Response Properties of Single Neurons in the Zebra Finch Auditory Midbrain: Response Patterns, Frequency Coding, Intensity Coding, and Spike Latencies

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Submitted 2 July 2003; accepted in final form 23 September 2003

Woolley, Sarah M. N. and John H. Casseday. Response properties of single neurons in the zebra finch auditory midbrain: response patterns, frequency coding, intensity coding, and spike latencies. J Neurophysiol 91: 136–151, 2004. The avian mesencephalic lateralis, dorsalis (MLd) is the auditory midbrain nucleus in which multiple parallel inputs from lower brain stem converge and through which most auditory information passes to reach the forebrain. Auditory processing in the MLd has not been investigated in songbirds. We studied the tuning properties of single MLd neurons in adult male zebra finches. Pure tones were used to examine tonotopy, temporal response patterns, frequency coding, intensity coding, spike latencies, and duration tuning. Most neurons had no spontaneous activity. The tonotopy of MLd is like that of other birds and mammals; characteristic frequencies (CFs) increase in a dorsal to ventral direction. Four major response patterns were found: 1) onset (49% of cells); 2) primary-like (20%); 3) sustained (19%); and 4) primary-like with notch (12%). CFs ranged between 0.9 and 6.1 kHz, matching the zebra finch hearing range and the power spectrum of song. Tuning curves were generally V-shaped, but complex curves, with multiple peaks or noncontiguous excitatory regions, were observed in 22% of cells. Rate-level functions indicated that 51% of nononset cells showed monotonic relationships between spike rate and sound level. Other cells showed low saturation or nonmonotonic responses. Spike latencies ranged from 4 to 40 ms, measured at CF. Spike latencies generally decreased with increasing sound pressure level (SPL), although paradoxical latency shifts were observed in 16% of units. For onset cells, changes in SPL produced smaller latency changes than for cells showing other response types. Results suggest that auditory midbrain neurons may be particularly suited for processing temporally complex signals with a high degree of precision.

INTRODUCTION

Acoustic communication is common among vertebrates. Birds produce calls to solicit contact and communicate distress. Male birds also produce songs to attract females and for male–male interactions. Song is particularly interesting because it is acoustically complex and must be learned by imitation; young males copy the mature songs of older males. Although the exact song must be copied, acoustic preferences for conspecific song guide what song material is learned (Adret 1993; Dooling and Searcy 1980; Eales 1987; Marler 1970; Marler and Peters 1977; ten Cate 1991). This preference is evident even in acoustically naïve birds (Braaten and Reynolds 1999) and is often referred to as the “innate auditory preference” (see Marler 1997). Because the behavioral acoustic preferences that guide song learning appear to exist independent of experience, such preferences must to some extent reflect the inherent acoustic tuning properties of neurons in the songbird auditory system.

The locus of neurons that contribute to conspecific song preferences is unknown. Although neurons in the song control system of the forebrain respond selectively to the bird’s own song, these neurons do not respond well to conspecific song (Doupe and Konishi 1991; Margoliash 1983). There are several reasons to indicate that the auditory midbrain region, mesencephalic lateralis, dorsalis (MLd) is a candidate locus for tuning to conspecific sounds. 1) The MLd is analogous to the mammalian inferior colliculus (IC) and the amphibian torus semicircularis (TS), where specialized acoustic tuning properties related to vocal signals have been demonstrated. Studies on vocalizing frogs, echolocating bats, and mice indicate that complex acoustic tuning properties matching the acoustic features of conspecific vocalizations arise in the auditory midbrains of these animals (Casseday and Covey 1992; Casseday et al. 1997; Diekamp and Gerhardt 1992; Feng et al. 1978; Given 1993; Mittmann and Wenstrup 1995; Narins and Capranica 1980; Pollak and Bodenhamer 1981; Rose and Capranica 1983, 1985; Suga 1969). 2) Virtually all of the multiple, parallel auditory brain stem pathways converge in the MLd, suggesting this as a major site of neural integration where convergent inputs could interact to produce signal transformations necessary for specialized tuning (Fig. 1; Akesson et al. 1987; Boord 1968; Conlee and Parks 1986; Correia et al. 1982; Karten 1967, 1968; Park and Pollak 1994; Parks and Rubel 1975; Takahashi and Konishi 1988; Wild 1995). 3) The songbird MLd may be hypertrophied (Cobb 1964; Rylander 1979). 4) The songbird MLd receives descending input from regions that are involved in the control of song behavior, providing a potential feedback loop for the integration of auditory information and vocal motor control (Fig. 1; Mello et al. 1998; Wild 1993).

To date, little attention has been paid to the processing of song by neurons of the ascending auditory system that is presynaptic to the song control system. Grace et al. (2003) recently showed that neurons in the ascending auditory forebrain region, field L (primary auditory cortex analog), respond preferentially to song over synthetic sounds with matched power spectra. Thus it seems possible that acoustic tuning in the ascending auditory nuclei could contribute to the mechanisms of auditory preference for conspecific vocalizations.
To begin to understand the role of the songbird auditory midbrain in the processing of acoustic information, we studied the basic auditory responses of single neurons in the zebra finch MLd. This is, to our knowledge, the first examination of the response properties of auditory midbrain neurons in a songbird. We describe here the tonotopy, temporal response patterns, frequency tuning, intensity coding, and latency relationships of MLd neurons in adult male zebra finches.

**Methods**

**Animals**

We used 23 adult male zebra finches that were purchased from a supplier (Magnolia Bird Farm, Anaheim, CA). Birds were housed in groups of 5 to 10 individuals and maintained on a 14:10 light/dark cycle in a temperature-controlled room. Food and water were available at all times. All animal procedures were approved by the University of Washington Animal Care Committee.

**Surgical preparation**

Birds were anesthetized with urethane (Sigma, St. Louis, MO; 2.5 mg/g, given in 4 intramuscular injections delivered at 20-min intervals). 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 thermometer 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, caudal to the eye and dorsal to the skull using dental acrylic. Another incision was made in the skin over the skull covering the optic tectum, caudal to the eye and dorsal to the skull using dental acrylic.

