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Developmental Experience Alters Information Coding in Auditory Midbrain and Forebrain Neurons

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Abstract

In songbirds, species identity and developmental experience shape vocal behavior and behavioral responses to vocalizations. The interaction of species identity and developmental experience may also shape the coding properties of sensory neurons. We tested whether responses of auditory midbrain and forebrain neurons to songs differed between species and between groups of conspecific birds with different developmental exposure to song. We also compared responses of individual neurons to conspecific and heterospecific songs. Zebra and Bengalese finches that were raised and tutored by conspecific birds, and zebra finches that were cross-tutored by Bengalese finches were studied. Single-unit responses to zebra and Bengalese finch songs were recorded and analyzed by calculating mutual information, response reliability, mean spike rate, fluctuations in time-varying spike rate, distributions of time-varying spike rates, and neural discrimination of individual songs. Mutual information quantifies a response's capacity to encode information about a stimulus. In midbrain and forebrain neurons, mutual information was significantly higher in normal zebra finch neurons than in Bengalese finch and cross-tutored zebra finch neurons, but not between Bengalese finch and cross-tutored zebra finch neurons. Information rate differences were largely due to spike rate differences. Mutual information did not differ between responses to conspecific and heterospecific songs. Therefore, neurons from normal zebra finches encoded more information about songs than did neurons from other birds, but conspecific and heterospecific songs were encoded equally. Neural discrimination of songs and mutual information were highly correlated. Results demonstrate that developmental exposure to vocalizations shapes the information coding properties of songbird auditory neurons.

Keywords

inferior colliculus; vocalization; songbird; neural coding; song

INTRODUCTION

A major function of auditory neurons is to identify behaviorally important sounds, including those produced by predators, prey, and social partners. In animals that depend on acoustic cues to communicate or echolocate, similarities between auditory tuning properties and the acoustics of vocalizations exist in midbrain and forebrain neurons, at the single unit and population coding levels (Pollak et al., 1977; Suga 1978; Rose and Capranica, 1983;

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Casseday et al., 1994; Reike et al., 1995; Wang et al., 1995; Covey and Casseday, 1999; Escabi et al 2003; Grace et al., 2003; Bass et al., 2005; Covey and Carr, 2005; Portfors and Sinex, 2005; Woolley et al., 2005, 2006). Several studies have shown that developmental exposure to simple, synthetic sounds such as clicks and pure tones alters frequency tuning in mammalian auditory forebrain neurons (Zhang et al., 2001, 2002; Chang and Merzenich, 2003; Nakahara et al., 2004; Norena et al., 2006; de Villers-Sidani et al., 2007) and midbrain neurons (Sanes and Constantine-Paton, 1985; Poon and Chen, 1992; Yu et al., 2007). But few studies have addressed how natural sound experience during ontogeny shapes the response properties of auditory neurons.

Songbirds that naturally flock are strong models for studying how developmental experience with natural sounds affects auditory development. For these animals, the sounds that are most important during development are known. Many estrildid finches, including zebra finches *Taeniopygia guttata*, live communally and learn their communication vocalizations using hearing and social interaction during ontogeny (Zann, 1996). In this regard, these songbirds are similar to humans. Estrildid finches can be raised in the laboratory and reared in families within colonies, experiencing the vocalizations of their parents and colony members. Juvenile song learning by imitation demonstrates the impact of developmental auditory experience on adult vocal communication in songbirds.

Little is known about what aspects of auditory responses differ across songbird species that use different acoustic signals to communicate and how auditory neurons encode different classes of natural sounds, such as conspecific and heterospecific vocalizations. Because the production and perception of conspecific vocalizations exert strong selective pressure on brain evolution, it is possible that the coding properties of auditory neurons are determined by species identity. It is also possible that developmental auditory experience influences the neural coding of songs. Behavioral studies on song preferences and mate choice suggest that developmental experience shapes auditory coding (Clayton, 1988, 1989; Campbell and Hauber, 2009a, b and c). Here, we measured species differences and the influence of developmental song experience on the auditory midbrain and forebrain coding of conspecific and heterospecific songs.

We studied the responses of single auditory neurons to conspecific and heterospecific song in two closely related species of songbird, zebra and Bengalese finches (*Lonchura striata* vars. *domestica*). Zebra and Bengalese finch songs contain spectral information covering the same frequency range, ~0.3 to ~10 kHz, but they differ in spectrotemporal structure. Zebra finch songs contain noisy and harmonic syllables of ~80 to 200 ms in duration (Immelmann, 1969; Price, 1979; Zann, 1996). Bengalese finch songs contain repeated, harmonic syllables that show low frequency spectral modulations, are ~50 to 150 ms in duration, and often show downward frequency-modulated energy (Immelmann, 1969; Woolley and Rubel, 1997). The influence of developmental experience on auditory coding was tested by cross-fostering juvenile zebra finches into Bengalese finch broods so that auditory responses could be compared across genetic zebra finches raised hearing zebra finch songs, genetic zebra finches raised hearing Bengalese finch songs and genetic Bengalese finches raised hearing Bengalese finch songs. Zebra and Bengalese finch audiograms are highly similar (Okanoya and Dooling, 1987). Therefore, species differences in general sensitivity to sound frequencies could be ruled out as potential influences on the results. Responses were analyzed first by comparing mutual information rates in responses from neurons in the three groups of birds and between song types. In the analysis of neural responses to sensory stimuli, mutual information is defined as the capacity of a response to encode information about a stimulus, in units of bits per second (Strong et al., 1998). Second, four specific response properties of single neurons were measured: response reliability, mean spike rate, fluctuations in time-varying spike rate, and the distribution of

time-varying spike rates. Finally, we compared the neural discrimination of individual songs across the three groups of birds, and between the two song types.

METHODS

Animals

All animal procedures were approved by the Animal Care and Use Committee at the University of California, Berkeley. Seventeen adult (>120 days of age) male zebra finches were used. Eleven birds were raised by their genetic parents in a zebra finch colony and tutored by their fathers. Six birds were cross-tutored into the nests of Bengalese finches as eggs or 1–2 day old hatchlings, and raised and tutored by their Bengalese finch foster fathers in a Bengalese finch colony. Eight adult male Bengalese finches that were raised by their genetic fathers in the Bengalese finch colony were also used. In both colonies, birds were raised in cages containing one family (mother, father, juveniles). During development, birds could hear but not see the birds in adjacent cages. Tutors' songs and the adult songs of the subject birds used were recorded and visually analyzed to ensure that tutor-specific song learning occurred in all birds, including those that were cross-tutored. Examples of tutor and tutee songs are shown in Figure 1. Attempts to cross-foster Bengalese finches into zebra finch families failed due to chick rejection (Hauber and Kilner, 2007).

