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Influences of song experience on the development of selectivity in the Zebra finch forebrain

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Influences of Song Experience on the Development of Selectivity in the Zebra Finch Forebrain

by

Michele M. Solis

ABSTRACT

Auditory neurons of the anterior forebrain (AF) of zebra finches become selective for song during song learning. In adults, these neurons respond more to the bird's own song (BOS) than to the songs of other zebra finches (conspecifics) or BOS played in reverse. In contrast, AF neurons from young birds (30 d) respond equally well to all song stimuli. If present in birds learning to sing, song selective neurons could process the auditory experiences of BOS and tutor song that are required for song learning. For example, neurons tuned by BOS experience could provide feedback about the current state of BOS, whereas neurons tuned by tutor song experience could store tutor song information. Thus, both determining when selectivity emerges and investigating the song experiences that shape selectivity could elucidate AF function during song learning.

AF selectivity was characterized at 60 d, an intermediate stage of song learning. Neural responses to song stimuli, including BOS and tutor song, were obtained from extracellular recordings of single neurons in anesthetized birds. By 60 d, selectivity was evident: on average, neurons responded more to BOS and tutor song than to conspecific or reversed songs. Thus, selectivity is found in juveniles learning to sing, and might function in vocal learning.

Because similarities normally arise between BOS and tutor song during learning, it is not straightforward to assess their respective contributions to selectivity development. To minimize these acoustic similarities, birds were induced to produce abnormal song by denervating the syrinx, the avian vocal organ. At 60 d, many AF neurons responded more to the abnormal BOS than to tutor song, indicating that BOS experience can influence

selectivity. Unexpectedly, many neurons also responded equally well to both the abnormal BOS and tutor song, despite their acoustic differences. These neurons were also selective, indicating that they were not simply immature. This dual selectivity suggests that both BOS and tutor song experiences can contribute to the selectivity of AF neurons; such neurons might mediate the comparison between BOS and tutor song that occurs behaviorally.

Michael P Stryker

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Chapter 1:

Background and Experimental Rationale

Songbirds are a promising model system for the study of learning and memory. Unlike many animals which serve as models for learning and memory, songbirds do not need to be trained to perform a certain behavior. Songbirds readily learn to sing, and their song is a complex behavior, involving the precise coordination of the vocal apparatus and respiration. Furthermore, song learning is readily manipulated experimentally. For example, studies that alter auditory experience during song development have revealed important behavioral requirements for learning. Animals specialized for a particular behavior often have highly differentiated nervous structures that are adapted for these behaviors. Studies of these specialized structures can reveal general principles of nervous system organization and function. Birds that learn to sing have a unique set of anatomically distinct brain nuclei, called the song system (Nottebohm et al, 1976). Such specialized nuclei are found in multiple orders of birds which learn to sing, including songbirds (Passeriformes), parrots (Psittaciformes), and hummingbirds (Apodiformes). In contrast, birds belonging to the same order as songbirds, but which do not learn their songs (suborder suboscine), lack these specialized nuclei (Kroodsma and Konishi, 1991; Nottebohm, 1980). Thus, the song system nuclei are likely locations for neural changes associated with song learning. Behavioral studies of song learning have revealed several requirements which constrain the properties of neurons that would mediate song learning. The basis of these requirements and the extent to which neurons of the song system meet them will be discussed in the following sections.

The anterior forebrain is crucial during song learning

The song system is involved in song learning and production. It consists of a motor pathway and the anterior forebrain (AF) pathway (Figure 1-1A). The motor pathway, which is necessary for normal song production throughout life, includes HVc, the robust nucleus of the archistriatum (RA), and the tracheosyringeal portion of the hypoglossal nucleus (nXIIts). The nXIIts contains the motor neurons controlling the muscles of the syrinx, the avian vocal organ. RA also projects to brainstem respiratory nuclei, which control the airflow through the syrinx during song production (reviewed in Wild, 1997). In contrast to the motor pathway, the AF is required for normal song development. This pathway consists of Area X (X), the medial nucleus of the dorsolateral thalamus (DLM), and the lateral magnocellular nucleus of the anterior neostriatum (LMAN). LMAN or X lesions in juvenile birds severely disrupt song learning, whereas the same lesions in adult birds do not affect their songs (Bottjer et al, 1984; Sohrabji et al, 1990; Scharff and Nottebohm, 1991). Thus, AF neurons may mediate song learning, perhaps by guiding motor output via the projection from LMAN to RA.

Song learning requires auditory experience

Like human speech, song learning requires auditory experience. Song learning occurs in two phases, known as the sensory phase and the sensorimotor phase. During the sensory phase, a young bird listens to and memorizes its tutor song; this memory is often called the tutor song "template". The sensorimotor phase begins when the young bird starts to sing, producing "plastic song." Plastic song is soft and rambling, has poor syllable morphology, and lacks a stereotyped syllable order. The juvenile bird listens to its own immature vocalizations and compares them to the memorized tutor song. In this way, the bird gradually modifies its plastic song, until it achieves a stereotyped "crystallized" song, which usually resembles the tutor song. Thus, the bird requires auditory experience of tutor song and the bird's own song. The importance of auditory feedback in both phases of learning was demonstrated with deafening experiments (Konishi, 1965; Price, 1979). Specifically, birds deafened during the sensory phase cannot memorize the tutor song and hence develop severely abnormal song. Birds deafened during the sensorimotor phase cannot compare their own vocalizations to the tutor song template, which also results in abnormal song. Zebra finches (*Taeniopygia guttata*) learn their songs very quickly, making them useful for both behavioral and neurophysiological studies. They complete song learning in only three months, and the two phases of learning overlap in time (Figure 1-1B). Their sensory phase lasts until 60 d of age, and the sensorimotor phase begins at ~30 d with the onset of singing, and ends at ~90 d, when a bird has attained a crystallized song. Most of the data discussed here were obtained from zebra finches.

Given the auditory requirements for song learning, neural mechanisms for auditory learning, and for auditory feedback-guided modification of song must exist in the songbird brain. Auditory input from Field L, the primary auditory area in birds, enters the song system through HVc (Fortune and Margoliash). AF neurons, somehow involved during learning, could process the important auditory experiences because they respond to auditory stimuli. In addition, LMAN and X neurons in adult zebra finches are extremely selective for the bird's own song (BOS) (Doupe and Konishi, 1991; Doupe, 1997). These neurons respond more to BOS than to songs of other zebra finches (conspecific song) and reverse songs; this indicates that AF neurons are extremely sensitive to both the spectral and temporal features of BOS. Song selective neurons seem well-suited to process the song experiences required for normal song development. Consistent with an auditory role for the AF, LMAN and X neurons from very young juveniles (30 d) in the process of learning song respond to song stimuli. They are not selective at this age, however, and respond equally well to tutor song, conspecific song and reverse tutor song (Doupe 1997). Thus, selectivity emerges in the AF during song learning.

Whether selective neurons develop at a later stage of song learning, or are found only once song learning is complete, was tested by characterizing the selectivity of neurons from birds at an intermediate stage of learning. Chapter 2 describes AF selectivity in 60 d old juveniles; at this age birds have had substantial experience of both tutor song and their own plastic song. AF neurons in these birds were clearly selective for both plastic BOS and tutor song, which is consistent with a role for AF selectivity sometime during song learning.

The AF could participate in both the sensory and sensorimotor phases of learning

Several studies have suggested a role for LMAN during sensory learning. LMAN specifically has been implicated in tutor song template acquisition. When NMDA receptor activation was blocked in LMAN during tutor song exposure, birds copied less from the tutor song than is normal (Basham et al, 1996). When birds are isolated from tutor song experience, their sensory phase is lengthened such that these isolates can acquire normal song when exposed to a tutor after 60 d (Eales, 1987; Morrison and Nottebohm, 1993; Aamodt et al, 1995; Jones et al, 1996). This late learning is also disrupted by LMAN lesions (Morrison and Nottebohm, 1993), suggesting that LMAN is important for sensory learning, regardless of when it happens during development. Anatomical data also suggest a role for LMAN during the sensory phase. AF circuitry (unlike motor) is in place by 15 d of age (Mooney and Rao, 1994), early enough to process tutor song experience. Another study has shown that although dendritic spine density in LMAN decreases during song learning, this decrease does not occur in isolate birds (Wallhäusser-Franke et al, 1995). Thus, LMAN seems sensitive to tutor song experience, and might be involved in template acquistion. Similar studies have not been conducted in X.

The AF could also mediate sensorimotor learning, although there is less work investigating this. Preliminary data show singing-related activity in LMAN of a juvenile bird singing plastic song (Hessler, unpublished data). Furthermore, immediate early gene expression is induced in X by singing in juvenile birds (Jarvis and Nottebohm, 1997; Jin and Clayton, 1997), suggesting that X neurons increase their activity during singing. Thus, AF involvement in both sensory and sensorimotor learning remains possible, but the exact role of these nuclei remains unclear.

AF function may be clarified with studies of selectivity

AF function during song learning could also be elucidated by knowing which song experience shapes AF properties. This could be revealed by determining the specific song experience responsible for AF selectivity. For example, neurons tuned by tutor song experience could store tutor song information acquired during the sensory phase. Neurons tuned by BOS experience could inform the brain about the current state of plastic song, and perhaps participate in the evaluation of BOS during the sensorimotor phase. The lack of selectivity for tutor song in 30 d juveniles might be used as evidence against a role for the AF in tutor song template acquisition; however, at this early age it is unclear what these birds had memorized of tutor song. Although one report finds that 35 d birds have sufficient tutor song information (Böhner, 1990), it is generally agreed that complete tutor song memorization requires at least 60 d of exposure to the tutor song (Eales, 1985; Immelman, 1969). Thus, studying the contributions of both tutor song and BOS experiences to AF selectivity in 60 d old birds is important, since at this age birds have had substantial experience of both songs. As described in Chapter 2, selectivity for both plastic BOS and tutor song was apparent in AF neurons from 60 d birds. Whether this indicates the contributions of both song experiences to AF selectivity is unclear, however. Because of the acoustic similarities that arise between BOS and tutor song, it is difficult to disentangle neural properties resulting from BOS experience from those resulting from tutor song experience. For example, neurons tuned by BOS experience only could also show selectivity for tutor song if the two songs were acoustically similar enough.

To clarify the respective contributions of BOS and tutor song to AF selectivity, the similarity between BOS and tutor song was minimized by transecting the NXIIts (ts) nerves of juvenile birds. Chapter 3 describes AF selectivity in 60 d birds with abnormal, ts cut song (tsBOS). Despite the acoustic differences between tsBOS and tutor song, neural selectivity for both tsBOS and tutor song was maintained. Moreover, many single neurons responded equally well to these two songs, and yet they were selective. This result suggests that both BOS and tutor song experience can influence the selectivity of single AF neurons.

Studies of birds with ts cut songs can also clarify the interpretation of neurons preferring BOS over tutor song found in the AF of normal 60 d zebra finches (Solis and Doupe, 1997) and in HVc of normal white-crowned sparrows (Margoliash, 1986; Volman, 1993). In these studies, strong neural preferences for BOS over tutor song were found in birds with the most abnormal songs. Because these abnormal songs did not resemble the tutor song, they may well indicate poor copying of the tutor song model during the sensory phase. If true, then BOS may resemble the template more than the actual tutor song model. In this case, a neural preference for BOS over tutor song could reflect sensory experience rather than BOS experience. NXIIts transections can help clarify the interpretation of BOS selectivity, because they induce a unique, abnormal song without interfering with tutoring. A population of neurons preferring tsBOS over tutor song is also described in Chapter 3, which clearly indicates a role for BOS experience in shaping AF neurons.

The evidence for both tutor song and BOS contributions to AF selectivity does not restrict AF participation to one particular phase of learning. Tutor song selectivity could store tutor song information acquired during the sensory phase. Neurons exhibiting tutor song selectivity exclusively were rare, however. This raises the possibility that tutor song information resides in neurons that also are selective for BOS. This may be especially useful if the AF is involved in comparing BOS to the template during sensorimotor learning, perhaps computing an error signal reflecting the mismatch between plastic song and tutor song. The final output of the AF could guide motor pathway activity, given the projection from LMAN to RA. While both LMAN and X may process song experience, they may differ in their respective functions. For example, while X lesions result in highly variable songs, LMAN lesions induce prematurely stereotyped songs comprising very few syllables (Scharff and Nottebohm, 1991). This suggests that X could reinforce specific song patterns produced during plastic song; in contrast, LMAN may promote plasticity within the song system.

AF function in adulthood

Studies of adult birds confirm and extend these ideas of AF function. The AF may mediate song perception in adults: both LMAN and X lesions impair their ability to discriminate between the bird's own song and conspecific songs (Scharff et al, 1998). A premotor role for the AF is suggested by chronic recordings of neural activity in LMAN and X in awake, singing adult birds. Neural activity in these areas is increased during singing, even in deafened birds (Hessler and Doupe, 1999; Dave et al, 1997), demonstrating that a portion of this activity is associated with song production. Because lesions of the AF do not affect song production in adults, this singing-related activity may represent an efference copy signal of premotor commands. That LMAN contributes to plasticity in the song system is also evident in adult birds. Upon deafening, adults experience song degradation; LMAN lesions can block this plasticity (Brainard and Doupe, 1997).