**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), then 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 measured at the beginning of each experiment with a 1/4-in. Larson-Davis microphone.

The stimuli were pure tones (0.1–7.0 kHz) of durations between 5 and 1,000 ms, presented at sound levels between 5 and 90 dB, depending on the test. Stimuli always started at the zero-crossing point of the sine wave. Rise–fall times varied across stimuli but were generally 2 ms. Stimuli were presented either singly, as pairs separated by a variable delay, or as sequences of multiple sounds. Presentation rates varied between 1 and 3 repetitions/s. Within a trial, stimuli were presented in a random sequence. Each stimulus was presented 15 to 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 tonotopic 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 collected only 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 multichannel 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. Dot raster displays, spike rates, spike latencies, and peristimulus time histograms (PSTHs), and excitatory frequency response areas (FRAs) were seen on-line. More 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), means and variability of spike latencies, and rate-duration functions were performed.

**Search stimuli**

White noise was used as a search stimulus while approaching MLd. Once significant multiunit activity was detected, a variety of search stimuli including pure tones, noise bands, white noise, FM sweeps, and bird 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 almost no cells in this region show spontaneous firing under urethane anesthesia.

**Data analysis**

To examine temporal response patterns, frequency coding, and intensity coding, complete excitatory FRAs were generated. FRA plots show a grid of PSTHs, one for each frequency/intensity combination across a range of frequencies and intensities. For every unit, an FRA was generated by recording the responses to 50-ms pure tones ranging in frequency between 0.5 and 7.0 kHz (within the frequency range of the zebra finch audiogram) and ranging in intensity between below threshold and 90 dB. Frequency steps were 0.5 kHz for intensity ranges above threshold and 0.1 kHz around threshold. Intensity steps were 5 dB at intensities above threshold and 2 dB around threshold. Frequencies were presented in random order. For most cells, multiple FRAs and additional tests such as RLFs were used to define spontaneous firing rate, response pattern (e.g., onset, primary-like, etc.), characteristic frequency (CF), threshold, width of frequency tuning, and first-spike latencies. Spontaneous activity rates were calculated from the 50 to 100 ms collection windows preceding each stimulus repetition. Threshold was defined as the lowest intensity to elicit a response on 10 out of 15 trials. CF was defined as the frequency with the lowest threshold. Frequency tuning was examined by calculating tuning curve bandwidths at 80 dB SPL and Q10 and Q30. Q10/Q30 is equal to the CF divided by the linear bandwidth measured at 10 dB above threshold; Q30 is the same but uses the bandwidth measured at 30 dB SPL. Examples of FRAs are shown in Fig. 4.

Temporal response patterns were classified on the basis of the cell’s responses to 50-ms tones at CF at several amplitudes. Therefore if a unit showed a consistent temporal response pattern at CF and across a range of intensities, it was assigned a temporal response type. If a unit did not show consistent temporal response characteristics across intensities or if there was too little data to determine a pattern with reasonable certainty, it was not assigned a response type. The temporal response patterns at frequencies other than CF were examined but were not used to classify cells. Rate-level functions using tones of 50 to 100 ms duration presented at CF were collected to determine
whether cells showed “monotonic,” “low saturation,” or “nonmonotonic” spike counts as a function of sound level. These labels describe 3 distinct classes of RLFs that we observed under conditions of sound intensities ranging between 5 and 90 dB SPL and are not intended to imply any relationship between sound level and spike rates at other intensities. Spike latencies were defined as the first-spike time arrivals across the full range of stimulus levels at CF. Mean ± SD first-spike latencies were calculated on-line and stored. First-spike latencies were examined across all frequencies to which cells would respond and intensities between threshold and 90 dB SPL. Depending on the cell, a variety of additional tests were used to confirm and further examine the temporal response patterns, CFs, thresholds, tuning widths, rate-level relationships, and duration tuning. The exact tests and the sequences of stimuli used depended on each cell’s response characteristics.

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 2 recording sites within a pass were marked. After recording sessions, birds were overdosed with Nembutal (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 30% sucrose in distilled water for 24 h and embedded in gelatin with formalin for 3–4 days. Frontal sections (40 μm) were cut on a freezing microtome and collected in 2 series into PBS. One series was mounted in avidin-biotin complex solution (ABC Vectastain Elite Kit, Vector Laboratories). After PBS rinses, BDA injection sites were visualized by incubation in diaminobenzidine (DAB) and 0.001% hydrogen peroxide in PBS. Sections were then rinsed in PBS, mounted onto chrome-alum slides, dried, and coverslipped with DPX.

Localization of auditory neurons in MLd/tonotopy

The borders between MLd and the surrounding nucleus intercollicularis (ICo), and the subregions of MLd have not been systematically defined in the zebra finch as they have in the barn owl (see Knudsen 1999). Therefore we used BDA injections (Fig. 2A) and Nissl-stained sections (Fig. 2B) to determine the borders of the area responsive to sound. Generally, two injections were made to label a pass, usually at the borders of the region responsive to sound. We then compared the locations of the injections with publications and brain atlases describing the MLd/ICo complex in numerous avian species including zebra finch to localize our recordings sites. The medial, dorsal, and ventral limits of MLd were often visible in Nissl sections. BDA injections that were placed to mark the medial and lateral borders of the auditory area matched the borders we identified using Nissl-stained sections. In the most rostral and most caudal portions of the MLd/ICo complex, the lateral borders of MLd were not visible in Nissl-stained sections. Because of this uncertainty, it is possible that some of the more rostral and lateral recording sites extended into ICo. As indicated by BDA injections labeling the borders of auditory responsive areas, the most lateral region from which we recorded was generally responsive to acoustic stimuli, and single units from this region were included in our analysis.

RESULTS

We recorded from 118 cells in the auditory midbrain (see Figs. 1 and 2). Seven of those cells did not respond well to tones and were excluded from the analysis.