Stimuli

Acoustic stimuli were unfamiliar songs from 20 adult zebra finches and 20 adult Bengalese finches. Zebra and Bengalese finch songs contain energy covering the same frequency range. Songs from the two species differ in their frequency power spectra (Fig. 2A and B), and spectrotemporal modulations (Fig. 2C). Each song sample (~ 2 s in duration) was recorded in a sound attenuated chamber (Acoustic Systems, Austin, TX), digitized at 32 kHz (TDT, Alachua, FL) and band-pass filtered between 250 and 8000 Hz. Each sample included 1–2 song motifs and introductory notes, whenever possible. For presentation, the intensity of each song sample was adjusted so that average levels in stimuli were at ~75 dB SPL. Song stimuli from each species were chosen to balance song sample duration and the summed duration of silence within a song sample. This was done to prevent potential response biases resulting from adaptation during ongoing sound presentation; some songbird auditory neurons respond less over time to ongoing sounds (Woolley and Casseday, 2004). The number and rate of silent intervals between song syllables within each song sample were measured but could not be balanced between the two species because Bengalese finch song naturally has shorter song syllables and therefore a higher rate of silent intervals.

All birds from which recordings were made learned the songs of the males that raised them (Fig. 1). Normal (i.e. not cross-tutored) zebra finches learned the songs of their zebra finch fathers. Bengalese finches learned the songs of their Bengalese finch fathers and sang normal Bengalese finch song. Cross-tutored zebra finches learned the songs of their Bengalese finch foster fathers.

Surgery

Two days before recording, a bird was anesthetized with Equithesin (0.03 ml i.m. of: 0.85 g chloral hydrate, 0.21 g pentobarbital, 0.42 g MgSO₄, 8.6 ml propylene glycol, and 2.2 ml of 100% ethanol to a total volume of 20 ml with H₂O). The bird was then placed in a custom stereotaxic with ear bars and a beak holder. Lidocaine (2%) was applied to the skin overlying the skull, and a midline incision was made. A metal pin was fixed to the skull with dental cement. The bird was then allowed to recover for 2 days.

On the day of recording, the bird was anesthetized with three injections of 20% urethane (3 i.m. injections, 30 ml each, 30 minutes apart) and was placed in the stereotaxic. The bird's head was immobilized by attaching the metal pin cemented to the bird's skull to a customized holder mounted on the stereotaxic. Lidocaine was applied to the skin overlying the skull regions covering the forebrain and optic lobe. After lidocaine application, small incisions were made in the skin over the skull covering the forebrain and optic tectum. Small openings were then made in the skull overlying the forebrain and optic tectum, and the dura was resected from the surface of the brain.

Electrophysiology

Electrophysiological recordings were made as in Woolley et al. (2005, 2006, 2009). Responses of single units in the auditory midbrain region, the mesencephalicus lateralis dorsalis (MLd), and in field L to zebra finch and Bengalese finch songs were recorded. Recordings were conducted while the bird was inside a sound-attenuated chamber (Acoustic Systems, Austin, TX). The bird was positioned ~ 20 cm in front of a Bose 101 speaker so that the bird's beak was centered horizontally and vertically with respect to the speaker cone. The output of the speaker was measured before each experiment with a Radio Shack electret condenser microphone (33–3013) and custom software to ensure a flat response (± 5 dB) from 250 – 8000 Hz. Sound levels were checked with a B&K sound level meter (RMS weighting B, fast) positioned 20 cm in front of the speaker at the bird's head. Body temperature was continuously monitored and adjusted to be between 38° and 39° C using a heater (FHC, Bowdoin, ME) with a thermistor placed under a wing and a heating blanket placed under the bird.

Recordings were obtained using epoxy coated tungsten electrodes (0.5 – 4.0 M Ω ; FHC, Bowdoin, ME). Electrodes were advanced into the brain with stepping microdrives at 0.5 micron steps (Newport, Irvine, CA). Extracellular signals were obtained with an extracellular amplifier (A-M Systems, Sequim, WA; X100 gain; high pass f_c 300 Hz; lowpass f_c 5 kHz), displayed on multi-channel oscilloscopes (Tektronix TDS 210, Beaverton, OR), and monitored on an audio amplifier/loudspeaker (Grass AM8, West Warwick, RI). Single unit spike arrival times were obtained by thresholding the extracellular recordings with a window discriminator and were logged on a computer running custom software (0.1 ms resolution). Neural recordings were assessed to be single unit by: 1) recording responses with a signal to noise ratio of >5 ; 2) monitoring the shape of the triggered action potentials on digital oscilloscopes with trace storage; and 3) calculating the spike auto-correlation functions post-hoc. All spike auto-correlation functions from what we determined to be single unit recordings showed the signature depression around 0 ms from post-spiking inhibition. Examination of the distribution of interspike interval (ISI) showed that the probability of finding ISIs < 1 ms was 3.4% and 3.8% for MLd and field L respectively (expected values for Poisson with same mean rates were 15.3% and 12.8%).

Zebra finch songs, Bengalese finch songs and noise were presented as search stimuli, with equal probability. When excitatory responses to any of these stimuli were detected (i.e. spike rate was higher than the baseline firing), we acquired 10 trials of responses to each of 20 zebra finch songs and 20 Bengalese finch songs. Presentation order of the stimuli was random within a trial. Background (spontaneous) activity was recorded for 2 s prior to the presentation of each stimulus. A random inter-stimulus interval with a uniform distribution between 4 and 6 s was used. At the end of a recording pass, 1–3 electrolytic lesions (100 μ A for 5 s) were made to verify the recording sites of that pass. Lesions were made well outside of any auditory areas unless it was the last pass. The same stereotaxic coordinates, electrode type, electrode impedances and equipment were used to make recordings in the three treatment groups.

The responses of 66 single MLd neurons to zebra finch and Bengalese finch song were recorded from 9 adult male zebra finches. Responses to the same stimuli were recorded from 47 MLd cells from 6 adult male zebra finches that had been raised and tutored by Bengalese finches (cross-tutored birds), and 57 MLd neurons in 8 normal adult male Bengalese finches. Concurrent recordings were made from single field L neurons in the same animals: 66 in 11 normal zebra finches, 35 in 6 cross-tutored zebra finches, and 42 in 8 normal Bengalese finches.

Histology

After recording, birds were overdosed with Nembutal and transcardially perfused with 0.9% saline followed by 3.7% formalin in 0.025 M phosphate buffer. The skullcap was removed and the brain was post-fixed in formalin for at least 5 days. The brain was then cryoprotected in 30% sucrose. Coronal sections (40 μ m) were cut on a freezing microtome and divided into two series. Sections were mounted on gelatin-subbed slides. One series was stained with cresyl violet and the other with silver stain. Electrolytic lesions were visually identified, and the distances between lesions within the same electrode tracks were used to calibrate depth measurements and reconstruct the locations of recording sites.

Calculating Mutual Information

Responses were analyzed and compared across stimulus types and cells from the three groups of birds by calculating the mutual information between the stimulus and the neural response to that stimulus. Mutual information (MI) measures the capacity of a neural response to encode information about the identity (or uniqueness) of a stimulus in a stimulus ensemble (stimulus type; e.g. zebra or Bengalese finch song). MI quantifies the average difference between the neural response to one particular stimulus in the ensemble and the neural responses to all other stimuli in the response. By taking the probability of all possible responses to any given stimulus into account, this information theoretic measure of “difference in neural responses” includes both neural tuning and neural noise (response variability). The MI is measured in bits/s. For example, if a hypothetical neuron yields a MI of 5 bits/s for conspecific song, then using the information embedded in a single spike trial response, one would be able to determine the identity of a one second segment of conspecific song as belonging to 1 of 32 (2^5) non-overlapping subsets of 1 s segments of song. The union of these non-overlapping subsets covers all possible segments of song, and each is equally probable. A neuron with a MI of 5 bit/s would theoretically have a coding capacity that can perfectly assign a stimulus to one of 32 distinct subsets.