Whether selectivity for tutor song is maintained into adulthood along with BOS selectivity can be tested in birds with abnormal song. This is difficult to test in normal adults, where the high degree of similarity between BOS and tutor song interferes with a clear assessment of the experience responsible for selectivity. Chapter 4 describes AF selectivity in adult birds that experienced NXIIts transections as juveniles. Equivalent responses to tsBOS and tutor song were apparent, and adult neurons were more selective than those from 60 d juveniles. These results confirm the ability of both BOS and tutor song to shape the selectivity of a single AF neuron, and suggest that tutor song information is retained into adulthood. However, neurons from ts cut adults were less selective than those from normal adults. Because of this, it is unclear whether the dual contributions of tsBOS and tutor song are particular for ts cut adults, or can be generalized to normal adult birds.

Encoding complex song stimuli

How the detailed spectral and temporal features of song are encoded by a neural circuit such as the AF is unclear. Ultimately, this is constrained by the capabilities of neurons downstream of LMAN and X. For this reason, the sensitivity to AF response variability and the integration times of downstream neurons are of interest. Chapter 5 describes measurements of LMAN and X response variability to behaviorally relevant song stimuli. Also, the dependence of AF selectivity on integration time is explored. We find that in addition to being some of the most selective sensory neurons, AF neurons are also some of the most variable. In addition, AF selectivity is highest when measured over long time windows (>250 ms). Not only do these analyses constrain our ideas about how the song system processes complex song stimuli, but they inform our decisions about how best to measure a neural response in the AF.

In summary, AF neurons could process auditory experiences important to song learning. This thesis finds evidence for contributions from both tutor song and BOS experience to AF selectivity. Furthermore, selectivity is found at an intermediate stage of song learning, and so itself may be a useful mechanism for song learning. Chapter 6 outlines future experiments suggested by these studies; as in this thesis, these future studies will be well served by song behavior, which guides our thoughts of what a neural correlate for learning would be.

Figure 1-1 A) The anatomy of the song system is illustrated. Motor pathway nuclei are gray; RAm refers nucleus retroambigualis, a respiratory premotor area. AF nuclei are black. B) Zebra finches learn to sing in two overlapping phases. The sensory phase ends at ~60d; the sensorimotor phase begins at ~30d and continues until 90 d+.

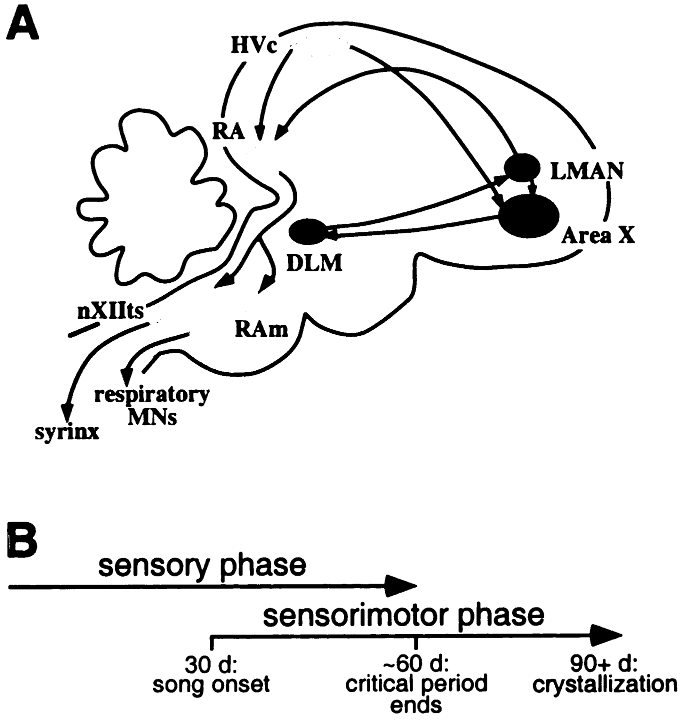

A
HVc
RA
LMAN
Area X
DLM
nXIIts
RAm
respiratory MNs
syrinx
B
sensory phase
sensorimotor phase
30 d: song onset
~60 d: critical period ends
90+ d: crystallization

Figure 1-1

Chapter 2:

Anterior Forebrain Neurons Develop Selectivity by an Intermediate Stage of Birdsong Learning

ABSTRACT

Auditory neurons of the anterior forebrain (AF) in adult zebra finches are highly selective for the bird's own song (BOS): they respond more to BOS than to songs of other zebra finches (conspecifics) and to BOS played in reverse. In contrast, juvenile AF neurons are not selective at 30 days of age, responding equally well to all song stimuli. Both BOS and tutor song experience are required by juveniles for normal song learning, and may produce the selective properties of adult neurons. Because such selectivity could subserve song learning, it is important to determine when it arises. Birds were therefore studied at an intermediate stage of learning, after substantial experience of both tutor song and their own developing (plastic) song.

Extracellular single neuron recordings in 60 day old zebra finches revealed that AF neurons had significant song and order selectivity for both BOS and tutor song. The degree of BOS selectivity was less than that found in adults, as indicated in part by 60 day neurons that were sensitive to the local order of elements within syllables, but not yet to the global order of syllables within a song. When responses to BOS and tutor song were compared, most neurons preferred BOS, some preferred tutor song, and others responded equally to both stimuli. The latter type of neuron was not simply immature, because many of these neurons responded significantly more to BOS and tutor song than to conspecific and reverse songs.

The selectivity of AF neurons at 60 days is markedly different from the unselective properties of neurons at 30 days, and may function in vocal learning at this stage. Moreover, the selectivity for both BOS and tutor song raises the possibility that both aspects of the birds' sensory experience during learning are reflected in properties of AF neurons.

INTRODUCTION

Auditory neurons of the songbird anterior forebrain (AF) are some of the most complex sensory neurons known, and acquire their selectivity during song learning. In adult birds, these neurons are highly selective for the bird's own song (BOS), preferring it to the songs of other zebra finches (conspecifics) and to BOS played in reverse (Doupe and Konishi, 1991). In contrast, neurons of 30 day old juveniles lack selectivity, responding equally well to all song stimuli (Doupe, 1997; Figure 2-1A). Because auditory experience of both BOS and tutor song are required for normal song development, these songs may shape AF neuron selectivity. Furthermore, selectivity may play a role in song learning, if present in a bird in the process of vocal development. Determining when selective neurons emerge will inform hypotheses about the purpose of selectivity, as well as AF function, during song learning.

Songbirds learn their songs in two phases, which overlap in zebra finches (Figure 2-1B). During the sensory phase, a young bird listens to and memorizes the tutor song; this stored memory is often called the "template". Later, during the sensorimotor phase, the juvenile bird begins to vocalize, producing "plastic song". Plastic song is soft and rambling, has poor syllable morphology, and lacks a stereotyped syllable order. The bird uses auditory feedback to gradually match its own immature vocalizations to the memorized tutor song. In this way the plastic song is modified until the bird produces a mature "crystallized" song, which is often a good copy of its tutor song.

Deafening birds in either the sensory or sensorimotor phase impairs learning (Konishi, 1965; Price, 1979), demonstrating the importance of auditory experience to song development. Specifically, birds deafened during the sensory phase cannot memorize their tutor song, and develop an abnormal song. Birds deafened during the sensorimotor phase cannot compare their own vocalizations to the tutor template, and also produce an abnormal song; this phase of learning is independent of ongoing tutor song experience.

The AF is a subset of the specialized nuclei unique to songbirds, all of which are likely locations for neuronal changes necessary for song learning and production (Figure 2-1C). Composed of Area X (X), the medial nucleus of the dorsolateral thalamus (DLM) and the lateral magnocellular nucleus of the anterior neostriatum (LMAN), the AF is required for normal song development (Bottjer et al., 1984; Sohrabji et al., 1990; Scharff and Nottebohm, 1991), perhaps processing important auditory information during learning. A separate motor pathway for song includes HVc (this acronym is used as the proper name, as proposed in Fortune and Margoliash, 1995), the robust nucleus of the archistriatum (RA), and the tracheosyringeal portion of the hypoglossal nucleus (nXIIts); nXIIts contains the motor neurons innervating the muscles of the syrinx, the avian vocal organ. This motor pathway is necessary for normal song production throughout life (Nottebohm et al., 1976), and might be influenced by its input from the AF.

To address whether selectivity is present in the AF of a learning bird, individual LMAN and X neurons were recorded and characterized in 60 day old zebra finches. At this age, birds are at an intermediate stage of song development: the sensory phase is ending, and the sensorimotor phase is underway (Figure 2-1B). These birds have probably memorized the tutor song, and have been singing plastic song for approximately one month (Immelmann, 1969; Eales, 1985). In this study both tutor song and BOS (in this case, plastic song) were among the stimuli used to characterize the selectivity of AF neurons, thus permitting the identification of neurons tuned by either BOS or tutor song experience.

MATERIALS AND METHODS

Experiments used male zebra finches (*Taeniopygia guttata*) of approximately 60 days of age (range 55-65). Birds were raised in individual cages with their parents and siblings from the same clutch. Juveniles learn from the tutor sharing a cage with them, even when other birds are within earshot or sight (Immelman 1969; Eales 1987; Williams, 1990). Although juveniles could hear songs of other conspecifics in the colony, they were visually isolated from them with opaque dividers placed between cages. Visually isolating juveniles from a tutor interferes with learning (Eales, 1989). Thus, keeping juveniles in the same cage as their tutor, combined with visually isolating them from nearby conspecifics, should restrict their learning to the tutor in their cage.

Song recording. One to two days prior to the experiment, the juvenile's plastic song and its tutor song were recorded, digitized and entered into a Sparc IPX computer (software by Larry Proctor and Michael Lewicki, California Institute of Technology). Because of the variable quality of plastic song, the song chosen as BOS was the song most frequently produced. This was decided subjectively, based on listening to many song renditions (>25 songs in most cases). For some experiments, multiple (2-3) versions of plastic song were used as stimuli. Neurons either responded equally well to all plastic song versions or responded more to the version most frequently produced (see Results).

Surgery. Two days prior to the experiment, birds were anesthetized with Equithesin (2ml/kg i.m.; 0.85 g of chloral hydrate, 0.21 g of pentobarbital, 0.42 g of $MgSO_4$, 2.2 ml of 100% ethanol, 8.6ml of propylene glycol to a total volume of 20 ml with water) and placed in a stereotaxic head holder (Herb Adams, Central Engineering, California Institute of Technology). Using stereotaxic coordinates, locations of song nuclei were marked on the skull, relative to the bifurcation of the mid-sagittal sinus. A stainless steel post was fixed to the skull over the mid-sagittal sinus with dental cement (Dentsply, Milford, DE).

On the day of the experiment, the bird was anesthetized with a 20% solution of urethane (5ml/kg, Sigma, St. Louis, MO; delivered in 3 injections, i.m., at 30 minute intervals). The bird was positioned into the stereotaxic apparatus, and its head was immobilized by fixing the head post to a bar overhead. Body temperature was maintained with a temperature controller (FHC, Brunswick, ME). A parylene-coated tungsten electrode (A-M Systems, Everett, WA) with resistance of 1-2MΩ was positioned over the mark designating nucleus location. A craniotomy was peformed around the area, the dura cut open, and the electrode lowered into the brain with a microdrive (FST, Foster City, CA). The animal was then placed into a double-walled anechoic sound-attenuated chamber (Acoustic Systems, Austin, TX) where acoustic stimuli were presented in free-field conditions by a speaker 25 cm away. The frequency response measured at the bird's location inside the chamber was flat (±5.0 dB) between 500Hz and 8kHz.

Stimuli. The stimuli presented included the plastic song of the experimental bird (BOS), the song of its tutor, the songs of other adult and juvenile (60 day) zebra finches (conspecifics), the songs of other species of estrildid finches (heterospecifics), broad-band noise bursts and tone bursts. Although HVc neurons (input nucleus to the AF) are relatively insensitive to intensity differences (Margoliash and Fortune, 1992), stimuli were presented at equivalent intensities. The mean peak intensity of song stimuli was 67.6 dB SPL (±0.43 SEM). These stimuli were presented in an interleaved fashion, with 6-8 sec between them to reduce the possibility of habituating or entraining the neurons. In some experiments, stimuli were presented in interleaved, random order. An effort was made to present each neuron with 15-20 trials of BOS, reverse BOS (song is completely reversed), reverse order BOS (temporal order of each syllable remains intact, but the sequence of syllables within the song is reversed), tutor song, reverse tutor song, reverse order tutor song, at least 2 different adult conspecific songs, at least 2 different juvenile conspecific songs, at least 2 different heterospecific songs, broad-band noise bursts and tone bursts; however, some neurons were lost before complete characterization.

Electrophysiology. Extracellular neuronal signals were amplified and filtered between 300Hz and 10KHz (A-M Systems, Everett, WA). Search stimuli used to locate auditory neurons included BOS, tutor song, conspecific song, heterospecific song, broad-band noise bursts, and tone bursts. Most single neurons were isolated with a window discriminator (Physiology Shop, UCSF); others were obtained from recorded waveforms using spike sorting software (Lewicki, 1994). Responses to acoustic stimuli were collected and analyzed by a Sparc IPX computer using software developed by Michael Lewicki and Larry Proctor (California Institute of Technology), and Frédéric Theunissen (UCSF). Data collected for each stimulus were displayed as raster patterns and summed peristimulus time histograms (30 ms binwidth) of 10-20 stimulus presentations. Electrolytic lesions were made at selected locations to enable reconstruction of recording sites after the experiment.