![Diagram of the generalized avian auditory system and the passerine song control nuclei. Ascending auditory circuits are shown in black. Dashed gray lines indicate the major descending song control circuit. Solid gray lines indicate the pathways providing descending input from the song nuclei HVC shelf and RA cup to MLd and Ov. Diagram shows the following brain regions: caudal hyperstriatum ventrale (cHV), dorsal nucleus of the mesencephalon (DM), HVC (used as a proper name), nucleus intercollicularis (ICo), field L, lateral lemniscal nuclei (LL nuclei), mesencephalicus lateralis dorsalis (MLd), nucleus angularis (NA); caudomedial neostriatum (NCM), nucleus interfascialis (NIF), nucleus laminaris (NL), nucleus magnocellularis (NM), nucleus ovoidalis (Ov), robust nucleus of the archistriatum (RA), superior olivary nucleus (SON), and nucleus uvaformis of the thalamus (Uva). MLd receives multiple parallel inputs from lower brain stem and provides the sole input to the auditory thalamus (Ov).](http://jn.physiology.org/abstract/jn.91.1.138)

J Neurophysiol • VOL 91 • JANUARY 2004 • www.jn.org
Figure 2 shows the location of a BDA injection within MLd. Figure 2B shows the adjacent Nissl-stained section. The CFs of cells systematically increased as electrode passes moved from dorsal to ventral positions. Figure 2C illustrates the anatomical locations and CFs of cells near the dorsal and ventral borders of MLd. The recording sites shown are from different birds. The recording locations were estimated in each bird using BDA injections and then projected onto a schematic drawing representing MLd. The tonotopic pattern showing an increase in CF in the dorsal to ventral orientation is the same as that found in the mammalian IC. No clear arrangement of temporal response patterns was found.

Spontaneous activity

Most (100 out of 111) MLd units showed no spontaneous activity (Fig. 3). Eleven of 111 cells showed low spontaneous activity (<7 spikes/s). No specific CF was associated with the presence of spontaneous firing, but each cell with some spontaneous firing showed either a “primary-like” or “sustained” response to tones (see following text).

Excitatory frequency response areas

Because we are ultimately interested in how cells in MLd process acoustic communication signals, we examined frequency and intensity coding together by collecting excitatory FRA plots (see above). Figure 4 shows examples of FRAs from 2 different cells to illustrate temporal response patterns and frequency tuning. Each cell fired with a consistent temporal response pattern across frequencies and intensities. One cell had an ongoing or sustained response pattern and was narrowly tuned to frequency (Fig. 4A). The other cell had an onset pattern and was broadly tuned to frequency (Fig. 4B).
Temporal response patterns

FRA plots were used to determine temporal response patterns for each cell. Additional responses to tones at CF between threshold and 90 dB SPL were used to confirm response pattern. Of 111 cells, 92 showed clear response patterns. The 19 cells for which a response pattern could not be assigned either had responses that were too variable to characterize or changed response patterns when intensity changed. Those cells with consistent responses were first classified as responding transiently with only 1–4 spikes per stimulus (onset) or responding throughout the duration of the stimulus (ongoing). Of the cells showing ongoing responses, 3 distinct temporal response patterns were observed. These 3 response patterns correspond to the “primary-like,” “sustained,” and “primary-like with notch” response patterns observed in single neurons of both mammalian and avian auditory nuclei (Koppl and Carr 2003; Pollak et al. 1978; Syka et al. 2000; Warchol and Dallos 1990). All consistent responses fell into 4 categories: 1) onset, in which a few spikes were locked to the onset of the stimulus; 2) primary-like, in which the response showed strong firing at the onset of the stimulus followed by significant but less firing throughout the rest of the stimulus; 3) sustained, in which the firing rate was essentially the same throughout the entire duration of the stimulus; and 4) primary-like with notch, a primary-like response but with a pause in firing just after an onset response and before a sustained response. Examples of these 4 response patterns are shown in Fig. 5.

Of the 92 cells for which response pattern could be assigned, 49% had an onset pattern, 20% had a primary-like pattern, 19% had a sustained pattern, and 12% had a primary-like with notch pattern. Some cells that were classified as primary-like showed chopper-like qualities (Fig. 5E). Of the cells with ongoing responses to pure tones (47 total), most (93%) had responses that matched the stimulus in duration (Fig. 5, B–D). Four percent of cells with ongoing responses showed firing that lasted significantly longer (>50%) than the stimulus duration (Fig. 5F), and 3% of ongoing cells fired longer than onset cells but stopped firing before the end of the stimulus.

FIG. 4. Examples of excitatory frequency response area (FRA) plots. Peristimulus time histograms (PSTHs) for one cell’s responses to each frequency/intensity combination are shown in a grid. Response patterns and tuning curves were determined using these plots and additional tests. A: example FRA plot showing the responses of a cell with the “primary-like” response pattern and narrow frequency tuning. B: FRA plot showing the responses of an onset cell that is widely tuned to frequency.
Characteristic frequency and threshold were not correlated ($r = -0.080$; Fig. 6A). Characteristic frequencies ranged between 0.9 and 6.1 kHz. Mean ($\pm$ SE) CF for all cells was 3.46 ($\pm$ 0.14) kHz. Thresholds ranged between 4 and 75 dB SPL, with a mean of 34.82 ($\pm$ 0.17). Figure 6B shows the distribution of thresholds for cells grouped by response pattern. The distribution of thresholds was slightly wider for ongoing responses (4 to 75 dB SPL) than for onset responses (15 to 70 dB). The mean threshold for cells with onset responses (40.33 ($\pm$ 2.09 dB SPL) was significantly higher (unpaired t-test; $P = 0.005$) than that for cells with ongoing responses (30.74 ($\pm$ 2.12 dB SPL). There were no significant differences among the mean thresholds for cells showing primary-like, sustained, and primary-like with notch type responses (single-factor ANOVA; $F = 1.714$; $P = 0.190$). Mean ($\pm$ SE) thresholds were 27.00 ($\pm$ 2.52) dB SPL for cells with primary-like responses, 35.92 ($\pm$ 5.31) for sustained responses, and 30.85 ($\pm$ 2.83) for primary-like with notch responses. Because many more cells with ongoing responses had lower thresholds (<25 dB SPL) than did cells showing onset responses (Fig. 6), and because all cells showing spontaneous firing had ongoing response patterns, it is possible that cells with ongoing responses are generally more excitable than cells with only onset responses.