To calculate information we examined the probability of all possible neural responses for segments of song. As proposed in Strong et al. (1998), we coded neural response trials as a succession of words where each word is composed of letters that are zeros and ones. One indicates a spike and zero indicates the absence of a spike. Both the length of the word and the temporal resolution of the letters are varied so that the actual value of the information rate can be obtained by extrapolating for long words and small letters. This approach has the advantage of making no assumptions about the nature of the neural code and its relevant time scale. Unfortunately, it also requires a large number of trials and spikes per stimulus to evaluate the probability of all possible words obtained in response to a given song segment. To overcome data limitation, we used the method proposed by Hsu et al (2004). We modeled the neural responses as inhomogenous gamma models and then used that parameterization to generate enough model spike trains to assess the probability of all possible words at any point in time in the stimulus presentation. This approach has been validated previously by comparing the MI obtained with the gamma model to the MI calculated directly from datasets in which a very large number of trials were recorded (Hsu et al., 2004). Here, we also validated the calculation by comparing the values obtained with

the Gamma model to information values obtained from spike rate values in varying time windows (data not shown), and to the neural discrimination performance of an ideal observer (see Results).

Response Properties Contributing to Information

To examine which aspects of neural responses affected MI, we measured four response parameters that further quantify differences in responses to different sounds and response variability. We examined response parameters that could be directly related to the MI rather than the frequency and/or temporal tuning properties described by linear spectrotemporal receptive fields, as has been done in other studies (e.g. Woolley et al, 2009). For each neuron's responses to each stimulus type, we calculated the: 1) gamma order to quantify response reliability; 2) mean spike rate to quantify the overall magnitude of the driven response; 3) response bandwidth to quantify how quickly and slowly the spike rate changed over time; and 4) spike rate distribution to quantify how frequently each spike rate occurred in responses.

The gamma order was obtained by modeling of the neural response as inhomogeneous gamma processes: an inhomogeneous gamma process is a random time series with a time-varying rate of events and with variance that is modeled by a gamma distribution. The gamma order specifies the amount of variance. A gamma order of 1 corresponds to a Poisson distribution with exponentially distributed inter-spike intervals. Higher gamma orders correspond to more regular spiking responses. At very high gamma orders, a neuron would approach a deterministic process, with a fixed interspike interval for a given rate. To calculate the gamma order, the time-varying mean firing rate was first obtained by convolving the spikes recorded from all trials with a varying-width kernel. The spike trains were then time-rescaled to obtain a constant firing rate process. The inter-spike interval distribution of the rescaled spike trains was then fit with a Gamma distribution to obtain its order.

The mean spike rate, the bandwidth and shape of the distribution of the time-varying rate are all factors that affect the information contained in responses (Hsu et al., 2004). Firing rate is the mean number of spikes per second during stimulus presentation, and was quantified by averaging the spike rate during song presentation over 10 presentations of 20 individual zebra finch or 20 Bengalese finch songs, and then averaging the mean firing rate for each song to obtain a mean firing rate for each song type, for each neuron. Mean spontaneous rate was calculated by averaging spontaneous spike rates recorded during the 2 s preceding stimulus onset. Mean spontaneous spike rates were subtracted from the mean driven spike rates. For coding time-varying stimuli, time-varying responses with higher mean spike rates have a higher potential for containing a higher number of distinct responses for different stimuli. In information theory, the mean rate is directly related to the power in the communication channel, a quantity that is called the channel capacity (Strong et al., 1998). The presence of higher firing rates (or higher channel capacity) does not necessarily have to lead to higher mutual information, however. For example, a saturated neuron may have high firing rates but low mutual information because the responses to all stimuli are similar.

Mutual information is affected by how firing rate changes over time such that different stimulus segments evoke different responses. We quantified the range and relative frequency of time-varying firing rates using two measures, the response bandwidth and the shape of the spike rate distribution. Response bandwidth is the range of frequencies in the power spectrum of the time-varying firing rate and measures how quickly spike rate changes across the duration of the response. Because bandwidth quantifies the range of slow to fast fluctuations in the time-varying rate, a large bandwidth indicates a high capacity to carry information (e.g. the ability for a neuron to quickly change between the letters 0 and 1 in a

word). The bandwidth was calculated from the power spectrum of the time-varying spike rate. The time-varying rate was estimated by convolving spike trains with varying width Gaussian kernels as in Hsu et al (2004). This width of the Gaussian kernels was chosen such that 5 SDs was the distance to the farther of two neighboring spikes. The power spectral density (psd) was estimated using multi-tapered methods with non-overlapping 128 ms windows. The bandwidth in Hz is:

$$bandwidth = \sqrt{\int f^2 psd(f)}$$

Figure 3 shows examples of power spectra from which bandwidths were measured for two midbrain neurons.

The shape of the distribution of firing rates in the responses of each neuron was measured because it affects the information about stimuli that is transmitted. For a fixed mean rate, the distribution of instantaneous firing rates with the largest entropy is the exponential distribution (Dayan, 2001). To compare the rate distributions in responses to an exponential distribution, we fit the histogram of instantaneous firing rates obtained for each neuron and each stimulus type with a gamma distribution. The gamma order was then used quantify the shape of this distribution. A gamma order of 1 corresponds to an exponential distribution and higher entropy values. A gamma order that is greater than 1 corresponds to a distribution with smaller variance, a more normal shape and lower entropy. For a fixed rate, the variance of a gamma distribution is given by the square of the rate divided by the gamma order. Note that this gamma distribution is different from the one used to model the variability in inter-spike intervals (above). Examples of the rate distributions for two field L neurons are shown in Figure 4B. Additional discussion of the effects of bandwidth and rate distribution on information is in Hsu et al. (2004).

Neural Discrimination

The *van Rossum distance metric* (van Rossum, 2001) was used to quantify neural discrimination of songs as measured by an Ideal Observer. Here, neural discrimination measures the ability of the Ideal Observer to correctly identify individual songs by comparing single stimulus-evoked spike trains to a “stored” template spike train that identifies each song. Following the method described in Wang et al. (2007), stored template spike trains were single responses, one for each song, chosen at random. Comparison spike trains were always different from the template spike trains but were otherwise randomly chosen. The similarity between a pair of spike trains was quantified as the Euclidian distance between the spike trains after smoothing the responses with a 30 ms hanning window. The distance was calculated with a 1 ms resolution and for the first 1 s of the response. This value was chosen because it was lower than the shortest song length. The 30 ms hanning window gave the best average performance (data not shown). A percent correct was then calculated by determining the distances between a test spike train evoked by one particular song and each of the template spike trains. If the test spike train and the closest template spike train were evoked by the same song, then the decision was counted as correct. By repeating this procedure 2000 times, an average percent correct was obtained. For 20 songs, chance level was 1/20 or 5%.