At the end of an experiment, animals were deeply anesthetized with Metofane (Pitman-Moore, Mundelein, IL), and intracardially perfused with 0.9% saline, followed by 3.7% formalin in 0.025M phosphate buffer. Brains were postfixed and cut into 40μm sections with a freezing microtome. Sections were stained with cresyl violet, and electrode tracks and lesions were located. Only neurons confirmed histologically to be in LMAN or X were used. A neuron's specific location within each nucleus was also documented.

Data analysis. Neuronal responses were quantified for the period during which a stimulus was presented, delayed by an estimate of the latency of the response. The latency for each neuron was usually measured by viewing its response to a broad-band noise or tone burst as a summed peristimulus time histogram with a binwidth of 5 ms. Latency was defined as the onset of the first 2 consecutive bins after stimulus onset in which the number of spikes/bin was at least twice the mean number of spikes/bin prior to stimulus onset. Because many LMAN neurons failed to respond to broad-band noise or tone bursts, it was often not possible to measure latency in this way. For these cases, the latency of another cell within the same nucleus of the same experimental bird was assigned. If there were none, then a default latency slightly longer than the latency characteristic of adult LMAN neurons was used (65ms).

To be considered auditory and included for analysis, a neuron had to have an average firing rate during at least one stimulus that was significantly different ($p<0.05$, paired t-test) from its average spontaneous rate. The average spontaneous rate was the mean firing rate of two time periods during each trial: 2 sec preceding stimulus onset and 2-3 sec beginning 1 sec after the end of the stimulus. For each neuron, the response strength (RS) to a stimulus was calculated by subtracting the spontaneous rate from the firing rate during the stimulus. RS was measured for each trial, then averaged across trials to get a mean RS to the stimulus, expressed in spikes/s. Data from different stimuli, but of same stimulus type, were also averaged in this way in order to obtain a value for a neuron's RS to a particular stimulus type (eg., individual RS values for each trial to two different adult conspecific songs were averaged together to give a mean RS value for adult conspecific song). A mean RS for each stimulus type for an entire nucleus was also calculated from all LMAN or X neuron responses.

An individual neuron's selectivity for one stimulus (A) over another stimulus (B) was described with a d' value (Green and Swets, 1966; Tolhurst et al., 1983) and a selectivity index (Volman, 1996; Doupe, 1997). Previously employed measures of selectivity using ratios (RS_A/RS_B or $\log(RS_A/RS_B)$; Margoliash, 1986; Volman, 1993) were not appropriate for this study because of the frequency of inhibitory responses in LMAN. Instead, a neuron's preference for stimulus A over stimulus B was described with a d' value, such that $d'_{A.B} = \dfrac{2\left(\overline{RS_A} - \overline{RS_B}\right)}{\sqrt{\sigma_A^{\,2} + \sigma_B^{\,2}}}$, where $\overline{RS_A}$ and $\overline{RS_B}$ are the mean RS to stimulus A and B, respectively, and σ^2 is the variance of each mean RS. If $d'_{A\text{-}B}$ is positive, then stimulus A elicited a greater response; if it is negative, then stimulus B elicited a greater response. Values of $d'_{A\text{-}B}$ around zero indicate no difference in the responses evoked by A and B. This d' value has an advantage over ratios of mean RS because the difference between two means is weighted by the variance of their distributions. The d' value distinguishes between two means that come from largely overlapping distributions and those that do not, and is thus a measure of discriminability between the two stimuli given the two responses. A d' value was only calculated for those neurons with significant responses to at least one of the two stimuli compared ($p<0.05$ for a paired t-test between the response during the stimulus and the spontaneous rate).

A disadvantage of the d' value is that it does not convey the magnitude of the difference between the mean RS values to two stimuli (d' normalizes this difference by a standard deviation which differs for each pair of means compared). Thus, the difference between mean RS to stimulus A and stimulus B was also expressed using a selectivity index ("SI"): $\frac{\overline{RS_A}}{\left(\overline{RS_A} + \overline{RS_B}\right)}$. Neurons preferring stimulus A over B have SI values near 1, neurons preferring stimulus B to A have values near 0, and those with no preference have values around 0.5. In general, there was good correspondence between d' and SI measures.

A d' value of $|0.5|$ was chosen as the criterion for a "selective" neuron. Cells with a d' value of $|0.5|$ or greater usually had a mean RS to the preferred stimulus which was at least twice as great as that to a non-preferred stimulus, as measured by SI (Figure 2-4B). Also, a d' value of $|0.5|$ corresponds to responses to two stimuli that are significantly different from each other by a paired t-test ($p=0.031$) when 20 trials of each stimulus are compared. In a previous study, the selectivity of an individual cell was characterized by whether it had significantly different responses to two test stimuli ($p<0.05$, paired t-test (Lewicki and Arthur, 1996). In this study, a $d'_{A\text{-}B}$ value of 0.5 or greater indicates a neuron selective for stimulus A over B. Likewise, $d'_{A\text{-}B}$ values of -0.5 or less reflect a selectivity for stimulus B over A.

Normalizing spike counts by the length of song stimulus (approximately 2 sec) to obtain the firing rate during a stimulus did not underestimate d' values. For a subset of neurons (12 from LMAN, 7 from X; from 3 birds) peak RS values during a stimulus were determined using a series of sliding windows, whose size ranged from 10 to 2000 ms. For each window size, d' values were calculated using the corresponding peak RS values. In general, d' values were smallest for short windows, rose with increasing window length, and reached a plateau for windows of 200-500ms duration and above. Thus d' values calculated with RS normalized by the length of a song also gave maximum d' values (data not shown).

Cluster analysis. A Monte Carlo analysis was used to determine whether the $d'_{BOS\text{-}TUTOR}$ values of cells recorded from each bird were more clustered than expected by chance. To do this, the variance of the $d'_{BOS\text{-}TUTOR}$ values obtained from each bird was compared to the distribution of variances that resulted from random draws of all $d'_{BOS\text{-}TUTOR}$ values from all experiments. This distribution was determined from 1000 Monte Carlo simulations which randomly selected n $d'_{BOS\text{-}TUTOR}$ values from the pool of $d'_{BOS\text{-}TUTOR}$ values, where n equals the number of cells recorded in each bird. The median of this simulated distribution of variances was then compared to the variance from each experiment. If the experimental variance was significantly less than the median of the simulated distribution (one-sample test, $p<0.05$), then it was considered "clustered"; otherwise, the experimental variance was marked "unclustered". A sign-test determined whether the frequency of "clustered" birds was greater than expected by chance. This procedure was completed for $d'_{BOS\text{-}TUTOR}$ values from LMAN alone, X alone, and both nuclei together.

To determine if clustered values were due to the particular stimulus conditions or duration of an experiment, ratios of stimulus intensity and duration were calculated and correlated to mean $SI_{BOS\text{-}TUTOR}$ of each bird (specifically, peak BOS intensity/(peak BOS intensity+peak tutor intensity), mean BOS intensity/(mean BOS intensity+mean tutor intensity), and BOS duration/(BOS duration+tutor duration).

Song analysis. Once electrophysiology experiments were completed, the BOS and tutor song stimuli themselves were analyzed. Song is composed of syllables, which are short, discrete vocalizations of 10-200 ms duration, separated by brief silent intervals. Syllables themselves are made of smaller components called "notes" which give a syllable its morphology. A repeated sequence of syllables is a motif. A song "bout" consists of introductory notes followed by one or more motifs. Two subjective tests were conducted on the songs: a similarity test and a stereotypy test. For each test, observers familiar with zebra finch song both listened to songs and looked at accompanying sonograms and oscillograms to decide on a score for each experimental animal. Observers were blind to the selective properties of neurons already obtained from each bird. In the similarity test, the plastic song's similarity to the tutor song was scored on a scale of 1-5, such that 1 described those plastic songs that bore no resemblance to the tutor song, and 5 described those plastic songs that were good copies of the tutor song. Observers were instructed to consider similarities in syllable morphology, syllable order, syllable and motif duration, and rhythm. In the stereotypy test, a plastic song's stereotypy was judged, based on multiple renditions of the bird's plastic song (10 songs analyzed per bird, except in three cases). Observers were instructed to decide whether consistent syllable morphologies, syllable order, durations, and motif structures were present in the different renditions of plastic song, and to score songs according to the consistency observed. A stereotypy score was given to each set of plastic songs: a score of 1 meant that the renditions of song were very different from each other, and a score of 5 described a set of very stereotyped song renditions. To control for slight differences in scoring between individuals, each observer's score was normalized by the mean score given by that observer, which resulted in a range of normalized scores between 0.3 and 1.9. Normalized scores from eight observers were then averaged to produce a final similarity score and a final stereotypy score for each experimental bird. Thus, a score of 0.3 corresponds to a song which did not resemble the tutor song, or which was not at all stereotyped, and a score of 1.9 corresponds to a song which strongly resembled the tutor song or which was highly

RESULTS

Selectivity of LMAN neurons at 60 days

Analysis of 61 LMAN neurons from 16 birds revealed that by 60 days, these neurons were song and order selective for BOS (in this case, plastic song) and tutor song. A song selective neuron responds more to BOS and/or tutor song than to other song stimuli; in this study, these other song stimuli included adult and juvenile (60 days) conspecific songs, and heterospecific songs. An example of a 60 day song selective neuron is shown in Figure 2-2A; although this neuron responded well to both BOS and tutor song, it responded less to an adult conspecific song. Song selectivity was a general feature of LMAN neurons: on average, BOS and tutor song evoked significantly greater responses than other stimulus types (2-2B, paired t-tests, $p<0.0001$ for BOS-adult conspecifics, $n=58$, BOS-juvenile conspecifics, $n=32$, BOS-heterospecifics, $n=60$, and tutor-heterospecifics, $n=60$; $p<0.0011$ for tutor-adult conspecifics, $n=58$). The song selectivity of individual LMAN neurons is illustrated with scatterplots where each cell's mean RS to BOS (Figure 2-2C) or tutor song (Figure 2-2D) is plotted against its mean RS to adult conspecific song. Many points lie below the diagonal lines, indicating those neurons with stronger responses to BOS or tutor song than to adult conspecific songs. The percentage of song selective LMAN cells (i.e., $d'_{\text{BOS-ADULT CON}} \geq 0.5$ and/or $d'_{\text{TUTOR-ADULT CON}} \geq 0.5$; see Methods) in each selectivity category is listed in Table 2-1.

LMAN neurons had also developed order selectivity by 60 days. An order selective neuron responds more to BOS and/or tutor song than when these songs are completely reversed. In these reverse stimuli, the song is entirely backwards, so that both the order of syllables and of the elements within them are reversed. An order selective LMAN neuron is shown in Figure 2-3A, with its strong response to BOS (first panel) significantly reduced upon reversing BOS ("BOS rev", second panel). On average, LMAN was order selective: the mean RS of all LMAN neurons to forward BOS and tutor song stimuli was significantly greater than that to the corresponding reverse stimuli (Figure 2-3B, paired t-test, $p<0.0001$ for BOS-reverse BOS, $n=55$; $p<0.0023$ for tutor-reverse tutor, $n=50$). The order selectivity of individual LMAN units is shown in scatterplots where each cell's mean RS to BOS (Figure 2-3C) or tutor song (Figure 2-3D) is plotted against its mean RS to the corresponding reverse stimulus. Many cells responded more to forward than to reverse stimuli, and thus fall below the diagonal line. Of the LMAN neurons tested, 81% were selective for forward over reverse stimuli ($d'_{\text{BOS-REV}} \geq 0.5$ and/or $d'_{\text{TUTOR-REV}} \geq 0.5$; for specific selectivity category percentages, see Table 2-1).

The order of a song stimulus can be altered in several ways. Reverse order is a stimulus in which the temporal order within each syllable remains intact, but the sequence of syllables is reversed. For example, if each syllable is represented by a letter, then forward vs. reverse order song is analogous to ABC vs. CBA. In adult neurons, responses to reverse order stimuli are significantly less than those to forward stimuli (Doupe, 1997). In contrast, many 60 day LMAN neurons responded strongly to the "reverse order" manipulation of BOS and tutor song. Figure 2-3A shows such a neuron with strong responses to both reverse order BOS ("BOS ro" third panel) and forward BOS. Of those LMAN neurons tested, 39% were similar to the neuron in Figure 2-3A; these neurons were selective for forward compared to reversed stimuli, but responded equivalently to forward and reverse order stimuli ($d'_{\text{FOR-REV}} \geq 0.5$ but $-0.5 < d'_{\text{FOR-RO}} < 0.5$). Thus, this subset of 60 day neurons was sensitive to the local order of elements within a syllable, but remained insensitive to the global order of syllables within a song. Other 60 day neurons were more adult-like: 66% were selective for forward relative to both reverse and reverse order song stimuli (see Table 2-1 for percentages of cells in the specific order selectivity categories). Overall, comparisons of mean RS from all LMAN neurons showed a small significant difference between forward BOS and reverse order BOS (paired t-test, $p<0.0021$, $n=41$), but not between forward tutor song and reverse order tutor song (paired t-test, $p<0.9728$, $n=29$).