The ranges and mean values of characteristic frequencies for neurons with different response types were similar (data not shown). CFs for cells with onset responses ranged between 1.5 and 6.1 kHz. CFs for cells showing ongoing responses ranged between 0.9 and 6.1 kHz.

Width of frequency tuning

Tuning curves were generated from FRA plots. Tuning curve widths were highly variable across the population of cells. The narrowest tuning curve we encountered showed a maximum width of 0.5 kHz at 50 dB above threshold and a minimum width of 0.1 kHz at just above threshold. The widest tuning curve spanned the 7.0-kHz range of test frequencies at

**CF and threshold**

FIG. 5. PSTHs showing representative examples of the 4 response patterns. A: “onset,” indicating a strong response to the onset of the sound stimulus and little or no response to the rest of the stimulus. B: “primary-like,” in which an onset response is followed by a progressively less vigorous response to the remainder of the stimulus. C: “sustained,” in which a constant response is given throughout the duration of the stimulus. D: “primary-like with notch,” in which an onset response is followed by no response for a fixed duration and then a sustained response to the remainder of the stimulus. E: some of the cells classified as “primary-like” showed transient periodicity that did not match the period in their responses to tones. An example of this “chopper-like” pattern is shown. F: some cells showed sustained responses that lasted longer than the stimulus.

FIG. 6. Plots showing the distribution and means $\pm$ SE of thresholds and characteristic frequencies (CFs) for cells grouped by response pattern. A: thresholds and characteristic frequencies were not correlated. B: threshold distributions and means are plotted for cells showing the onset and ongoing response patterns. Range of thresholds for onset cells was smaller than that for cells with ongoing responses. Cells with ongoing responses showed significantly lower thresholds. Each diamond represents the value for one cell. Horizontal bars indicate mean values. Vertical bars on the sides of the horizontal bars indicate 1 SE. **$P < 0.01$.
maximum and 1.0 kHz at threshold. Examples of tuning curves are shown in Fig. 7.

Because we are interested in how these neurons process vocalizations, we examined the relationship between the frequency range to which a neuron is sensitive (bandwidth) and CF at a fixed intensity that represented a “behavioral sound level.” For this analysis, a sound level of 80 dB SPL was chosen because that is the peak sound level at which songs are presented in many physiological studies (Sen et al. 2001; Theunissen and Doupe 1998). Tuning curve bandwidths were measured at 80 dB SPL and compared with temporal response patterns, thresholds, and CFs. There was no clear relationship between tuning curve bandwidth at 80 dB SPL and temporal response pattern or threshold. Characteristic frequencies were positively correlated with tuning curve bandwidth at 80 dB ($r = 0.356$, $P = 0.0005$); cells with wider tuning curves had higher CFs (Fig. 8). There was no difference in correlation between bandwidth at 80 dB SPL and CF in cells with onset responses versus cells with ongoing responses.

Tuning curve shapes varied widely. Seventy-eight percent of cells showed V-shaped tuning curves, with the slope of the low frequency side of the curve usually being less than the slope on the high-frequency side of the curve. V-shaped curves were found across a wide range of CFs and thresholds (Fig. 7). The remaining 22% of cells showed more complex tuning curve shapes. Curves showing square, columnar, or tilted shapes as well as multiple excitatory peaks or multiple, noncontiguous excitatory regions were considered to be complex. Figure 9 shows examples of complex tuning curves. Although some cells showed nonmonotonic responses at CF (see following text), no closed tuning curves [also called type IV (Sachs and Sinnott 1978; Young and Brownell 1976) or type O (Ramaechandran et al. 2000)] were observed.

The relationship between threshold and tuning curve shape was examined by calculating a $Q_{10}$ and $Q_{30}$ for each cell (Fig. 10). The correlation between $Q_{10}$ and threshold was $0.064$, indicating that there was no relationship between threshold and sharpness of tuning at 10 dB above threshold. The relationship between threshold and $Q_{10}$ was similar for cells showing onset responses and those showing ongoing responses (Fig. 10A). The correlation between threshold and $Q_{30}$ was $-0.294$, indicating a slight negative relationship between threshold and tuning curve width at 30 dB above threshold. The relationship between threshold and $Q_{30}$ was different for cells grouped by temporal response type; cells with ongoing responses showed lower thresholds and higher $Q_{30}$s (Fig. 10B) than onset units. Onset units showed a more limited range of $Q_{30}$s. Tuning curve width was also compared with CF (Fig. 10, C and D). The correlation between $Q_{10}$ and CF was $0.182$, indicating a weak positive relationship between sharpness of tuning near threshold and CF. The relationship between CF and $Q_{10}$ was not different between cells with onset and ongoing responses (Fig. 10C). Despite the linear relationship between CF and $Q_{10}$ in some cells (Fig. 10C), there was only a weak positive correlation between CF and $Q_{10}$ overall. The correlation between $Q_{30}$ and CF was $0.183$, indicating a similar positive relationship between sharpness of tuning and CF as the level increased (Fig. 10D). The $Q_{30}$s of cells with ongoing responses were higher than those of onset cells across a wide range of CFs (Fig. 10D).

**Intensity coding**

To examine intensity coding, we collected RLFs in response to pure tones at CF. Cells that showed ongoing responses ($n = 47$) were the focus of this analysis because there was little or no change in spike rate as a function of intensity (above threshold) for cells showing only onset responses. Cells with ongoing responses fell into 3 classes in terms of their RLFs: 1)
monotonic, in which spike rate continually increased as intensity increased (Fig. 11A); 2) low saturation, in which the spike rate increased with increasing intensity at lower intensities but then reached a plateau at which spike rate remained constant with further increases in intensity (Fig. 11B); and 3) nonmonotonic, in which spike rate increased with intensity up to some point, but then decreased with further increases in intensity (Fig. 11C). Of those cells showing ongoing temporal responses, 51% showed monotonic RLFs, indicating sensitivity to a wide range of changes in intensity, and 39% showed low saturation RLFs. Ten percent of cells (5/47) showed nonmonotonic RLFs. These cells had either primary-like or sustained temporal response patterns.