Statistics

For comparisons of information values and response properties across bird groups we used two-way ANOVAs with Tukey’s Honestly-Significant-Difference *post hoc* tests to determine differences between bird groups. Within-cell comparisons of response differences to zebra finch and Bengalese finch songs were made using paired t-tests. The statistical

significance of correlations between information rates in responses to zebra finch and Bengalese finch songs and between information and the four response properties was calculated by testing r against the null hypothesis of random association.

RESULTS

Midbrain and forebrain neurons recorded from birds in all three groups produced robust, stimulus-locked responses to conspecific and heterospecific songs. Figure 3 shows the responses of a normal zebra finch midbrain neuron and a Bengalese finch midbrain neuron to Bengalese finch song. Figure 4 shows the responses of a normal zebra field L neuron and a cross-tutored zebra finch field L neuron to zebra finch song. Quantitative analysis of response spike trains showed systematic and significant differences in information coding between species, and between normal and cross-tutored zebra finches.

Mutual Information - Midbrain

In MLd neurons, information rates calculated from responses to zebra and Bengalese finch song were higher in normal zebra finch neurons than in cross-tutored zebra finch and normal Bengalese finch neurons (Fig. 5A; $p = 0.0017$ for zebra finch song and $p = 0.0017$ for Bengalese finch song between normal zebra finch and cross-tutored zebra finch neurons; $p = 0.000023$ for zebra finch song and $p = 0.000002$ for Bengalese finch song between normal zebra finch and Bengalese finch neurons). Information rates did not significantly differ between cross-tutored zebra finches and Bengalese finches ($p = 0.71$ for zebra finch song and $p = 0.074$ for Bengalese finch song). Information rates for responses to zebra finch song were 5.89 ± 3.5 (mean $\pm$ SD) bits/s in zebra finches, 4.06 ± 2.04 bits/s in cross-tutored zebra finch neurons and 3.62 ± 2.39 bits/s in Bengalese finch neurons. Information rates for responses to Bengalese finch song were 6.70 ± 3.6 bits/s in normal zebra finch neurons, 4.81 ± 2.40 bits/s in cross-tutored zebra finch neurons and 3.57 ± 1.8 bits/s in Bengalese finch neurons. Therefore, the species differences in the representation of both conspecific and heterospecific songs by midbrain neurons can be eliminated by altering developmental experience.

Information rates in midbrain neurons did not differ between responses to zebra and Bengalese finch song ($p = 0.21$ for zebra finch neurons, $p = 0.11$ for cross-tutored zebra finch neurons, $p = 0.89$ for Bengalese finch neurons). Information rates within a single neuron but between responses to the two types of song were highly correlated for neurons recorded from all three groups of birds ($r = 0.86$, Fig. 5A). Therefore, in the midbrain neurons of both zebra and Bengalese finches, conspecific and heterospecific songs are coded with similar information rates.

Mutual information - Forebrain

In the primary forebrain region field L, species and experience-dependent differences in information rates were also significant (Fig. 5B; $p = 0.000021$ for zebra finch song and $p = 0.0028$ for Bengalese finch song between normal zebra finch and cross-tutored zebra finch neurons; $p = 0.000012$ for zebra finch song between normal zebra finch and Bengalese finch neurons). For the responses of single field L neurons to zebra finch and Bengalese finch song, information rates were higher in normally raised zebra finches (6.16 ± 2.82 and 6.52 ± 3.23 bits/s, respectively) than in cross-tutored zebra finches (3.97 ± 1.49 and 4.46 ± 1.94 bits/s) and in Bengalese finches (4.04 ± 1.99 and 5.24 ± 3.28 bits/s). Therefore, as in midbrain neurons, responses of primary forebrain neurons to songs were strongly affected by developmental experience. Differences in information rates between normal zebra finch and Bengalese finch neurons in responses to Bengalese finch song specifically were not statistically significant, however ($p = 0.073$). As in the midbrain, information rates

calculated from forebrain responses to zebra and Bengalese finch song did not significantly differ ($p = 0.21$ in zebra finch neurons; $p = 0.12$ in cross-tutored zebra finch neurons; $p = 0.89$ in Bengalese finch neurons). Also as in the midbrain, information rates in responses to zebra and Bengalese finch song were highly correlated within a neuron ($r = 0.90$).

Information rates in responses to zebra finch song were compared between midbrain and forebrain neurons. Rates did not significantly differ between midbrain and forebrain neurons in zebra finches (unpaired t-test; $p = 0.63$), in cross-tutored zebra finches ($p = 0.84$) or in Bengalese finches ($p = 0.36$). Therefore, although the specific song features that are encoded by single neurons differ between the midbrain and forebrain (e.g. Woolley et al., 2009), the *information bearing capacity* of the song features encoded by single neurons from both regions did not differ. Information rates for the responses of zebra finch neurons to zebra finch songs were comparable to previously reported information rates measured for responses of zebra finch auditory neurons to zebra finch song (Hsu et al., 2004).

Response Properties Contributing to Information - Midbrain

Response properties such as response reliability, mean firing rate, response bandwidth and rate distribution determine the mutual information in auditory responses (Methods; Hsu et al., 2004).

In midbrain neurons, reliability did not differ between normal zebra finch and cross-tutored zebra finch neurons (Fig. 6A; $p = 0.093$ for zebra finch song and $p = 0.121$ for Bengalese finch song) or between normal zebra and Bengalese finch neurons ($p = 0.43$ for zebra finch song and $p = 0.394$ for Bengalese finch song). Reliability was significantly higher in Bengalese finch neurons than in cross-tutored zebra finch neurons ($p = 0.0055$). Reliability did not differ between responses to the two song types ($p = 0.96$ for zebra finch neurons, $p = 0.39$ for cross-tutored zebra finch neurons, and $p = 0.84$ for Bengalese finch neurons). Therefore, the variability of the spike trains evoked by multiple presentations of the same stimulus did not differ between zebra finches raised hearing zebra finch song and zebra finches raised hearing Bengalese finch song, but did differ slightly between birds of different species that were raised hearing the same songs.

Mean firing rate was significantly higher in normal zebra finch neurons than in cross-tutored zebra finch neurons ($p = 0.0058$ for zebra finch song and $p = 0.0018$ for Bengalese finch song). Neurons in normal zebra finches responded to zebra finch and Bengalese finch song with mean spike rates of 8.4 ± 4.8 and 8.2 ± 4.0 spikes/s, while neurons in cross-tutored zebra finches responded to zebra and Bengalese finch song with mean spike rates of 5.6 ± 3.2 and 6.0 ± 2.9 spikes/s. Mean firing rate was also significantly higher in normal zebra finch neurons than in normal Bengalese finch neurons ($p = 0.0000019$ for zebra finch song and $p = 0.0000017$ for Bengalese finch song). In Bengalese finch neurons, mean spike rates were 4.6 ± 3.4 spikes/s in response to zebra finch song and 4.1 ± 2.4 spikes/s in response to Bengalese finch song. Mean firing rates of responses from cross-tutored zebra finch neurons and Bengalese finch neurons did not differ for zebra finch song ($p = 0.42$), but did differ for Bengalese finch song ($p = 0.0071$). As with mutual information, the manipulation of developmental acoustic experience significantly affected firing rates in response to both zebra and Bengalese finch song. Species differences were significant in normally raised birds but were not consistently different between birds that were raised with the same developmental experience (i.e. cross-tutored zebra finches and normally-raised Bengalese finches). Mean firing rates in responses of the same neurons to zebra and Bengalese finch song did not differ in any of the three bird groups ($p = 0.83$ for normal zebra finch neurons, $p = 0.55$ for cross-tutored zebra finch neurons, and $p = 0.38$ for Bengalese finch neurons).