LMAN responses to BOS and tutor song

The availability of both tutor song and the bird's own developing song allowed a direct comparison of neural responses to these two behaviorally important stimuli. LMAN neurons at 60 days had a variety of preferences for BOS vs. tutor song. Some LMAN neurons preferred BOS to tutor song, others preferred tutor song to BOS, and many showed no preference, responding equally well to both stimuli (Figure 2-4A). To describe a neuron's preference, a $d'_{BOS\text{-}TUTOR}$ value was calculated. Neurons with greater responses to BOS than to tutor had values greater than 0, those with greater responses to tutor song than to BOS had values less than 0, and those responding equally to both songs had values around 0. A plot of $d'_{BOS\text{-}TUTOR}$ values obtained from LMAN neurons shows the range and distribution of preferences (Figure 2-4B). Although neurons were significantly responsive to BOS and/or tutor song, 34% of them had no strong preference for either stimulus ($-0.5<d'_{BOS\text{-}TUTOR}<0.5$, marked in grey). White circles signify neurons whose average response to the preferred stimulus was at least twice as great as to the non-preferred stimulus (ie., $SI \geq 0.67$ or ≤ 0.33; see Methods for details). Most white circles lie outside of the grey unselective region and most black circles lie within the grey region, indicating a good correspondance between these two measures of selectivity. Although individual neurons varied in their BOS vs. tutor song preference, BOS elicited a greater average response than tutor song in LMAN (see black circles in Figure 2-8E; paired t-test, $p<0.0038$, $n=61$).

Because of the variable quality of plastic song, it was possible that neurons without a strong BOS preference occurred when the version of plastic song presented as BOS was one to which neurons were unresponsive. To test this, 2-3 different versions of BOS were presented in six experiments (19 neurons): neurons either responded equally well to all versions of plastic song, or preferred the song that was most frequently produced, called "BOS 1" (Figure 2-4C). This song was used as BOS in all comparisons with other song stimuli in all experiments. On average, BOS 1 elicited a slightly greater response than other plastic song renditions (ANOVA, $F_{2,47}=3.861$, $p<0.0281$). Thus, selectivity measurements were unlikely to be biased by inappropriate BOS choice.

LMAN cells responding equally well to BOS and tutor song were not simply unselective neurons, because they also showed song or order selectivity. Figure 2-5A plots the BOS vs. tutor song preference ($d'_{\text{BOS-TUTOR}}$) of individual neurons against their degree of song selectivity ($d'_{\text{BOS-ADULT CON}}$ and $d'_{\text{TUTOR-ADULT CON}}$): even neurons without a strong BOS vs. tutor song preference (grey region) exceeded d' values of 0.5 in these song selectivity measures. Figure 2-5B shows the result of categorizing neurons based on their d'values for different measures of selectivity. If a neuron had a $d'\geq 0.5$ for any one of four selectivity categories (BOS-adult conspecific, tutor-adult conspecific, BOS-BOS reverse, tutor-tutor reverse), it was considered selective. Classified in this way, 68% of neurons that responded equally well to BOS and tutor song were selective, and only 5 neurons in this class completely lacked such selectivity. This small fraction of cells recorded (9%) resembled 30 day neurons, which respond equally well to all song types.

The graph in Figure 2-5A also shows that neurons preferring BOS over tutor song are also strongly selective for BOS relative to adult conspecific song; likewise, neurons preferring tutor song relative to BOS also maintained this preference when comparing tutor responses to those of other adult conspecifics. Thus, a neuron's preference for BOS or tutor song was consistent across comparisons with other song stimuli (eg., conspecfic, heterospecific, reverse, and reverse order song; all data not shown). This supports the idea that these neurons respond specifically to BOS or tutor song.

Song and order selectivity of X neurons at 60 days

To investigate circuitry that contributes to the selectivity of LMAN, neurons were recorded in X, the first nucleus in the AF pathway (Figure 2-1C). As in LMAN, these neurons (n=56) also exhibited song and order selectivity at 60 days. Figure 2-6A illustrates the song selectivity of a single X neuron, which had robust responses to BOS and tutor song, and weak responses to an adult conspecific song. The mean RS of all X neurons is shown for each stimulus type in Figure 2-6B: BOS and tutor songs evoked significantly greater responses than adult and juvenile conspecific songs, and heterospecific songs (paired t-tests, $p<0.0001$ for BOS-adult conspecific, $n=49$, for BOS-heterospecific, $n=53$, and for tutor-heterospecific, $n=53$; $p<0.0026$ for BOS-juvenile conspecific, $n=35$; $p<0.0005$ for tutor-adult conspecific, $n=49$). On average, there was no significant difference between BOS and tutor song responses (see white circles in Figure 2-8E; paired t-test, $p<0.2950$, $n=52$). The song selectivity of individual X neurons is illustrated with scatterplots that compare each neuron's mean RS to BOS (Figure 2-6C) and tutor song (Figure 2-6D) to its mean RS to adult conspecific song. All points below the diagonal lines represent X neurons that responded more to BOS or tutor song than to adult conspecific songs. The percentages of selective X cells in each song selectivity category are listed in Table 2-1.

X neurons were also order selective, responding more to BOS or tutor song than to completely reversed stimuli. An example of an order selective X neuron is shown in Figure 2-7A (first two panels). Comparisons of mean RS of all X neurons showed that forward BOS and tutor song elicited significantly stronger responses than the corresponding reverse stimuli (Figure 2-7B; paired t-tests, $p<0.0001$ for BOS-reverse BOS, $n=45$; and for tutor-reverse tutor, $n=46$). Most X cells responded more to forward than to reverse stimuli: when each neuron's mean RS to BOS (Figure 2-7C) or tutor song (Figure 2-7D) is compared to the corresponding reverse stimulus, most cells lie below the diagonal line. 82% of X neurons tested were selective for forward relative to reverse stimuli (see Table 2-1 for specific selectivity category percentages).

As in LMAN, some X neurons had strong responses to reverse order stimuli (Figure 2-7A, "BOS ro", third panel). Of those X neurons tested, 30% were selective for forward stimuli relative to reverse, but responded equally well to forward and reverse order stimuli ($d'_{FOR-REV} \geq 0.5$ but $-0.5 < d'_{FOR-RO} < 0.5$). In contrast, 63% resembled adult neurons, being selective for forward stimuli over both reverse and reverse order song stimuli ($d'_{FOR-REV} \geq 0.5$ and $d'_{FOR-RO} \geq 0.5$; see Table 2-1 for percentages of cells in specific selectivity categories). On average, X neurons responded significantly less to reverse order stimuli when compared to forward responses (Figure 2-7B; paired t-test, $p<0.0003$ for BOS-reverse order BOS, n=35; $p<0.0264$ for tutor-reverse order tutor, n=38). Thus, sensitivity to the global order of song is in the process of developing at 60 days.

Of those X neurons with equal responses to BOS and tutor song ($-0.5 < d'_{BOS-TUTOR} < 0.5$), 63% exhibited at least one aspect of song or order selectivity (specifically, $d'_{BOS-ADULT\ CON} \geq 0.5$, $d'_{TUTOR-ADULT\ CON} \geq 0.5$, $d'_{BOS-REV} \geq 0.5$, or $d'_{TUT-REV} \geq 0.5$). Only 10% of X cells recorded responded equally well to all of these song stimuli. As in LMAN, the BOS or tutor song preference of an X neuron was consistent across different song comparisons (data not shown).

A comparison of LMAN and X responses

Although they shared song and order selectivity, LMAN and X differed from each other in some respects. LMAN neurons had a significantly lower mean spontaneous rate than X neurons did (Figure 2-8A; mean and SEM, 1.60 ± 0.25 spikes/s for LMAN, 40.04 ± 3.84 spikes/s for X; unpaired t-test, $p<0.0001$). Also, broad-band noise bursts often inhibited LMAN neurons, whereas they elicited robust firing from X neurons. The mean RS to a broad-band noise burst (300 ms) of LMAN neurons was significantly less than that of X neurons (Figure 2-8B; unpaired t-test, $p<0.0001$).

LMAN and X did not differ significantly in their degree of selectivity, whether measured with d' or SI values (Figure 2-8C). Although LMAN tended to have higher mean SI values than X did, paired comparisons between nuclei found only one significant difference: LMAN had greater BOS-adult conspecific selectivity than X (paired t-test between mean SI, $p<0.0092$, n=16). No significant difference was found for any selectivity category when mean d' values were compared between LMAN and X. In general, LMAN and X shared the same range of BOS vs. tutor song preferences (Figure 2-8D). Like LMAN, many X neurons responded equally well to both stimuli (compare 33% in X to 34% in LMAN with $-0.5 < d'_{\text{BOS-TUTOR}} < 0.5$). On average, LMAN responded more to BOS than to tutor song, whereas in X, the mean RS values to these two stimuli were equivalent (Figure 2-8E). This difference is likely due to the greater number of tutor-preferring neurons in X than in LMAN (ie., neurons with $d'_{\text{BOS-TUTOR}} \leq -0.5$).

The number of selective neurons in LMAN was significantly different from X for four selectivity categories (Table 2-1): LMAN had significantly more BOS>adult conspecific and BOS>heterospecific neurons than X did, whereas X had more tutor>BOS and tutor> reverse order tutor neurons than LMAN. These two nuclei also differed in the frequency of inhibitory responses: of all selective responses found in each nucleus (ie., all $d' \geq 0.5$), significant inhibition to non-preferred stimuli ($p<0.05$, paired t-test between the firing rate during a stimulus and spontaneous rate) occurred in 41% of LMAN cases and in only 6% of X cases. Thus, inhibition was a component of LMAN's selective responses more frequently than those of X (chi-square test, $p<0.0001$).

Analyses between individual birds

The $d'_{\text{BOS-TUTOR}}$ values of LMAN neurons from individual birds clustered in certain regions of the preference range, rather than spanning the full range (ordinate of Figure 2-9A; mean and SEM of $d'_{\text{BOS-TUTOR}}$ values for each bird are also shown in Figures 2-9B and C). Experimental $d'_{\text{BOS-TUTOR}}$ values were considered as clustered in 10/12 birds (see Methods), and this frequency of clustering was greater than expected by chance (sign test, $p<0.0161$). Such clustering was also apparent for X neurons, and for LMAN and X neurons considered together (for X, 10/13 birds were clustered, $p<0.036$; for X and LMAN together, 14/16 birds were clustered, $p<0.0019$). Thus, something specific to each bird could explain the BOS vs. tutor song preference of its neurons.

These differences between each bird's mean $d'_{BOS-TUTOR}$ could not be accounted for by conditions that varied between experiments. Slight intensity differences between BOS and tutor song stimuli did not affect the BOS vs. tutor song preference of neurons from an individual bird (see Methods; for peak intensity, $r^2=0.033$ for LMAN, $r^2=0.041$ for X; for relative values of mean intensity, correlations were also insignificant). Similarly, stimulus duration did not strongly correlate with a bird's BOS vs. tutor song preference ($r^2=0.001$ for LMAN and $r^2=0.055$ for X). The duration of the experiment at the time when individual neurons were recorded also did not affect their $d'_{BOS-TUTOR}$ values ($r^2=0.010$ for LMAN, $r^2=0.053$ for X). Because differences in anesthesia depth between experiments could cause clustering of $d'_{BOS-TUTOR}$ values from each bird, every neuron's $d'_{BOS-TUTOR}$ value was correlated with its spontaneous rate and maximum RS to a song stimulus, both potential indicators of anesthesia depth. No association between a neuron's spontaneous rate and its $d'_{BOS-TUTOR}$ value existed in LMAN or in X ($r^2=0.049$ for LMAN $r^2=0.005$ for X). Similarly, a neuron's maximum RS to a song stimulus was not strongly correlated with its $d'_{BOS-TUTOR}$ value ($r^2=0.019$ for LMAN, $r^2=0.044$ for X). Recording from different locations within LMAN could have biased the $d'_{BOS-TUTOR}$ values obtained if a topography of selectivity existed. Yet, comparisons of each neuron's location along the dorsal-ventral, medial-lateral or anterior-posterior axes of LMAN to its $d'_{BOS-TUTOR}$ value yielded no strong correlations ($r^2=0.085$ for dorsal-ventral, $r^2=0.033$ for medial-lateral, and $r^2=0.045$ for anterior-posterior). Thus, no evidence for a topography of selectivity was found in LMAN. Together, these results indicate that clustering of $d'_{BOS-TUTOR}$ values in individual birds could not be explained by these particular differences between experiments.

To test whether the BOS vs. tutor song preference of a bird depended on some aspect of its experience, the mean $d'_{BOS-TUTOR}$ value of each bird was compared to different measures of the bird's maturity. A comparison between a bird's mean $d'_{BOS-TUTOR}$ value and its age revealed no significant correlation (Figure 2-9A; $r^2=0.055$ for LMAN; data not shown for X, $r^2=0.018$). A bird's maturity can also be measured in terms of its song development. The song maturity of each experimental bird was estimated using a similarity test and a stereotypy test (see Methods). The similarity test rated the similarity between BOS and tutor song. Comparing the final similarity score for each bird to the mean $d'_{BOS-TUTOR}$ of LMAN cells obtained from each bird produced a moderate, significant correlation (Figure 2-9B, $r^2=0.453$, $p<0.0059$). The more BOS differed from tutor song, the more neurons preferred BOS; the more BOS resembled tutor song, the more neurons responded equally to BOS and tutor song or more to tutor song. Because birds do not always produce perfect copies of tutor songs in adulthood, song similarity between the BOS and tutor song is an incomplete description of song maturity. Thus, another measure of maturity was used, which judged each bird's song stereotypy based on several renditions of its plastic song. Comparing the final stereotypy score for each bird to its mean $d'_{BOS-TUTOR}$ value of LMAN neurons resulted in a small but insignificant correlation (Figure 2-9C, $r^2=0.273$, $p<0.0553$). The similarity scores and stereotypy scores for each bird covaried, indicating that these two features of maturity develop together (Figure 2-9D, $r^2=0.668$, $p<0.0002$). Thus, for this experimental group of birds, lack of similarity to the tutor song was often related to the immaturity of the song, rather than to the occurrence of learning from other conspecifics in the colony. The $d'_{BOS-TUTOR}$ values of individual cells were also compared to song test scores in order to weigh each bird's contribution to the correlation by the number of cells recorded from that bird. These comparisons yielded similar results (dotted lines in Figures 2-9A, B, and C; for age $r^2=0.075$, $p<0.0410$, for similarity test $r^2=0.257$, $p<0.0001$, and for stereotypy test, $r^2=0.150$, $p<0.0035$, respectively). These correlations with song maturity suggest that a bird's stage of song learning and the selectivity of its neurons may be related.

