where responses in the other birds phase lock well to stimulus modulations ≤2,000 Hz (Gleich and Narins 1988; Hill et al. 1989; Sachs et al. 1974). The MLd temporal BMFs are similar to the temporal BMFs found in mammalian auditory midbrain neurons, suggesting that temporal processing speed in the midbrain may be conserved across taxa (Krishna and Semple 2000; Langner and Schreiner 1988; Liang et al. 2002; Rees and Palmer 1989; Reimer 1987), perhaps matching the rates of biologically generated sounds (Casseday and Covey 1996). Additionally, the temporal modulations to which MLd units synchronize best match well with the modulations that are found in zebra finch song. Songs of this species contain temporal modulations ≤100 Hz, with most of the energy between the lowest temporal frequencies and 50 Hz (Singh and Theunissen 2003). The majority of temporal BMFs that we measured in MLd neurons fell just above 50 Hz. And most synchronizing neurons were able to follow the periodicity of SAM stimuli ≤100 Hz. This match in both range and preferred modulation rates suggests that zebra finch MLd cells are well suited for encoding the temporal modulations that characterize zebra finch song. Further analysis of the temporal tuning properties of MLd neurons in zebra finches and other species will determine how specialized temporal modulation tuning is for the processing of particular natural sounds such as conspecific vocalizations.

In summary, in the zebra finch MLd, we found few neurons with evidence of selective tuning mechanisms such as those observed in the bat IC using modulated sounds. Instead, most MLd neurons are opportunistic, responding strongly to a wide variety of stimuli. The findings here and those reported in Woolley and Casseday (2004) suggest that most MLd units are well suited for temporal processing. There is a preponderance of precisely spiking onset neurons, as well as neurons showing...
reliable temporal responses throughout stimulus durations, even to complex stimuli such as white noise bursts. The element selective responses to white noise reported here suggest that some MLd neurons can distinguish one noise stimulus from another. This encoding capacity may play a role in encoding and discriminating among different zebra finch songs, which are composed of individually distinct, noise-like syllables. The onset responses and spiking precision observed in most MLd responses suggest that complex sounds may be encoded with a high degree of temporal precision such that the unique temporal characteristics of individual sounds are preserved in the neural code that is sent on to the auditory thalamus. The responses to SAM sounds suggest that MLd neurons may respond well to temporal modulations of zebra finch vocalizations.

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