Response bandwidth in midbrain neurons did not differ significantly between neurons from normal and cross-tutored zebra finches (Fig. 6C; $p = 0.65$ for zebra finch song and $p = 0.99$ for Bengalese finch song). Response bandwidth did differ between species in normal birds (Fig. 3B and Fig. 6C; $p = 0.0000031$ for zebra finch song, and $p = 0.0000045$ for Bengalese finch songs) and between cross-tutored zebra finches and Bengalese finches (Fig. 6C; $p = 0.0000021$ for zebra finch song, and $p = 0.000024$ for Bengalese finch song). Mean bandwidth for responses to zebra finch song was 63.8 ± 18.4 Hz in normal zebra finch neurons, 67.1 ± 21.9 Hz in cross-tutored zebra finch neurons and 46.2 ± 18.5 Hz in Bengalese finch neurons. Mean bandwidth in responses to Bengalese finch song was 63.1 ± 16.0 Hz in normal zebra finch neurons, 62.8 ± 16.1 Hz in cross-tutored zebra finch neurons and 47.5 ± 19.6 Hz in Bengalese finch neurons. Figure 3B shows power spectra of the time-varying spike rates for two midbrain neurons (responses are shown in panel A). Although the two neurons had similar mean firing rates, the time-varying rates of the responses recorded from the normal zebra finch exhibited faster fluctuations than did the responses of the Bengalese finch neuron. The bandwidth of the response was 78 Hz for the zebra finch neuron, but only 31 Hz for the Bengalese finch neuron. This aspect of response dynamics was not affected by the development manipulation of cross-tutoring. The large differences in response bandwidth between neurons from zebra finches (both normal and cross-tutored) and Bengalese finches indicate that this response property is species-specific and is likely independent of specific acoustic experience during development.

Response bandwidth did not differ in responses to zebra and Bengalese finch song (Fig. 6C; $p = 0.83$ for zebra finch neurons, $p = 0.29$ for cross-tutored zebra finch neurons, and $p = 0.71$ for Bengalese finch neurons).

The shape of the spike rate distribution is determined by the range and relative probabilities of instantaneous firing rates in responses that show time-varying firing rates during stimulus presentation, and quantifies how much the instantaneous spike rate can vary from one stimulus segment to another (Fig. 6D). The shape of the spike rate distribution for normal and cross-tutored zebra finch midbrain neurons did not differ ($p = 0.62$ for zebra finch song and $p = 0.16$ for Bengalese finch song). The shape of the spike rate distribution also did not differ between normal birds of both species ($p = 0.76$ for zebra finch song and $p = 0.87$ for Bengalese finch song), or between cross-tutored zebra finches and Bengalese finches ($p = 0.24$ for zebra finch song and $p = 0.39$ for Bengalese finch song). Therefore, the relative probabilities of time-varying spike rates across responses were not affected by differences in developmental experience and were consistent between species. The gamma order of the rate distribution was higher for zebra finch song than for Bengalese finch song in the responses of neurons from all three groups of birds ($p = 0.0087$ for zebra finch neurons, $p = 0.050$ for cross-tutored zebra finch neurons, and $p = 0.0081$ for Bengalese finch neurons). A correlation analysis of the response reliability, mean firing rate, response bandwidth and spike rate distribution versus information showed that the primary response property contributing to differences in mutual information among the three groups of birds was mean firing rate (see below).

Response Properties Contributing to Information - Forebrain

In field L, response reliability did not differ across bird groups or between song types (Fig. 7A). Gamma constants of the interspike interval distributions between normal and cross-tutored zebra finch neurons did not differ ($p = 0.20$ for zebra finch song and $p = 0.35$ for Bengalese finch song). Gamma constants of the interspike interval distributions between normal zebra finch and Bengalese finch neurons did not differ ($p = 0.077$ for zebra finch song and $p = 0.18$ for Bengalese finch song). And gamma constants did not differ between Bengalese finch neurons and cross-tutored zebra finch neurons ($p = 0.95$ for zebra finch song and $p = 0.96$ for Bengalese finch song). Responses to zebra and Bengalese finch songs

also did not differ in any of the three groups of birds ($p = 0.79$ for normal zebra finch neurons, $p = 0.60$ for cross-tutored zebra finch neurons, and $p = 0.69$ for Bengalese finch neurons).

Mean firing rates in field L responses to both types of song were significantly higher in neurons from normal zebra finches than in neurons from cross-tutored zebra finches (Fig. 7B; $p = 0.0012$ for zebra finch song and $p = 0.037$ for Bengalese finch song), and neurons from Bengalese finches ($p = 0.0000021$ for zebra finch song and $p = 0.022$ for Bengalese finch song). Mean firing rates in responses to zebra finch song were 9.13 ± 4.3 spikes/s for normal zebra finch neurons, 6.4 ± 3.2 spikes/s for cross-tutored zebra finch neurons, and 5.3 ± 3.0 spikes/s for Bengalese finch neurons. Mean rates in responses to Bengalese finch song were 8.9 ± 4.3 spikes/s for normal zebra finch neurons, 6.8 ± 3.0 spikes/s for cross-tutored zebra finch neurons, and 6.8 ± 4.2 spikes/s for Bengalese finch neurons. Firing rates in responses to song were therefore significantly different in neurons from birds of the same species (zebra finches) but that had different developmental acoustic experience. Firing rates in response to song did not differ between cross-tutored zebra finches and Bengalese finches, indicating that neurons from different species but with similar developmental acoustic experience responded to song with the same spike rates (Fig. 7B; $p = 0.39$ for zebra finch song and $p = 0.99$ for Bengalese finch song). Therefore, responses did not differ in mean firing rate between neurons from birds of different species that had highly similar developmental acoustic experience. There were no differences in firing rates between responses to zebra finch song and Bengalese finch song in neurons from normal ($p = 0.28$) or cross-tutored zebra finches ($p = 0.081$). Firing rates were, however, higher to Bengalese finch song than to zebra finch song in field L neurons from Bengalese finches ($p = 0.00042$). Therefore, in terms of mean firing rate, field L neurons showed no selectivity for conspecific over heterospecific song in zebra finches but did show selectivity for conspecific over heterospecific song in Bengalese finches.

In contrast to midbrain neurons, primary forebrain neurons did not show differences across bird groups in response bandwidth (Fig. 7C; $p = 0.69$ between normal and cross-tutored zebra finch neurons, $p = 0.99$ between normal zebra and Bengalese finch neurons, and $p = 0.73$ between cross-tutored zebra finch and Bengalese finch neurons). Response bandwidth did not differ between song types in any group of birds ($p = 0.63$ for normal zebra finch neurons, $p = 0.17$ for cross-tutored zebra finch neurons, and $p = 0.47$ for Bengalese finch neurons).