The same analyses were completed for X neurons; weaker correlations in the same direction as those for LMAN resulted. A comparison between a bird's song similarity score and the BOS vs. tutor song preference of its X neurons produced a small, insignificant correlation (data not shown; mean $d'_{BOS-TUTOR}$ value vs. similarity score:

$r^2=0.173$, $p<0.1090$; individual $d'_{BOS-TUTOR}$ values vs. similarity score: $r^2=0.140$, $p<0.0080$). Comparing a bird's song stereotypy to the BOS vs. tutor song preference of its X neurons also yielded a small but insignificant correlation (data not shown; mean $d'_{BOS-TUTOR}$ value vs. stereotypy score: $r^2=0.107$, $p<0.2336$; individual $d'_{BOS-TUTOR}$ value vs. stereotypy score: $r^2=0.049$, $p<0.1304$).

DISCUSSION

This study demonstrates that in one month, AF neurons have diverged from the unselective properties of neurons in 30 day old birds. By 60 days, these neurons showed significant song and order selectivity for BOS and tutor song. On average, neurons responded more to BOS or tutor song than to conspecific and heterospecific songs. AF neurons also decreased their responses to BOS and tutor song when these songs were reversed, demonstrating an ability to discern fine temporal properties of song. Comparisons of responses to BOS and tutor song revealed a range of preferences in LMAN and X: most neurons preferred BOS, some preferred tutor song, and others responded equally to both. Many neurons with equal responses to BOS and tutor song were not simply immature, because they exhibited song or order selectivity.

Intermediate selectivity at 60 days

AF neurons were selective in 60 day birds, who are still in the process of learning their song. The song and order selectivity for tutor song apparent at this age is not found in 30 day juveniles. Yet, the degree of selectivity for BOS at 60 days was less than that of adults: cumulative distributions of d' values for song selectivity (specifically, $d'_{\text{BOS-ADULT CON}}$ and $d'_{\text{TUTOR-ADULT CON}}$) at different ages show that 60 day values were intermediate to 30 day and adult values in both LMAN and X (Figures 2-10A and 2-10B, respectively; 30 day and adult data from Doupe, 1997 is reanalyzed here). 60 day neurons were also intermediate in their degree of order selectivity (data not shown); this was also evident when comparing average responses to forward, reverse and reverse order stimuli at different ages. Figure 2-10C shows that the average difference in LMAN responses between forward and reverse order stimuli at 60 days was less than that recorded in adults, implying that further selectivity for forward relative to reverse order stimuli will develop. Thus, neurons seem initially sensitive to the local temporal order within a syllable, and only later distinguish the global order of syllables within the song. This differential time course suggests that distinct mechanisms, differing in temporal integration times, generate these two kinds of order selectivity. Moreover, this sequence of order selectivity acquisition is analogous to sensory learning in zebra finches: juveniles memorize individual syllables first and their sequence later (Immelmann, 1969).

The presence of song and order selectivity by 60 days in LMAN and X is consistent with a role for selectivity during song learning, but what this specific function is remains unclear. In other systems, selective neurons subserve perceptual discriminations (Riquimaroux et al., 1991; Newsome et al., 1990). Similarly, song and order selective neurons may permit young birds to discriminate their own song from other auditory stimuli, or may evaluate and guide developing song behavior.

Selectivity differences between LMAN and X

LMAN and X shared equivalent degrees of selectivity when mean d' values for different selectivity categories were compared (Figure 2-8C); however, there was a trend for LMAN to be more selective than X when comparing mean SI. The difference between these two measures reflects the greater variance in LMAN responses relative to X responses: the greater difference in RS between preferred and non-preferred stimuli in LMAN than in X (as detected by SI) was minimized in the d' measure, which is normalized by response variance. Whether SI measures of selectivity are physiologically relevant depends on the decoding capabilities of neurons downstream of LMAN or X. If downstream neurons average activity from many LMAN or X cells, then the difference in selectivity between the two nuclei as measured by SI could be important, and selectivity might increase as one progresses from X to LMAN. Alternatively, if downstream neurons are sensitive to the variance of LMAN or X responses, then d' values would be more appropriate selectivity measures. In this situation, the similarity of LMAN and X d'values suggests that the same information is available from LMAN and X responses.

Potential mechanisms behind selectivity development

Selectivity development in the AF is likely to reflect changes occurring within the AF as well as in its input nucleus, HVc. Although HVc in 60 day finches has not been characterized, adult HVc neurons are also song and order selective (Margoliash, 1983; Margoliash and Konishi, 1985; Margoliash and Fortune, 1992; Volman 1996; Lewicki and Arthur, 1996). Furthermore, this selectivity develops during sensorimotor learning in white-crowned sparrows (Volman, 1993). The selective properties of HVc inputs could underlie AF selectivity. Yet, differences between LMAN and X imply that changes occurring within the AF may also contribute to the selectivity of these nuclei. For example, more LMAN neurons were inhibited to non-preferred stimuli than were X neurons in both 60 day and adult zebra finches (Doupe, 1997). This indicates the presence of additional inhibitory circuitry within or between AF nuclei.

Increases in selectivity during development could be created by increasing responses to preferred stimuli and/or decreasing responses to non-preferred stimuli. Which of these occurs might be determined by comparing the mean RS of neurons to these stimuli at different ages; however, interpreting the data available for this comparison is limited by the fact that 30 day, 60 day, and adult data have been collected in different experiments, and thus in potentially varied conditions. Nonetheless, with this caveat in mind, comparisons of mean RS between different ages can point to possible events underlying selectivity development. For LMAN, comparisons of 30 day to adult data (Doupe, 1997; Figure 2-1A) suggest that both increased responses to preferred stimuli and decreased responses to non-preferred stimuli produce selectivity. At 60 days, however, selectivity seemed attributable primarily to reduced responses to non-preferred stimuli relative to 30 day responses (Figure 2-11A; unpaired t-tests, $p<0.0010$ for adult conspecific; $p<0.0001$ for tutor reverse). In contrast, 60 day responses to tutor song were not significantly different from those at 30 days (unpaired t-test, $p<0.1841$). Figure 2-11B shows that 60 day LMAN neurons approximated adult responses to non-preferred stimuli (unpaired t-tests, $p<0.6671$ for adult conspecific; $p<0.0420$ for BOS reverse). Yet, the excitatory component of selectivity remained undeveloped: 60 day neurons responded less to a preferred stimulus (BOS) than adult neurons did (unpaired t-test, $p<0.0001$). Furthermore, responses to BOS at 60 days did not differ significantly from 30 day responses to all song stimuli (ANOVA, $F_{3,92}=0.349$, $p<0.7898$). In contrast, X neuron selectivity at 60 days might be due to increased responses to preferred stimuli relative to 30 day responses (Figure 2-11C). Between 60 days and adulthood, responses to non-preferred stimuli may well decrease, thus augmenting the selectivity of X neurons (Figure 2-11D).

Although this study does not resolve the site of plasticity, several cellular events have occurred in LMAN and X by 60 days that could subserve the development of selectivity. In LMAN, NMDA receptors (Aamodt et al., 1992; Carrillo and Doupe, 1995), spine densities (Nixdorf-Bergweiler et al., 1995), synapse number (Nixdorf-Bergweiler, 1995), and DLM arborization density (Johnson and Bottjer, 1992) all decrease between 35 days and adulthood; this suggests that an initial reduction of connections could increase selectivity of a post-synaptic cell, perhaps followed by synaptic strengthening and/or growth of other inputs. A similar sequence occurs during the development of ocular dominance in the visual cortex (Antonini and Stryker, 1993). In X, the number of interneurons increases by ~50% between 20 and 55 days (Nordeen and Nordeen, 1988; Sohrabji et al., 1993). Also, tyrosine hydroxylase and catecholamine staining in X has increased to nearly adult levels by 60 days (Soha et al., 1996).

Song experience responsible for selectivity

This study begins to address the nature of the experience involved in generating 60 day selectivity. Because of their importance to song learning, acoustic experiences of BOS and tutor song are likely candidates. Neurons shaped by either song experience should display a sensitivity to temporal and spectral features of that song. Neurons shaped by BOS experience could provide feedback to premotor targets of the AF about what the bird is singing. Neurons shaped by tutor song might act as a "template": they could provide information about how well a bird's vocalizations match its memorized song model.

Several results here indicate that BOS experience is important for AF selectivity. Despite the immature quality of plastic song, neurons were sensitive to its complex properties: they had significant song and order selectivity for BOS, even when compared to the plastic songs of other juveniles. Also, neurons with strong preferences for BOS over tutor song predominated in both LMAN and X. This is consistent with a previous study in white-crowned sparrows showing that selectivity in HVc is due to BOS experience (Volman, 1993). If the selectivity of the AF pathway is derived completely from such BOS-tuned HVc neurons, then LMAN and X neurons would likewise be tuned by BOS experience. Such neurons might be useful during song learning for identifying the current state of BOS, which would then be evaluated and changed accordingly; in adulthood birds might rely on BOS selective neurons for conspecific recognition (Margoliash, 1986).

If BOS experience alone generates the selectivity of AF neurons, then those neurons with equal responses to BOS and tutor song, as well as the selectivity for tutor song found here, could be explained by acoustic similarity between BOS and tutor song. This idea is consistent with the observation that an adult white-crowned sparrow with equivalent HVc responses to BOS and tutor song also had a song very similar to its tutor (Margoliash, 1983). Furthermore, neurons with strong BOS preferences came from birds with songs least resembling the tutor in both adult (Margoliash, 1983) and juvenile (Volman, 1993) white-crowned sparrows, as well as in 60 day old zebra finches (Figure 2-9B). However, results from the similarity song test in the present study indicate that neurons with equal responses to BOS and tutor song were not always associated with acoustic similarity between these songs: some of these neurons came from birds whose songs had little similarity to the tutor song (Figure 2-9B). Whether acoustic similarity accounts for neurons with equal responses to BOS and tutor song, as well as the selectivity for tutor song, could be clarified experimentally with birds induced to sing abnormal song by manipulating the syrinx early in development. Such birds' plastic songs would be extremely different from the tutor song. If they lacked neurons that responded equally to both stimuli, as well as song and order selectivity for tutor song, then acoustic similarity is a likely cause of these properties.

Alternatively, the selectivity for both BOS and tutor song as well as the frequency of neurons with equal responses to BOS and tutor song could reflect the contributions of both songs to AF selectivity. Although HVc is shaped by BOS experience in white-crowned sparrows, it is possible for the AF to derive its selectivity independently of the BOS-tuned neurons described in HVc. HVc has two populations of projection neurons (Sohrabji et al., 1989) which have never been examined separately in physiological experiments; hence, X-projecting neurons could develop selectivity separately from RA-projecting neurons. Also, the BOS preference of HVc neurons was determined from multi-unit recordings: such studies can potentially miss other kinds of selective cells if they are few, or may ascribe a single quality to a recording site that actually comprises neurons with heterogeneous preferences. Finally, selectivity could be generated independently of HVc by the circuitry within the AF pathway. Thus, AF selectivity may not be fully explained by the BOS-tuning described for HVc. If both BOS and tutor song experience contribute to AF selectivity, they could exert their respective influences simultaneously or sequentially; in the latter case, neurons with equal responses to BOS and tutor song could be making a transition from tuning for one song to another. An analogous shift in tuning has been characterized in the optic tectum of the barn owl. When the visual field of an owl is displaced horizontally with prismatic spectacles, the interaural time difference (ITD) selectivity of neurons shifts to an ITD that corresponds to the displaced visual field (Brainard and Knudsen, 1993; Knudsen and Brainard, 1991). As selectivity changes, multi-unit sites pass through a transition state in which both the normal and shifted ITDs elicit equivalent responses (Brainard and Knudsen, 1995).

Finally, LMAN and X neurons that preferred tutor song over BOS are also inconsistent with an exclusive contribution of BOS experience to selectivity. Such tutor-preferring neurons were unlikely to have resulted from inappropriate choice of plastic song as the BOS stimulus: song analysis showed that these neurons came from birds with the most mature songs, making song selection straightforward. These neurons were not as numerous as BOS-preferring neurons, however. Their lower number was unlikely to be due to presentation of the wrong tutor song: juveniles shared their cage with the tutor, and were visually isolated from other adult male birds in the colony, which should have restricted their learning to the tutor in their own cage (Immelman, 1969; Eales, 1987; Eales, 1989; Williams, 1990). If tutor song experience contributes to the selectivity of these neurons, the AF could store important information about the tutor song. A neural representation of memorized tutor song, or "template", is hypothesized to guide song learning (Konishi, 1965; Immelmann, 1969), and it is expected that by 60 days, juveniles have acquired this template (Eales, 1985).