The dynamic range is defined as the total range of sound
level over which the spike rate changed, measured between a cell’s threshold level and the maximum sound intensity tested. Cells with the different types of RLFs showed differences in dynamic range. Figure 12 shows the distributions of dynamic ranges for the 3 RLF types. The dynamic ranges for cells with monotonic responses were broad, between 15 and 80 dB (means ± SE: 53.28 ± 3.71 dB). Dynamic ranges for all cells with low saturation RLFs were between 7 and 40 dB, with a mean of 19.44 ± 2.16 dB. Such limited dynamic ranges suggest that these cells are not good at encoding intensity change because they show sensitivity for changes at lower intensities only. For cells with nonmonotonic RLFs, the mean dynamic range was 58.75 ± 11.23 dB, with a range of 32 to 86 dB. Nonmonotonic cells cannot code intensity unambiguously because the same spike rate can be elicited by 2 different intensities.

**Response latencies**

First-spike latencies were measured for responses to tones presented at CF, 20 dB above threshold. Under these conditions, the minimum response latency observed for the cells in our sample was 4 ms and the maximum latency was 40 ms. The mean first-spike latency for all cells was 11.36 ± 0.9 ms.

Figure 13 shows the distribution of first-spike latencies for onset and ongoing responses, and the relationship between first-spike latency and CF. Most latencies were clustered around 10 ms (Fig. 13, A and B). No relationship between first-spike latency and CF was found (Fig. 13 C).

Response latencies were slightly different between cells with onset response patterns and cells showing ongoing responses. The mean latency of onset cells was 10.31 ± 0.44 ms. This was significantly shorter than that of cells with ongoing responses (primary-like, sustained, and primary-like with notch), which had a mean of 13.8 ± 1.05 ms. These differences suggest that onset cells on average respond slightly faster to tones than do cells with ongoing responses.

For 15% of the cells in our sample there were no significant changes in spike latency across intensity. For the other 85%, response latency varied with both intensity and frequency. For 69% of all cells, latencies decreased with increasing sound level. Figure 14A shows a representative example of how response latency changed with sound level for an onset cell. In this example, the mean first-spike latency decreased from 8.1 to 6.0 ms as the sound level increased from 35 to 90 dB SPL. Figure 14B shows data from a cell with an ongoing response. For this cell, the mean first-spike latency decreased from 15.2 to 4.8 ms between 30 and 90 dB. For 16% of cells, latency decreased with increased sound level up to some point, but then increased with further increases in sound level. This effect is called a paradoxical latency shift because it is the opposite of the relationship usually observed between intensity and latency (Sullivan 1982). Figure 14C shows an example of paradoxical latency shift. Note that latency does decrease with increased sound level near threshold, but the pattern reverses at higher levels.

To determine whether cells with onset responses are more temporally resistant to level differences than cells with ongoing responses, we compared the variability of spike latency across the entire range of test intensities (threshold to 90 dB SPL) in cells showing onset responses with the same measurement for cells showing ongoing responses. To control for differences in dynamic range that could bias the measures of latency changes, this comparison was done using a subset of cells with onset responses and a subset of cells with ongoing responses. Each subset was balanced for average threshold by randomly select-
Changes in subset showed an average threshold of 37 dB. Results showed
of each subset matched. This match was achieved when each
ing cells of each response pattern until the average thresholds
of each subset matched. This match was achieved when each
subset responded only (A) and
cells with ongoing responses (B). C: first-spike latency as a function of CF. Each symbol indicates the value for an individual cell.
Cells with onset and ongoing responses are distinguished by different symbols. No correlation between spike latency and CF was
longest

The response latency of most cells also varied across sound level.

As might be expected, only cells with midwidth and wide
tuning had a large enough frequency range to show this
interaction between latency and frequency.

Inhibition

Because MLd cells in the anesthetized zebra finch show little
or no spontaneous activity, we were not able to observe direct
evidence of how inhibition shapes frequency and intensity
coding in these neurons. However, the influence of inhibition
on response characteristics was indirectly observed. First, the
observation of complex tuning curves suggests the conver-
gence of multiple excitatory inputs and or competing excitatory
and inhibitory inputs onto a cell. Second, some cells showed
nonmonotonic RLFs (Fig. 11), suggesting that higher sound
levels recruited inhibitory inputs that suppressed the excitation
observed at lower sound levels. Third, the observation of
paradoxical latency shift suggests that inhibition can suppress
the early part of the response at high intensities. Figure 16
shows a single cell’s responses to tones presented at CF, at
varying sound levels. The response exhibits both a nonmono-
tonic RLF and a paradoxical latency shift, suggesting the
influence of inhibition on spike rate and response timing. At
higher intensities, firing occurs after the offset of the stimulus.
A cessation of firing accompanies the stimulus offset and is
then followed by a resumption of firing, suggesting inhibition
at the stimulus offset and the subsequent release from inhibi-
tion.

Duration testing

We tested cells at different sound durations to determine
whether their responses were locked to the onset or offset of
sound. Responses to sound offset are a characteristic of those
cells in the mammalian IC that respond selectively to narrow
ranges of sound duration. Pure tones of durations between 5
and 300 ms were presented at CF and at other frequencies. We
found neither cells that responded to sound offset only nor cells
that responded selectively to sound duration.

Discussion

To our knowledge, this is the first examination of the tuning
properties of auditory midbrain neurons in a songbird. Our goal
was to describe the temporal response patterns, frequency
coding, and intensity coding of these neurons to simple stimuli.
This characterization is intended to provide a basic understand-
ing of tuning properties in the auditory midbrain, which can be
helpful in designing studies to address the processing of com-
plex sounds, including biologically important sounds such as
songs and communication calls.