The shape of the rate distribution did differ significantly across bird groups, in field L (Fig. 7D). For both types of song, the gamma order describing the distribution was slightly but significantly higher in cross-tutored zebra finch neurons than in neurons from normal zebra finches ($p = 0.0018$ for zebra finch song and $p = 0.0035$ for Bengalese finch song). Figure 4B shows histograms of response rates for one zebra finch field L neuron (left) and one cross-tutored zebra finch neuron (right). Each histogram was fit with a gamma distribution. The distribution of responses for the normal zebra finch neuron is close to an exponential (gamma order 1.17), while the distribution for the cross-fostered zebra finch neuron has smaller variance and is more normal in shape (gamma order 2.31). The gamma order was also higher for cross-tutored zebra neurons than for Bengalese finch neurons ($p = 0.031$ for zebra finch song and $p = 0.0099$ for Bengalese finch song). In responses to zebra finch song, the gamma order was 1.42 ± 0.39 for zebra finch neurons, 1.69 ± 0.34 for cross-tutored zebra finches and 1.47 ± 0.43 for Bengalese finch neurons. In response to Bengalese finch song, the gamma order was 1.32 ± 0.40 for zebra finch neurons, 1.59 ± 0.40 for cross-tutored zebra finches and 1.32 ± 0.41 for Bengalese finch neurons. A higher gamma order results in smaller variance and entropy. These differences indicate that the range of responses of neurons from cross-tutored zebra finches is smaller than for the other groups of

birds, resulting in lower response entropy and therefore lower mutual information rates. For all three bird groups, gamma order was slightly higher for responses to zebra finch song than for responses to Bengalese finch song (Fig. 7D; $p = 0.000072$ for zebra finch neurons, $p = 0.0088$ for cross-tutored zebra finch neurons and $p = 0.000017$ for Bengalese finch neurons).

Of the four information-related response properties that we measured, mean firing rates differed the most in neurons from the three bird groups and showed the same general patterns as mutual information rates. Therefore, differences in information rates appeared to be driven largely by differences in firing rates. To test this further, we calculated the correlations between information rates and each of the four response parameters - reliability, mean firing rate, response bandwidth and spike rate distribution. In midbrain neurons, information and firing rate were highly correlated ($r = 0.92$). In primary forebrain neurons, information and firing rate were also highly correlated ($r = 0.88$). As expected, information rates and spike rate distribution gamma order were inversely correlated in midbrain neurons ($r = -0.64$) and forebrain neurons ($r = -0.56$). Information rates were not well correlated with reliability or bandwidth measures.

Neural Discrimination of Songs

The van Rossum distance metric was used to calculate percent correct scores for the neural discrimination of each neuron's responses to 20 zebra finch songs and 20 Bengalese finch songs. In the midbrain, percent correct values were significantly higher in normal zebra finch neurons than in cross-tutored zebra finch neurons (Fig. 8A upper; $p = 0.0045$ for zebra finch song and $p = 0.0094$ for Bengalese finch song) and between normal zebra and Bengalese finch neurons ($p = 0.011$ for zebra finch song and $p = 0.00030$ for Bengalese finch song). These results indicate that midbrain neurons from normally-raised zebra finches produced spike trains that more accurately discriminated among songs than did neurons from the other two groups of birds. The percent correct values for neurons from cross-tutored zebra finches and Bengalese finches did not differ ($p = 0.91$ for zebra finch song and $p = 0.75$ for Bengalese finch song). Differences across neurons from the three groups of birds were significant for responses to both types of song but were larger for responses to Bengalese finch song than for zebra finch song. Within a neuron, percent correct scores were highly correlated with information (Fig. 8A lower; $r = 0.84$; for responses to zebra finch song; $r = 0.83$; for responses to Bengalese finch song), validating these two independent measures of neural discrimination.

In the primary forebrain area field L, the variability in the percent correct discrimination of songs was higher among cells than in the midbrain (Fig. 8B upper). Differences for the discrimination of zebra finch songs but not Bengalese finch songs were significantly higher in normal zebra finch neurons than in cross-tutored zebra finches ($p = 0.0055$ for zebra finch song and $p = 0.12$ for Bengalese finch song). There were no other differences in % correct measures of neural discrimination across bird groups. As was the case for midbrain neurons, neural discrimination and information were highly correlated (Fig. 8B lower; $r = 0.83$; for responses to zebra finch song and $r = 0.88$; for responses to Bengalese finch song).

DISCUSSION

This study demonstrates that the manipulation of experience with vocalizations during ontogeny significantly alters sensory coding in adult midbrain and forebrain auditory neurons. Here, we examined the neural coding of vocalizations by measuring the mutual information in the responses of single neurons, response reliability, mean spike rate, the dynamics of time-varying responses and neural discrimination. The spike train measures described here take all spikes and all spike patterns into account, thereby avoiding the potential limitations associated with using models of neural responses that may not fully

capture a neuron's response properties. Mutual information, firing rate and neural discrimination at the single neuron level were significantly higher in normal zebra finches than in cross-tutored zebra finches, indicating that developmental experience with different types of natural sounds significantly shapes the information coding capacity of auditory neurons. In zebra finches, the experience of heterospecific vocalizations only and/or the lack of experience with conspecific vocalizations resulted in diminished information coding in adulthood. Information values for midbrain and forebrain neurons from Bengalese finches were lower than those for neurons from zebra finches but the same as from those for cross-tutored zebra finches. Considering that cross-tutored zebra finches and normal Bengalese finches had comparable developmental acoustic and social experience, the experience of Bengalese finch songs rather than zebra finch songs may have limited the information coding capacity of the auditory neurons studied here. Because we were not able to cross-foster juvenile Bengalese finches into zebra finch families, we did not determine whether experience with zebra finch songs could result in higher information coding capacity in Bengalese finch neurons.

Effects of Experience on Neural Responses

Our findings indicate that manipulations in the vocalizations that are heard and learned during ontogeny shape the information coding capacity of adult midbrain and forebrain neurons by affecting firing rates and some response dynamics. Information coding was not higher for the type of song that birds had experienced. Zebra finch song did not evoke responses with higher information rates or firing rates from normal zebra finch neurons than from the other birds. Bengalese finch song did not evoke responses with higher information or spike rates from midbrain neurons in cross-tutored zebra finches or Bengalese finches, or from forebrain neurons in cross-tutored zebra finches. These results indicate that experience with a specific type of song (e.g. zebra or Bengalese finch) does not result in greater responsivity to songs of that type. Further, with the exception of Bengalese finch primary forebrain neurons, no response biases for conspecific song were observed.

Because responses to more than two types of sounds were not tested, the generality of the response differences between normal and cross-tutored birds across many types of sounds remains unknown. However, the higher firing rates associated with developmental experience of zebra finch song were observed in responses evoked by sounds that birds heard during ontogeny and sounds that birds had never heard; responses of neurons from zebra finches that were raised with one or the other song type did not differ between zebra finch song and Bengalese finch song. Therefore, the higher information coding associated with zebra finch song exposure and the decreased information coding associated with Bengalese finch song exposure in both zebra and Bengalese finches were not due to familiarity with the sound stimuli. This suggests that the higher information coding capacity of neurons in normally-raised zebra finches may be applicable to a wider range of sounds than those that we tested. None of the individual songs that we used to collect auditory responses were familiar to birds. Thus, we did not test the effects of stimulus-specific experience on information coding.