Neurons with strong BOS preferences are not necessarily the product of BOS experience, because it is never known exactly what the bird has stored as a template: the only assay of this is what the bird eventually sings. For example, if a bird mismemorizes the tutor song, and produces a perfect copy of its inaccurate template, then BOS itself could be a closer approximation of the template than tutor song. If this were the case, birds with accurate templates would have neurons tuned to the tutor song and would produce good copies of the tutor song, whereas birds with inaccurate templates would have neurons that are apparently tuned to BOS, and would produce poor copies of the tutor song. A similar correlation occurred in this study (Figure 2-9B); however, it was only moderate, and perhaps derived from too small a population of neurons from each bird.

To confirm the role of BOS experience in shaping BOS-preferring neurons, it would again be informative to study birds induced to sing abnormal songs. If BOS-preferring neurons existed in such birds, they would be more likely due to BOS experience rather than a possibly inaccurate template. Whether tutor song also contributes to this selectivity could be revealed in studies of muted birds. Because these animals would not develop BOS, any resulting selectivity would have to be due to tutor song experience.

The selectivity observed here in normal 60 day birds demonstrates the rapid and marked changes that occur in the AF during learning. Moreover, investigation of these neurons with BOS and tutor song stimuli revealed that both types of song experience may influence the properties of this circuit during learning.

Figure 2-1 A) LMAN neurons become song and order selective between 30 days and adulthood. At 30 days neurons have equal response strengths (RS) to tutor song (TUT), conspecific song (CON) and tutor reverse (REV), whereas in adulthood, BOS elicits greater RS than CON or BOS reverse (REV; data from Doupe, 1997). B) The time course of zebra finch song learning is shown. Juveniles learn their song in two overlapping phases: the sensory phase ends at ~60 days, and the sensorimotor phase begins around 30 days and continues to adulthood (90+ days). C) The anatomy of the song system is illustrated, with the nuclei of the anterior forebrain in black and those of the motor pathway in grey.

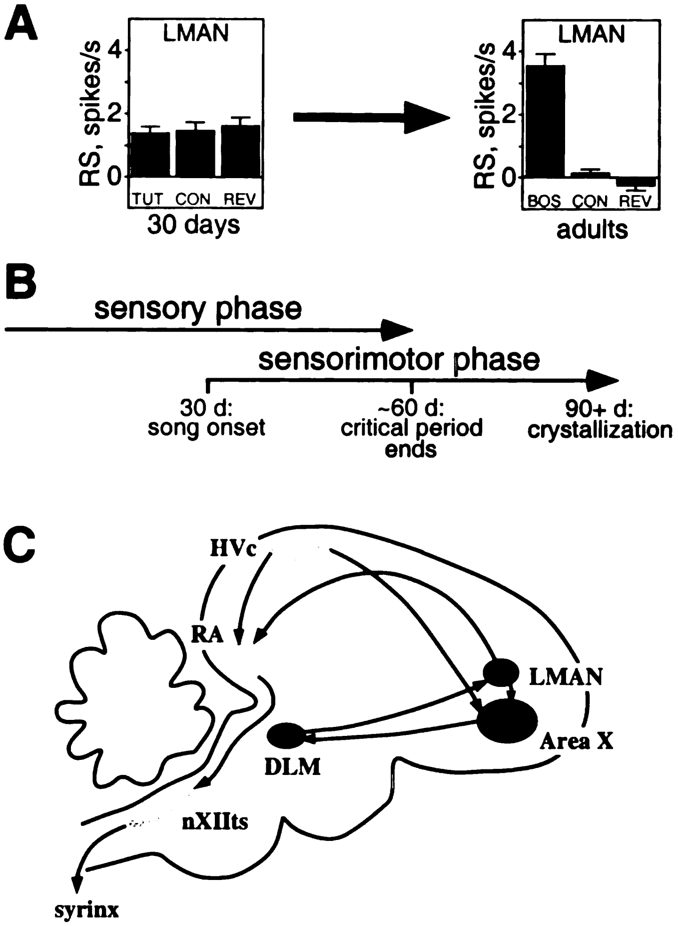

A
LMAN
RS, spikes/s
4
2
0
TUT CON REV
30 days
LMAN
RS, spikes/s
4
2
0
BOS CON REV
adults
B
sensory phase
sensorimotor phase
30 d:
song onset
~60 d:
critical period
ends
90+ d:
crystallization
C
HVc
RA
LMAN
Area X
DLM
nXIIts
syrinx

Figure 2-1

Figure 2-2 Song selectivity of LMAN neurons at 60 days. A) A peristimulus time histogram shows a single LMAN neuron's cumulative responses to multiple trials of song stimuli. This neuron had strong responses to BOS and tutor song and reduced responses to an adult conspecific song. Below each histogram, the sonogram (frequency vs. time plot, with the energy in each frequency band indicated by darkness) and oscillogram (amplitude waveform vs. time plot) of the song is shown. B) Mean RS values from all LMAN neurons are shown for several stimulus types; bars are SEM. Paired comparisons show that mean RS to BOS (black) and tutor song (white) are greater than those to adult conspecific ("adult con"), heterospecific ("het"), and juvenile conspecific ("juvenile con"). Asterisks mark significant differences (black for BOS comparisons, white for tutor comparisons). C) Each LMAN neuron's mean RS to BOS is plotted against its mean RS to adult conspecific song ("adult con"). The diagonal line marks where cells lie if they respond equally to both stimuli; black circles indicate cells with significantly greater responses to the stimulus depicted on the abscissa ($p<0.05$, unpaired t-test between abscissa stimulus trials and all adult conspecific trials). D) Each neuron's mean RS to tutor song is plotted against its mean RS to adult conspecific song. Conventions are as in C.

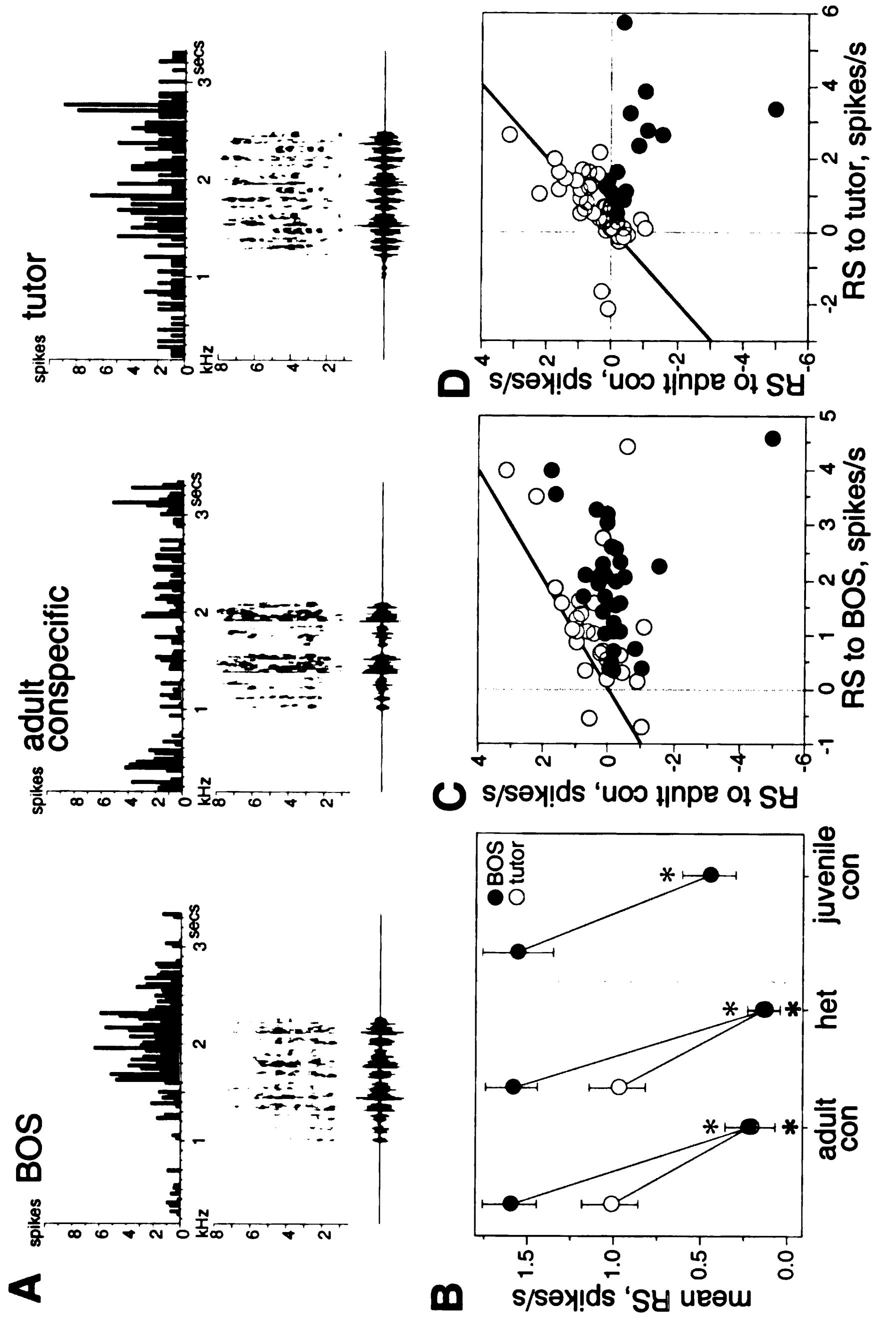

A
BOS
adult conspecific
tutor
spikes
8
6
4
2
0
kHz
8
6
4
2
1
2
3 secs
B
mean RS, spikes/s
1.5
1.0
0.5
0.0
adult con
het
juvenile con
● BOS
○ tutor
*
C
RS to adult con, spikes/s
4
2
0
-2
-4
-6
RS to BOS, spikes/s
-1
0
1
2
3
4
5
D
RS to adult con, spikes/s
RS to tutor, spikes/s
-2
0
2
4
6

Figure 2-2

Figure 2-3 Order selectivity of LMAN neurons at 60 days. A) A peristimulus time histogram of a single LMAN neuron shows a strong response to BOS (first panel), and a decreased response to BOS when the stimulus was reversed ("BOS rev"; middle panel). This cell responded well to the reverse order stimulus ("BOS ro"; third panel). B) Mean RS of all neurons to forward, reverse, and reverse order stimuli are shown; paired comparisons show significantly greater responses to forward than to reverse song stimuli for both BOS (black) and tutor song (white) comparisons. Mean RS to reverse order BOS was slightly less than forward BOS in paired comparisons, whereas no significant difference existed between forward tutor song and reverse order tutor song. Asterisks indicate significant differences (black for BOS comparisons, white for tutor comparisons), and bars are SEM. C) Each neuron's mean RS to BOS is plotted against its mean RS to reverse BOS ("BOS rev"). The diagonal line indicates where cells lie if they respond equally to both stimuli. Cells with significantly greater responses to forward than to reverse stimuli are marked with black circles ($p<0.05$, unpaired t-test between forward song trials and corresponding reverse song trials). D) Each neuron's mean RS to tutor song is plotted against its mean RS to reverse tutor song ("tutor rev"). Conventions are as in C.

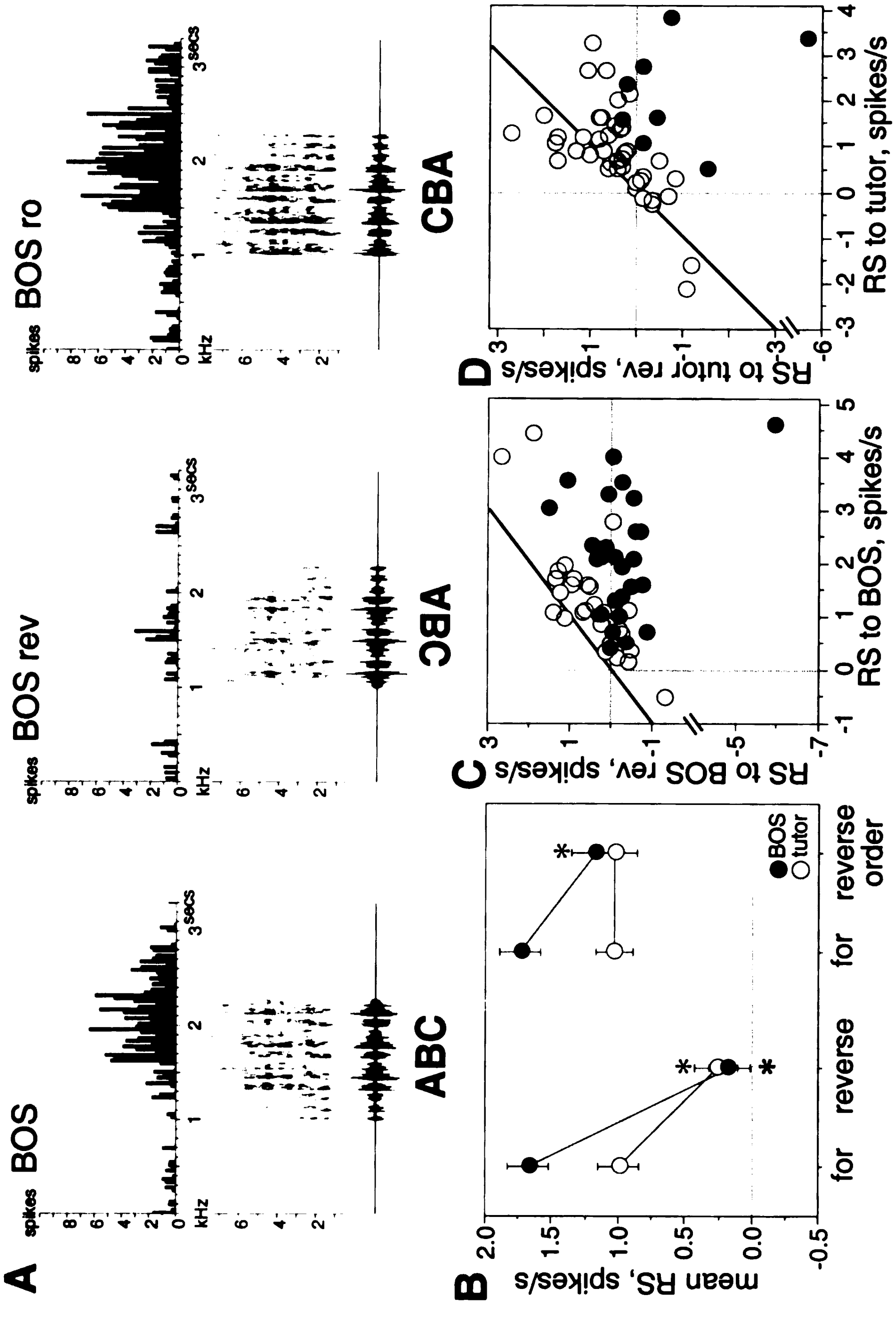

A
BOS
BOS rev
BOS ro
spikes
8
6
4
2
0
kHz
6
4
2
1
2
3 secs
ABC
ƆBA
CBA
B
mean RS, spikes/s
2.0
1.5
1.0
0.5
0.0
-0.5
for
reverse
for
reverse order
● BOS
○ tutor
C
RS to BOS rev, spikes/s
3
1
-1
-5
-7
-1
0
1
2
3
4
5
RS to BOS, spikes/s
D
RS to tutor rev, spikes/s
3
1
-1
-3
-6
-3
-2
-1
0
1
2
3
4
RS to tutor, spikes/s