Single MLd units in the zebra finch showed numerous tem-
poral response patterns and a wide range of frequency tuning,
with some cells showing highly complex frequency coding. A
general lack of sensitivity to intensity changes was observed.
Many cells showed only onset responses that have small dy-
namic ranges and 39% of the cells with ongoing responses
showed low-saturation RLFs. These findings suggest that the
songbird auditory midbrain may be particularly good at pro-
cessing temporally complex signals that show a high degree of
AM. Here, we compare the tuning properties of zebra finch
MLd units with those of auditory neurons in the midbrains of
other birds, the inferior colliculus of mammals and the lower
brain stem nuclei of birds. The implications of pure tone
**Comparison with MLd studies in other birds**

Among birds in general, very little study has been devoted to auditory midbrain and thalamus (see Koppl et al. 2000). The one exception to this paucity of information is the auditory space map in the MLd (called the IC) of barn owls (Knudsen and Konishi 1978; see Knudsen 1999 for review). Most of the work in owls has focused on mechanisms of sound localization, but some work has examined frequency and temporal tuning properties (Keller and Takahashi 2000; Knudsen and Konishi 1978; Koppl 1997, 2001; Sachs et al. 1980; Wagner 1990; Wagner et al. 1987, 2002). Neurons in the region of the barn owl midbrain that receives direct input from the cochlear nuclei, the ICCcore, share numerous tuning characteristics with the cells from which we recorded. Neurons in this region are organized into isofrequency layers (Takahashi and Konishi 1988) such that CFs increase along the dorsoventral axis (Knudsen and Konishi 1978; Wagner et al. 1987) and show single-peaked, V-shaped excitatory tuning curves (Wagner et al. 2002). Also consistent with our findings is the fact that the CFs of neurons in the ICCcore are distributed throughout the bird’s entire hearing range (Wagner et al. 2002). This is in contrast to neurons in the ICx, a midbrain region just lateral to the ICCcore where cells respond only to higher frequencies and are tuned to specific interaural time differences (Knudsen and Konishi 1978; Mazer 1998; Takahashi and Konishi 1986). Response patterns in owl IC neurons can vary depending on factors such as changes in

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**FIG. 14.** Dot rasters showing responses to tests examining changes in latency as a function of sound level. Stimuli are tones of either 20 (A) or 50 (B and C) ms in duration, as indicated by the bold line below the x-axis. A: dot raster for the responses of an onset cell, showing that latency decreases slightly with increased sound level. B: responses of an ongoing cell to varying sound levels. Latency decreases with increases in sound level. C: responses of a cell showing a paradoxical latency shift at sound levels of ≥50 dB.

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**FIG. 15.** Differences in the mean longest (max) and shortest (min) first-spike latencies for cells grouped according to response pattern. Ongoing cells show a greater range of latencies and therefore greater latency differences as sound level varies. First-spike latencies are more stable across intensities in onset cells. Error bars indicate 1 SE. **P < 0.01.

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**FIG. 16.** Dot raster showing an example of a paradoxical latency shift. First-spike latency increases with increased sound level above 50 dB, suggesting that increased sound level evokes stronger inhibition and the initial response seen at lower sound levels is blocked. Note also the pause and then reinitiation of firing after the end of the stimulus at higher intensities.
interaural time differences (ITDs; Wagner 1990). The sounds
we used were presented free-field, from a speaker in front of
the bird. Therefore we were not able to examine the effects of
varying sound source location on temporal response patterns.
Consistencies between the tuning properties of ICCcore units
and our findings suggest that the cells from which we recorded
in zebra finch MLd may correspond only to the ICCcore and
not to the additional, more specialized regions in the barn owl
auditory midbrain.

Two studies have examined tuning in MLd neurons of
terrestrial birds. Coles and Aitkin (1979) examined response
patterns, frequency tuning, and spike latencies in the chicken
MLd. Similar to our findings, they found that onset and pri-
mary-like response types were common whereas offset re-
 sponses were rare. As with the majority of our cells, latencies
generally decrease with increasing intensity. Minimum laten-
cies in the chicken were measured at 5 ms, agreeing well with
our minimum latencies of 4 ms. Frequency tuning appears to
be more complex in the zebra finch. Although the majority of
MLd neurons in both species show V-shaped tuning curves,
zebra finch neurons show complex tuning curves more fre-
quently and with more complicated patterns. Coles and Aitkin
found that 12% of chicken units showed double-peaked or
“O-type” curves. We found complex tuning curves with mul-
tiple peaks, noncontiguous excitatory regions, and narrow, tild,
and columnar shapes in 22% of zebra finch units. Assuming
that the coevolution of auditory and vocal systems affected
their physiological functioning, differences in the complexity
of frequency tuning could be related to differences in the
acoustics of vocal signals. Chickens have relatively simple
vocal repertoires that do not include learned components,
whereas zebra finch vocalizations are complex in both fre-
quency and amplitude, and are shaped by learning. Alterna-
tively, it is possible that other factors that are unrelated to vocal
behavior explain differences in frequency tuning. For example,
chickens have smaller hearing frequency ranges than do song-
birds. It is therefore possible that a decreased range of potential
tuning frequencies results in simpler tuning curves.

Scheich and colleagues (1977) examined the responses of
MLd neurons to vocalizations and some tonal stimuli in the
guinea fowl. Their birds were unanesthetized and most of
the neurons they investigated showed spontaneous activity. They
divided cells into “simple” and “complex” in terms of how well
a neuron’s frequency tuning predicted its responses to vocal-
izations. Similar to many cells from which we recorded, guinea
fowl units showed single- and multipeaked tuning curves, with
tuning bandwidths generally widening with increased sound
level. Cells that were nearly purely inhibitory, based on sup-
pression of spontaneous activity, were also found. We would
not have detected such inhibitory neurons in our sample be-
cause our cells had no spontaneous rate. Characteristic fre-
quencies fell between 1 and 4 kHz, indicating correlations
between CF range, hearing frequency range, and the power
spectra of guinea fowl vocalizations. Similarly, the range of
CFs in our cells (0.9–6.1 kHz) match both the zebra finch
audiogram (Okanoya and Douling 1987) and the power spec-
trum of zebra finch song (S.M.N. Woolley, personal observa-
tion). The fact that Scheich et al. found “complex” units for
which tonal responses could not predict responses to natural
calls provides evidence in favor of the idea that the MLd may
contain nonlinear tuning properties that correspond to a bird’s
vocal patterns.