The developmental experience of normally-raised and cross-tutored zebra finches differed in both auditory and social exposure. What acoustic differences between zebra finch and Bengalese finch song may promote differences in auditory responsivity? Even though the test stimuli used during electrophysiological recording were balanced in intensity and amount of silence, developmental experience was under the control of the vocalizing birds in the single-species colony-rearing environment. Therefore, both the acoustic differences in the two song types and other potential differences in the acoustic environment such as "amount" of sound heard should be considered. The range of frequencies in zebra and Bengalese finch songs is the same (Immelmann, 1969; Woolley and Rubel, 1997, 2002;

Wang and Woolley, 2007), but the frequency power spectra and spectrotemporal modulations of zebra and Bengalese finch songs differ (Fig. 2). The two song types also differ in acoustic features such as syllable repetition rate and the occurrence of frequency-modulated sweeps. These acoustic differences in song may contribute to the experience differences in normally-raised and cross-tutored zebra finches that resulted in differences in auditory responses (see Campbell and Hauber, 2009c).

Another consideration is differences in the amount of total sound birds experienced during development. The single-species colony design of this study did not permit control over the amount or intensity of sound exposure between birds raised by zebra finches and Bengalese finches. It is therefore possible that the amount or intensity of sound exposure during development influenced auditory responsivity. Even though the specific effects of each aspect of the developmental manipulation used here could not be identified, the finding that firing rates did not differ between cross-tutored zebra finches and Bengalese finches in three of the four comparisons (zebra finch songs, Bengalese finch songs, midbrain neurons, forebrain neurons) supports the idea that the rearing differences between zebra finch and Bengalese finch families altered auditory responsivity.

Birds raised in conspecific or heterospecific families also experienced social differences in rearing. Behavioral effects of cross-fostering beyond the obvious exposure and imitation of tutor song do occur (Immelmann, 1969; Clayton, 1989). For example, both male and female zebra finches are more likely to select Bengalese finches as mates if they have been raised by Bengalese finches (Clayton, 1987, 1988; Bischof and Clayton, 1991). Thus, social and acoustic exposure to heterospecific birds during development results in decreased behavioral selectivity. One study has examined how developmental social experience in particular affects auditory responsivity to songs in field L. Cousillas et al. (2006) raised male European starlings (*Sturnus vulgaris*) in groups with adult tutors, pairs without adult tutors and alone. All birds had auditory but not social exposure to the vocalizations of the group-raised birds. When birds reached adulthood, the auditory responses of multi-unit neuronal sites to starling song were recorded. Responses of sites from socially-reared birds were less selective, suggesting that social conditions during development can influence auditory responsivity. It is possible that social differences in rearing between normally-raised and cross-tutored zebra finches contributed to the differences in auditory responsivity that we observed. Nevertheless, because cross-fostering/tutoring does not occur between our study species in nature, the value of our experimental manipulation lies in the demonstration that developmental experience can play a role in adult neural coding of song and, in nature, may shape how the brain processes species-specific vocalizations. A recent study showing that behavioral preferences for conspecific over heterospecific songs in zebra finches are decreased by cross-fostering supports this conclusion (Campbell and Hauber, 2009b).

Species Differences

The mutual information in responses to the same twenty zebra and twenty Bengalese finch songs was significantly higher in neurons from normally-raised zebra finches than in neurons from Bengalese finches. In midbrain neurons, the mean and range of firing rates in individual responses were higher in zebra finch neurons than in Bengalese finch neurons. Bandwidth differences were maintained between midbrain neurons in Bengalese finches and cross-tutored zebra finches even though both groups of birds received comparable experience during development. Therefore, the differences in auditory experience and, eventually, in song production between birds raised by zebra finches and birds raised by Bengalese finches did not affect this response property. Because response bandwidths were similar between zebra finches that developed with very different experience and were different between birds of two different species that were raised with highly similar

experience, the differences in that aspect of auditory coding may reflect intrinsic species differences in cellular or circuit properties.

With the exception of response bandwidth, species differences in information coding were driven largely by differences in experience; the responses of neurons from birds of different species but that were raised with similar experience were highly similar. Forebrain neurons did differ between species in terms of response biases for conspecific over heterospecific songs, however. In zebra finch midbrain and forebrain neurons and Bengalese finch midbrain neurons, firing rates in response to zebra and Bengalese finch song did not differ. In Bengalese finch forebrain neurons, however, firing rates in response to Bengalese finch songs were higher than firing rates in response to zebra finch songs. Surprisingly, this was the only evidence of response biases for conspecific songs. Despite clear species differences in response bandwidth and conspecific song bias in Bengalese finch forebrain neurons, the major response differences among groups of birds were mean firing rates between neurons from birds that developed hearing zebra finch songs and birds that developed hearing Bengalese finch song. These findings demonstrate a strong effect of developmental acoustic experience on adult auditory coding.

Differences between Midbrain and Forebrain

The overall similarity in mutual information, mean firing rates and response dynamics between midbrain and forebrain neurons suggests that the signal transformations that occur between the midbrain and forebrain minimally affect information coding capacity. Woolley et al. (2009) directly compared the spectrotemporal tuning properties of auditory midbrain and forebrain neurons to examine transformations in tuning along the ascending pathway. Analysis of spectrotemporal receptive fields (STRFs) of zebra finch MLd and field L neurons showed that the three major STRFs types defined based on spectrotemporal tuning properties were the same between the midbrain and the forebrain. This corresponds well with the information coding similarities between the two brain regions described here. But, Woolley et al. also found that forebrain neurons showed a wider distribution of STRF shapes within a STRF type, and two STRF types that were not found in MLd. This larger diversity in STRF shapes across individual forebrain neurons compared to individual midbrain neurons corresponds well with the larger range of mutual information and neural discrimination values among forebrain neurons compared to midbrain neurons found in this study.

The information about a complex stimulus that neurons are capable of coding is greatly affected by the number of neurons that respond to the sound and the diversity of the single neuron responses that contribute to an ensemble response (Woolley et al., 2006). Here, we analyzed mutual information and response dynamics for single neurons independently. Information coding capacity for an ensemble of neurons is not simply given by the sum of the single neuron information. For example, redundancy in responses could lead to smaller ensemble information, and synergistic responses could lead to higher ensemble information. Therefore, the effects of redundant and/or synergistic information coding in different brain regions could affect discrimination of song because a decrease in information at the single neuron level could be compensated for by an increase in synergy at the ensemble level. Initial assessments of ensemble information in songbird auditory neurons, however, show strong correlations between single neuron and ensemble information (Gill et al, 2009). Future studies will directly compare mutual information in single neurons and neuronal ensembles to determine the effects of redundant and synergistic coding on neural information coding capacity.