Figure 2-3

Figure 2-4 BOS vs. tutor song preferences of LMAN neurons at 60 days. A) Pairs of peristimulus time histograms for three different neurons show the range of preferences for BOS vs. tutor song encountered in LMAN. The first pair shows a cell that responded more to the tutor song than to BOS, the second pair is from a neuron that responded equally well to both stimuli, and the third pair is from a cell that responded more to BOS than to its tutor song. The $d'_{BOS\text{-}TUTOR}$ value for each pair of responses is indicated. B) The cumulative distribution of $d'_{BOS\text{-}TUTOR}$ values from 56 LMAN neurons is shown. A white circle refers to a neuron whose response to the preferred stimulus was at least twice as great as that to the non-preferred stimulus, as determined from the SI. Grey shading indicates the region of d' values considered to be unselective ($-0.5<d'<0.5$). C) The responses of 19 cells to different versions of plastic song, named BOS 1, BOS 2, and BOS 3. BOS 1 is the song most frequently produced by the bird. The mean RS of all cells to each version is shown with thick black lines.

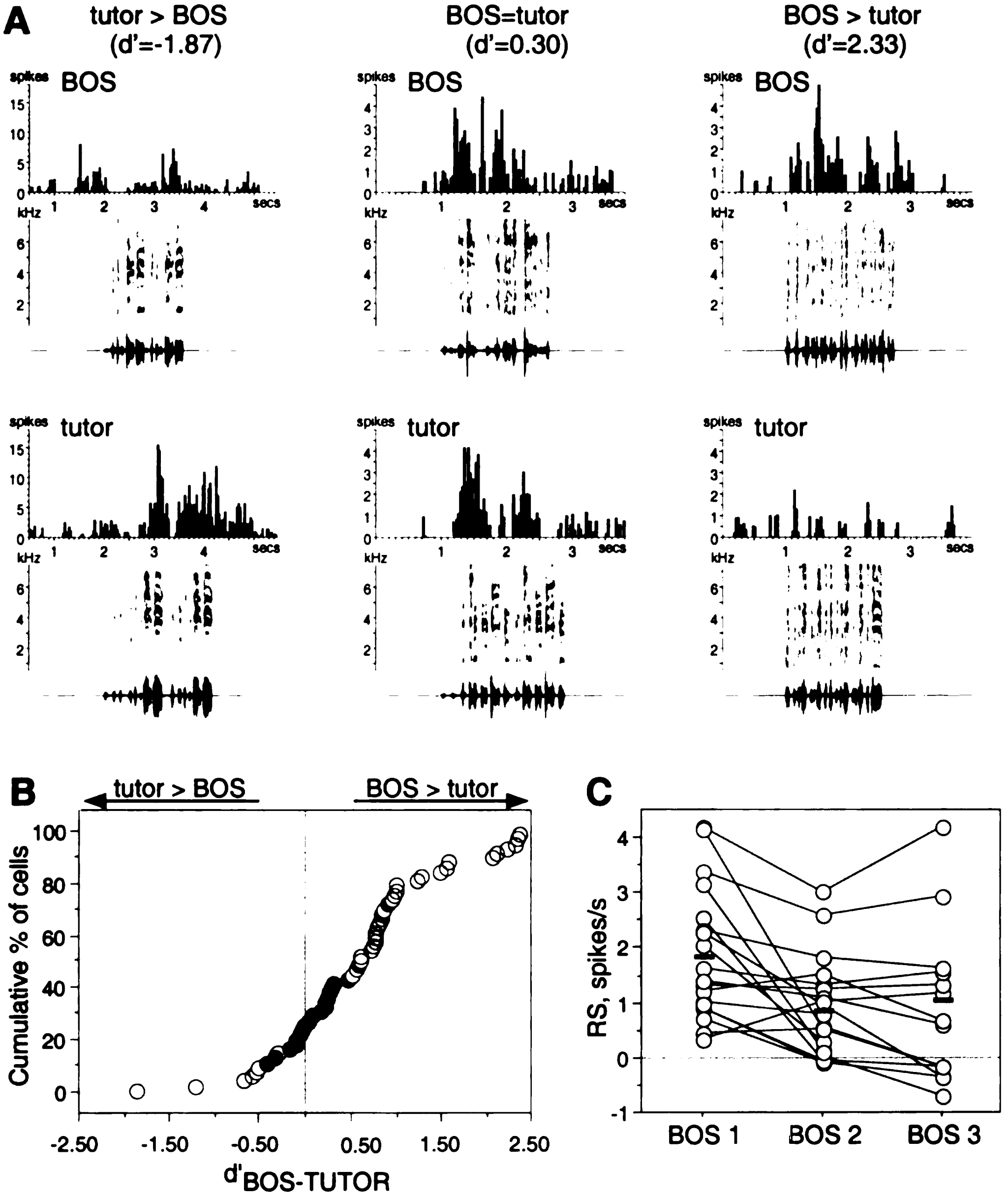

A
tutor > BOS
(d'=-1.87)
BOS=tutor
(d'=0.30)
BOS > tutor
(d'=2.33)
spikes
BOS
tutor
kHz
secs
B
tutor > BOS
BOS > tutor
Cumulative % of cells
d'BOS-TUTOR
-2.50
-1.50
-0.50
0.50
1.50
2.50
C
RS, spikes/s
BOS 1
BOS 2
BOS 3

Figure 2-4

Figure 2-5 Selectivity of LMAN neurons with equal responses to BOS and tutor song. A) The BOS vs. tutor song preference of each neuron ($d'_{BOS\text{-}TUTOR}$) is plotted against its degree of song selectivity, as measured by $d'_{BOS\text{-}ADULT\ CON}$ (black) and $d'_{TUTOR\text{-}ADULT\ CON}$ (white). The grey horizontal band highlights those cells with equal responses to BOS and tutor song. Note that many of these cells exceed 0.5 along the song selectivity axis, thus displaying significant song selectivity. B) Each LMAN neuron was classified according to its d' values for various comparisons of selectivity. BOS>tutor neurons had $d'_{BOS\text{-}TUTOR} \geq 0.5$, BOS=tutor neurons had $-0.5 < d'_{BOS\text{-}TUTOR} < 0.5$, and BOS<tutor song neurons had $d'_{BOS\text{-}TUTOR} \leq -0.5$. To be counted as selective, a neuron had to have $d' \geq 0.5$ in at least one of the following four selectivity categories: BOS-adult conspecific, tutor-adult conspecific, BOS-BOS reverse, or tutor-tutor reverse.

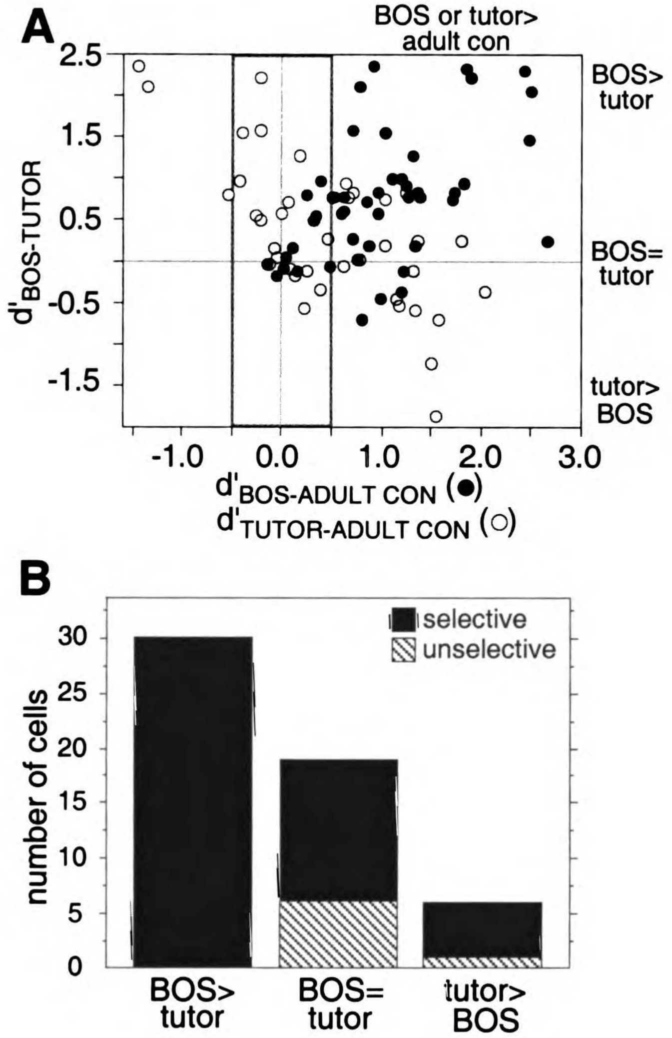

A
BOS or tutor>
adult con
BOS>
tutor
BOS=
tutor
tutor>
BOS
2.5
1.5
0.5
-0.5
-1.5
d'BOS-TUTOR
-1.0
0.0
1.0
2.0
3.0
d'BOS-ADULT CON (●)
d'TUTOR-ADULT CON (○)
B
selective
unselective
number of cells
30
25
20
15
10
5
0
BOS>
tutor
BOS=
tutor
tutor>
BOS

Figure 2-5

Figure 2-6 Song selectivity of X neurons at 60 days. A) Peristimulus time histograms of a single neuron's response to three different stimulus types. The neuron responded strongly to BOS and tutor song, but less well to an adult conspecific song. B) Mean RS values calculated from all X neurons are shown for several stimulus types; bars are SEM. Paired comparisons between mean RS of BOS (black) or tutor song (white) and mean RS to other stimulus types are shown. Responses to BOS and tutor song were significantly greater than to adult and juvenile conspecific and heterospecific songs. Black asterisks identify significant differences for BOS comparisons; white asterisks for tutor song comparisons. C) Song selectivity of individual X neurons is illustrated in scatterplots comparing each cell's mean RS to BOS to its mean RS to adult conspecific songs ("adult con"). The diagonal line marks where a cell lies if it responded equally to the stimuli compared. Cells with significantly greater responses to the stimulus depicted on the abscissa are marked with black circles ($p<0.05$, unpaired t-test between abscissa stimulus trials and all adult conspecific trials). D) Each cell's mean RS to tutor song is compared to its mean RS to adult conspecific song. Conventions are as in C.