Comparison with mammalian auditory midbrain—
is the bird different?

The mammalian auditory midbrain region, the inferior col-
liculus (IC), has been extensively studied in a variety of species
including cats, chinchilla, and mice. Here, we compare what is
known about response patterns, frequency tuning, and interac-
tions between latency and sound level in the IC to our
findings in the MLd. Clear similarities among the response patterns
found in the mammalian IC and MLd exist. As in MLd, some
mammalian IC cells exhibit only onset responses to tones; the
percentages of total cells showing only onset responses varies
across species and studies (Egorova et al. 2001; Nuding et al.
1999; Pollak et al. 1978; Rose et al. 1963; Skya et al. 2000).
However, the IC appears to be less dominated by onset cells
than the MLd (although see Wagner 1990). Tonic responses of
the primary-like, sustained, and primary-like with notch types
(as defined here) are also included in the mammalian IC
(Pollak et al. 1978; Skya et al. 2000). Some studies have shown
that additional response patterns such as onset–offset, offset,
and purely inhibitory are present in the IC (Casseday et al.
We did not find onset–offset or offset units. Mammalian onset
units respond with latencies that are shorter and more tempo-
rally constrained than those of cells with ongoing responses
(Nuding et al. 1999). The fact that onset cells appear to be more
temporally consistent in both birds and mammals suggests that
onset cells in general may be best at encoding some aspects of
time-varying signals because the timing of changes in both
frequency and amplitude can be accurately preserved in the
neural response.

Our findings suggest that the zebra finch MLd shows the
same general pattern of tonotopy as is found in the mammalian
IC (Casseday and Covey 1992; Nuding et al. 1999; Semple and
Aitkin 1979). Consistent with observations in the MLd of the
guinea fowl (Sheich et al. 1977) and the IC of cats and bats
(Casseday and Covey 1992; Pollak and Bodenhamer 1981;
Pollak et al. 1978), CFs in the zebra finch correspond well with
the frequency range and power spectra of species-typical vocal
signals and species-specific audiograms. Many units in both cat
and bat IC show V-shaped tuning curves similar to those that
we observed in the zebra finch. However, the bat IC contains
neurons that show more specialized frequency tuning charac-
teristics than any we observed in the zebra finch. Some bat IC
neurons show “narrow-filter” tuning curves, with extremely
limited excitatory frequency response areas. Narrow filter neu-
rons are found in the brain region that encodes frequencies
contained in the narrowband echolocation signals that the bat
uses when searching for prey (Casseday and Covey 1992;
Pollak and Bodenhamer 1981). Some MLd units did show
level-tolerant “columnar” frequency tuning curves that were
similar to the narrow-filter units of bats but broader. Such
tuning differences may reflect differences in the acoustics of
the vocal communication signals in bats and zebra finches; bats
use narrow-frequency signals and zebra finches use broadband
signals.

J Neurophysiol • VOL 91 • JANUARY 2004 • www.jn.org
Comparison with tuning properties of cochlear nucleus neurons—what properties may emerge in MLd?

The ascending inputs to MLd are the brain stem nuclei, nucleus magnocellularis (NM), nucleus laminaris (NL), and nucleus angularis (NA). These areas have been well described both anatomically and physiologically in chicks and owls (Carr and Konishi 1988; Koppl 1997, 2001; Koppl and Carr 2003; Parks and Rubel 1978; Rubel and Parks 1975; Sachs and Sinnott 1978; Sullivan 1985; Takahashi and Konishi 1988; Warchol and Dallos 1990). One study examined the frequency tuning found in NM and NA of a songbird, the red-winged blackbird (Sachs and Sinnott 1978). Some basic response characteristics are shared between MLd and the cochlear nuclei of other birds. A match between hearing range and the range of CFs measured in the MLd is also found in the cochlear nuclei (most clearly in NA) of chicks, red-winged blackbirds, and barn owls (Koppl and Carr 2003; Rubel and Parks 1975; Sachs and Sinnott 1978; Warchol and Dallos 1990). The wide range of thresholds found in MLd is consistent with the range of thresholds found in NM and NA (Koppl and Carr 2003; Warchol and Dallos 1990). The average threshold sensitivity of MLd neurons appears to match better with NA than NM; NM thresholds are generally higher. In the cochlear nuclei and in MLd, no correlation between CF and threshold is found.

In all of these bird species, NM contains only one, primary-like, response pattern. NA contains primary-like and onset responses (Koppl and Carr 2003; Sachs and Sinnott 1979; Sullivan 1985; Warchol and Dallos 1990) as well as chopper-transient responses (Koppl and Carr 2003; Sullivan 1985; Warchol and Dallos 1990). We recorded some chopper-transient responses in MLd of the zebra finch (see Figs. 5E and 14B), but the periodicity we observed was not generally as pronounced as that reported for NA. Although we performed many tests on an individual cell, each test typically contained only 15 to 25 trials. This number of repetitions provided enough data to classify units into major categories (given that multiple tests were used), but the data were not sufficient to distinguish chopper-like from primary-like response patterns. Therefore these responses, which showed the overall shape of primary-like responses, were classified as primary-like. The sustained responses found in 20% of MLd units and the primary-like with notch response types found in 12% of MLd units have not been described in avian cochlear nuclei, although they are present in the mammalian auditory midbrain, nuclei of the lateral lemniscus, and cochlear nucleus (Covey and Casseday 1991; Pollak et al. 1978; Skya et al. 2000). This suggests that, in birds, the sustained and primary-like with notch response types that are typical throughout the mammalian auditory system are first seen in the MLd.

Another notable difference between MLd and the cochlear nuclei in terms of temporal response patterns is the preponderance of temporally precise, onset responses in MLd. Although onset responses are present in NA, they do not occur in high numbers (Koppl and Carr 2003; Sullivan 1985; Warchol and Dallos 1990). Additionally, NA cells do not give a purely onset response; responses are usually strong to the onset of a stimulus and weakly continued to the rest of the stimulus. Nearly all onset cells in MLd respond only to the stimulus onset and do not spike again until a second stimulus is presented. In this way, MLd onset units may be particularly good at marking the temporal relationships between sequential stimuli with high fidelity, potentially firing and recovering quickly to encode rapid amplitude modulations.