Although no studies have directly compared responses to conspecific vocalizations between the inferior colliculus (IC) and primary auditory cortex (A1) in mammals, some comparisons

of vocalization coding in IC and A1 are available for guinea pigs, cats and bats. Bats and guinea pigs have spectrotemporally rich repertoires of more than 10 types of communication vocalizations (Bohn et al., 2008; Šuta et al., 2008). Bat IC neurons appear to exhibit much more selective responses to vocalizations than guinea pigs or finches, with inhibition-based complex tuning properties such as combination-sensitivity and delay tuning (Mittman and Wenstrup, 1995; Klug et al., 2002; Nataraj and Wenstrup, 2006; Sanchez et al., 2008). In the guinea pig IC, responses to calls are based largely on the spectrotemporal features of the calls, and the temporal patterns of calls are coded by time-varying firing rates (Šuta et al., 2003). Many guinea pig IC neurons fail to respond to slow temporal modulation, showing bandpass temporal modulation tuning (Rees and Palmer, 1989). In these respects, guinea pig and zebra finch auditory midbrain neurons show similar coding of spectrotemporally complex sounds, including vocalizations (Woolley and Casseday, 2005; Woolley et al., 2006). Chechik et al., (2006) compared the mutual information of responses to bird calls in cat IC and A1 neurons and found that information was higher in IC neurons than in A1 neurons. This difference between IC and A1 is inconsistent with our finding that mutual information was similar between the midbrain and forebrain in songbirds. Such differences in the comparison of information coding between midbrain and forebrain may reflect either species differences between songbirds and cats, or differences that are due to stimulus complexity; the acoustics of bird songs are much more complex than those of bird calls. Consistent with our analysis of differences in information and mean spike rates between groups of birds, Chechik et al. found that higher average information values were due to higher mean spike rates in IC neurons. The results of that study and the results described here indicate that, in both avian and mammalian auditory neurons, spike rate and information coding capacity are correlated. Chechik et al. also found that when neuronal ensemble information is estimated, cortical neurons show lower response redundancy than do IC neurons. It will be interesting to examine whether the same principle is present in the avian auditory system.

Single neurons in auditory cortex have been shown to represent the specific acoustic features of vocalizations less faithfully than subcortical neurons (Wang, 2007; Suta et al., 2008). Some cortical neurons appear to be tuned for perceptual features, such as pitch (Bendor and Wang, 2005), or highly selective for longer complex sounds including specific vocalizations (e.g. Rauschecker, 1995). Thus, the selectivity of neurons for vocalizations may remain constant, with single neurons responding better to specific acoustic features in lower brain regions and classifying vocalizations based on higher order features at higher brain regions. Our results are consistent with this idea because we found only small differences between midbrain and primary forebrain neurons in measures of mutual information. Future studies will examine how spectrotemporal tuning properties that determine what acoustic features drive responses contribute to species and experience-based differences in the midbrain and forebrain coding of songs.

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Figure 1.

Spectrograms show the acoustic features of zebra finch, Bengalese finch and cross-tutored zebra finch songs. Cross-tutored zebra finches copied the song syllables of their tutors' songs but produced songs with some zebra finch-like temporal patterns. Color indicates sound intensity. Red is high and blue is low. Tutor songs are on the left and the learned songs of the tutees are on the right.

Figure 2.

Zebra and Bengalese finch songs differ in spectral and temporal acoustics. A. Spectrograms of zebra (left) and Bengalese (right) finch songs. B. Frequency power spectra of zebra and Bengalese finch songs. C. Modulation spectra of zebra and Bengalese finch songs show the spectrotemporal modulations that characterize the songs of each species. Color indicates power. Red is high and blue is low. Contour lines on the color plots outline 80% of the total power for each spectrum.

Figure 3.

Responses of midbrain (MLd) neurons to song. **A.** Responses of one normal zebra finch neuron (left) and one Bengalese finch neuron (right) to a Bengalese finch song. The top row shows the song spectrogram. The middle row shows the spike rasters obtained for 10 trials. The bottom row shows the poststimulus time histograms of the responses. **B.** Power spectra of the time-varying spike rates for each neuron show that, despite similar mean spike rates, the responses of the zebra finch neuron (left) cover a wider bandwidth than do the responses of the Bengalese finch neuron (right). The power spectra include responses to all of the 20 Bengalese finch songs.

Figure 4.

Responses of forebrain (field L) neurons to song. A. Responses of one normal zebra finch neuron (left) and one cross-tutored zebra finch neuron (right) to a zebra finch song. The top row shows the song spectrogram. The middle row shows the spike rasters obtained for 10 trials. The bottom row shows the poststimulus time histograms of the responses. B. The distributions of time-varying spike rates for each neuron show that the responses of the normal zebra finch neuron (left) were distributed across rates with a shape that was closer to that of an exponential distribution than were the responses of the cross-tutored zebra finch neuron (right). The distributions were obtained from the responses to all 20 zebra finch songs. Because the mean spike rate was different between the two neurons, the distributions were rescaled to a mean of 10 spikes/s. The distribution for the normal zebra finch neuron was fit with a gamma order of 1.17. The distribution for the cross-tutored zebra finch neuron was better fit with a gamma order of 2.31. The cross-tutored distribution has smaller variance and smaller entropy.

Figure 5.

Mutual information values differ for neurons from normal and cross-tutored birds within species and between species. Average mutual information values for responses to songs are shown for neurons from normal zebra finches (ZF), cross-tutored zebra finches (xZF) and normal Bengalese finches (BF). Values for responses to zebra finch song are on the left. Values for responses to Bengalese finch song are on the right. Error bars are one SD, ** is $p < 0.01$, and *** is $p < 0.001$. Correlation plots (right) show that information values for responses to zebra finch song and Bengalese finch song are highly correlated, within a neuron. Each point represents the average information rate in responses to 20 zebra finch songs on the x axis and the average information rate in responses to 20 Bengalese finch songs on the y axis, for a single neuron. A. Midbrain (MLd). B. Primary forebrain (field L).

Figure 6.

Mean firing rate and response bandwidth differ significantly between zebra finch groups with different developmental experience and between species, in midbrain neurons. Reliability and rate distribution do not differ across groups. Average values for the four response properties are shown for neurons from normal zebra finches (ZF), cross-tutored zebra finches (xZF) and normal Bengalese finches (BF). Values for responses to zebra finch song are on the left. Values for responses to Bengalese finch song are on the right. Error bars are one SD, ** is $p < 0.01$ and *** is $p < 0.001$.

Figure 7.

Mean firing rate and spike rate distribution differ significantly between zebra finch groups with different developmental experience and between species, in primary forebrain neurons. Reliability and bandwidth do not differ across groups. Average values for the four response properties are shown for neurons from normal zebra finches (ZF), cross-tutored/tutored zebra finches (xZF) and normal Bengalese finches. Values for responses to zebra finch song are on the left. Values for responses to Bengalese finch song are on the right. Error bars are one SD, ** is $p < 0.01$ and *** is $p < 0.001$.

Figure 8.

Percent correct neural discrimination among songs differs between zebra finch groups with different developmental experience and between species in midbrain neurons, and with experience but not between species in forebrain neurons (upper). Percent correct values are more variable among forebrain neurons than among midbrain neurons. Information values and % correct are highly correlated within a neuron (lower). A. Midbrain (MLd). B. Forebrain (field L). Error bars are one SD. * is $p < 0.05$, ** is $p < 0.01$ and *** is $p < 0.001$.