Tuning curves for MLd cells appear to differ significantly from those found in the cochlear nuclei. Tuning curves in cochlear nucleus neurons are V-shaped, apparently without a wide variety of bandwidths. We observed V-shaped frequency tuning curves in 78% of units, but the $Q_{10dB}$, $Q_{20dB}$, and bandwidths at 80 dB SPL varied greatly. Our V-shaped curves showed maximum frequency bandwidths as limited as 1 kHz and as broad as 7 kHz. Additionally, 22% of the MLd tuning curves were complex, showing multiple peaks and/or multiple excitatory regions, columnar shapes, and tilt shapes. The fact that these types of complex frequency tuning curves have not been found at lower levels suggests that they emerge in MLd.

The presence of GABAergic neurons has been documented in the avian MLd (Carr et al. 1989; Fujita and Konishi 1991; Granda and Crosland 1989), suggesting that GABAergic inhibition might play a role in generating complex frequency tuning through the interplay of excitatory and inhibitory inputs. In mammals, especially in bats, multiple peaked tuning curves are found in IC neurons that respond optimally to 2 tones that differ in specific frequency and temporal spacing from one another (Dear and Suga 1995; Feng et al. 1978; Portfors and Wenstrup 1999, 2001; Simmons 1971, 1973). This raises the question of whether similar frequency-combination, delay-sensitive neurons exist in MLd.

MLd units were found to have limited dynamic ranges, meaning that they were relatively insensitive to changes in sound level. Onset cells (49% of MLd cells) inherently show small dynamic ranges because of the fact that very few spikes are elicited by any one stimulus. Many of the MLd cells with ongoing responses showed RLFs that saturated at relatively low intensities, with spike rates plateauing between 40 and 50 dB SPL. In total, only 26% of all the cells from which we recorded showed rate-level functions that did not saturate at our highest test intensities. The level insensitivity to sounds presented at intensities over approximately 50 dB SPL that was exhibited by most MLd neurons is in contrast to the RLFs described for neurons in the cochlear nuclei. Few cells in NM and NA are reported to show low-saturation RLFs. The functional consequences of an emergent insensitivity to intensity changes in MLd remain to be examined. Such cells may function best while coding signals with significant AM. It is possible that consistent responses regardless of overall intensity differences could act to stabilize the precise encoding of temporally complex signals that have the same meaning regardless of intensity. It is important to note, however, that the frequency bandwidth to which an MLd cell responds is highly dependent on intensity. Thus complex interactions between the intensity and frequency modulations that are typical of biologically meaningful sounds would still be expected to occur in MLd.

Implications for the processing of natural, complex sounds

The limitations of using simple, synthetic stimuli such as pure tones to examine how auditory circuits process sound in general have been recognized for decades (Casseday and Covey 1996; Pollak et al. 1978; Scheich et al. 1977; Theunissen and Doupe 2000). Nonlinear tuning properties that repre-
sent sensory adaptations (specializations) for coding vocal signals may be revealed only by approaching the system using complex stimuli, preferably containing the acoustic properties of the natural sounds under which the auditory system has evolved. In some systems, a unit’s responses to 2 tones presented separately cannot predict the response to those same tones presented simultaneously or sequentially (Dear and Suga 1995; Jen et al. 2002; Nieder and Klump 1999), much less within a complex signal (Klug et al. 2002; Scheich et al. 1977). Thus studying the responses of MLd neurons to pure tones may fail to reveal tuning complexities in the songbird midbrain. With this caveat in mind, it is helpful to have a basic understanding of the responses of these neurons to simple stimuli in terms of response patterns, and frequency/intensity coding before attempting to understand their responses to complex stimuli. Here, we draw 3 conclusions about the processing of simple stimuli that allow us to make predictions about how these neurons may function in processing complex signals such as songs and calls. In this way, we can ask what information within natural sounds may be transmitted to higher levels through MLd.

Half of the cells we encountered showed only onset responses to free-field sound presentation, with precise timing regardless of intensity changes. These cells could be optimal for encoding the temporal patterns of complex signals that are characterized by amplitude modulations such as songs. For example, a broadband onset cell could detect the onsets of any vocal element that is preceded by silence, such as a song syllable, thereby preserving the amplitude envelope of the song through a distinctly patterned sequence of spikes. Narrowband onset cells could detect the onsets of particular vocal elements that are not necessarily preceded by silence but that contain frequencies falling within the excitatory tuning curve of that cell. Second, we found a large variety of frequency tuning characteristics across cells. A population of cells showing highly different frequency tuning could be beneficial for generating activity in a unique constellation of neurons in response to each call, song note, song syllable, and so forth. In this scenario, the concurrent firing of a specific group of midbrain neurons could serve as the neural representation of an individual vocalization. Finally, most of the neurons we characterized were surprisingly insensitive to intensity changes at and around “behavioral levels.” The inherent nature of an onset response makes it poor at resolving intensity, showing the same phasic spike pattern regardless of sound level. Such level tolerance has also been observed in the region of the bat IC that encodes echolocation frequencies (Pollak and Bodenhamer 1981). In MLd, many of the cells with ongoing responses also showed limited dynamic ranges, suggesting that intensity discrimination above 50–60 dB SPL is not accomplished well. This insensitivity could function to maintain the constancy of response patterns that code signals, such as song, that have the same meaning regardless of their overall intensity. To investigate this issue, the responses to simple stimuli and to complex, natural stimuli would, ideally, be examined in the same cells.

ACKNOWLEDGMENTS

We thank B. Warren for programming expertise and K. Miller for assistance with histological procedures. We also thank E. Covey and K. Miller for comments on an earlier version of this manuscript.

GRANTS

This work was supported by National Institute on Deafness and Other Communication Disorders Grants DC-00287 and DC-04661 and the University of Washington Royalty Research Fund.

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