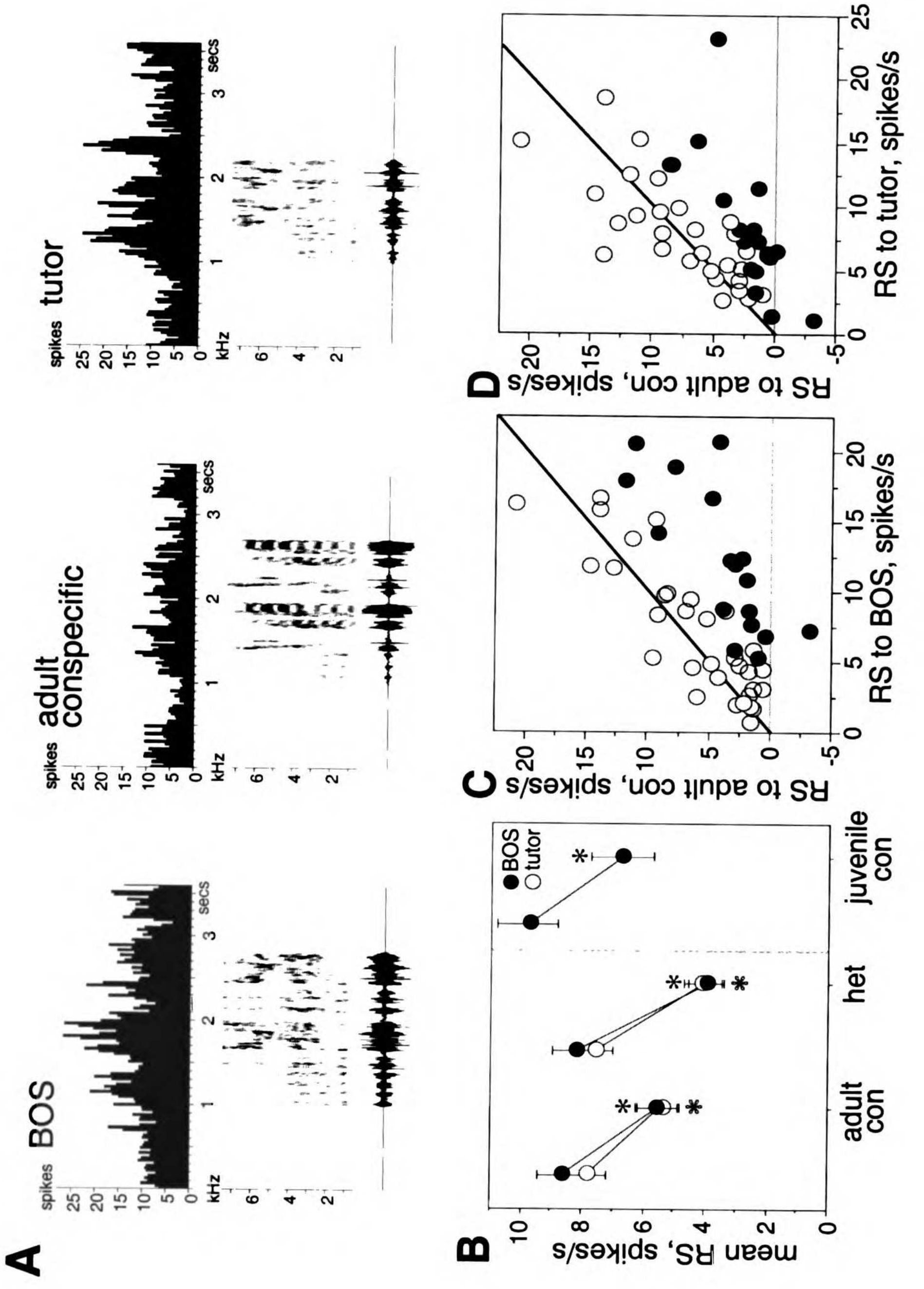

A
BOS
adult conspecific
tutor
spikes
kHz
secs
B
mean RS, spikes/s
BOS
tutor
adult con
het
juvenile con
C
RS to adult con, spikes/s
RS to BOS, spikes/s
D
RS to tutor, spikes/s

Figure 2-6

Figure 2-7 Order selectivity of X at 60 days. A) Peristimulus time histograms of a single X neuron's responses to three different stimuli are shown. The BOS response was greatly reduced by completely reversing the song stimulus ("BOS rev"). However, its response was not reduced by the reverse order stimulus ("BOS ro"). B) The mean RS of all X neurons to forward, reverse, and reverse order stimuli are shown; paired comparisons show significantly greater responses to forward than to reverse and reverse order stimuli for both BOS (black) and tutor song (white) comparisons; bars are SEM. Asterisks mark significant differences (black for BOS comparisons, white for tutor comparisons). C) Order selectivity of individual X units is displayed in scatterplots comparing each neuron's RS to BOS to its RS to reverse BOS ("BOS rev"). The diagonal line marks where cells lie if they responded equally to both stimuli; cells with significantly greater responses to forward than to reverse are marked with black circles ($p<0.05$, unpaired t-test between forward stimulus trials and corresponding reverse stimulus trials). D) Each neuron's RS to tutor song is compared to its RS to reverse tutor song ("tutor rev"). Conventions are as in C.

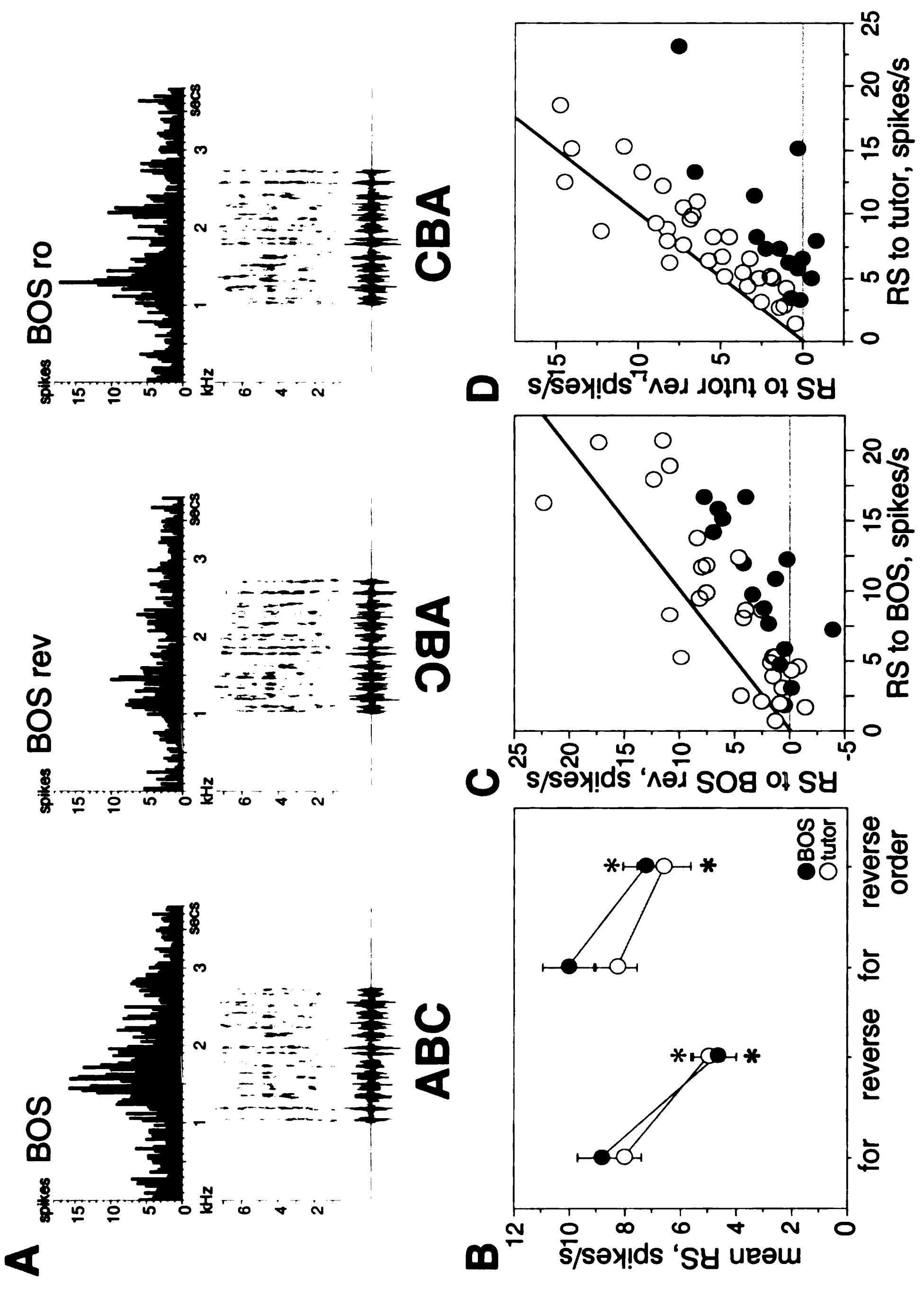

A
B
C
D
BOS
BOS rev
BOS ro
ABC
CBA
spikes
15
10
5
0
kHz
6
4
2
1
2
3
secs
mean RS, spikes/s
12
10
8
6
4
2
0
for
reverse
for
reverse order
BOS
tutor
RS to BOS rev, spikes/s
25
20
15
10
5
0
-5
RS to BOS, spikes/s
RS to tutor rev, spikes/s
RS to tutor, spikes/s
*

Figure 2-7

Figure 2-8 Comparisons of LMAN and X properties. A) Mean spontaneous firing rates for each LMAN and X neuron recorded are shown. B) A histogram compares the mean RS of all LMAN neurons to a 300 ms broad-band noise burst to that of all X neurons. C) Mean d' value (left graph) and mean SI (right graph) for each selectivity category is shown for LMAN (black) and X (white); bars are SEM. Means were calculated from the average SI or d' value of each nucleus from each bird. The dotted line at 0.5 in the left-hand graph marks the criterion value for a selective response. In the right-hand graph, means plotted to the right of the dotted line ("2x") denote average responses to the preferred stimulus that were at least two times greater than average responses to the non-preferred stimulus. The asterisk marks the only significant difference between the nuclei. D) The cumulative distribution of $d'_{BOS-TUTOR}$ values is shown for neurons in LMAN (black) and X (white). E) The mean RS of each nucleus to BOS and tutor song is shown; the left-hand axis corresponds to LMAN values (black circles), the right-hand axis corresponds to X values (white circles). Bars are SEM. The difference in RS between BOS and tutor song was significant for LMAN only (black asterisk).

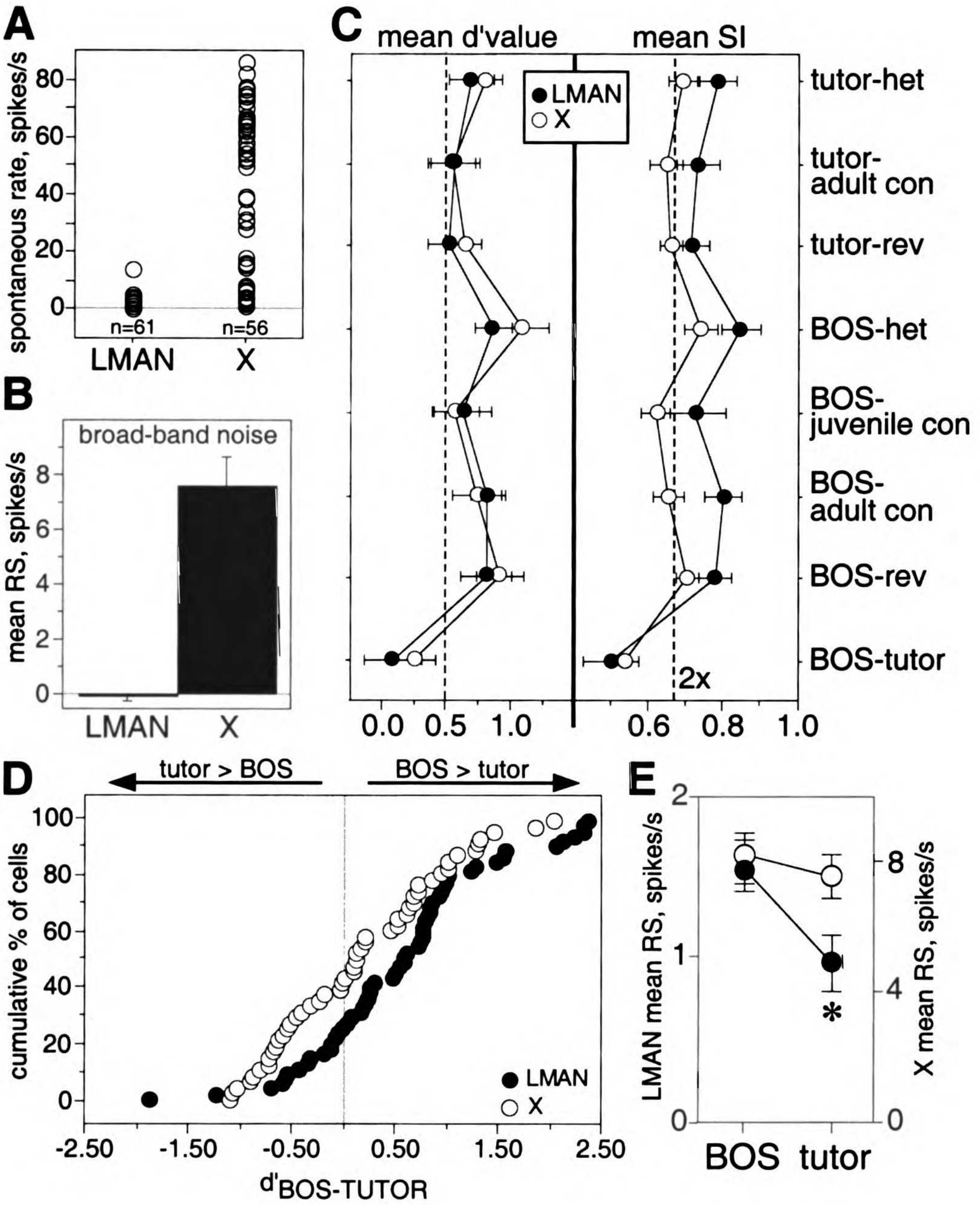

A
spontaneous rate, spikes/s
0
20
40
60
80
n=61
n=56
LMAN
X
B
broad-band noise
mean RS, spikes/s
0
2
4
6
8
LMAN
X
C
mean d'value
mean SI
● LMAN
○ X
tutor-het
tutor-
adult con
tutor-rev
BOS-het
BOS-
juvenile con
BOS-
adult con
BOS-rev
BOS-tutor
2x
0.0
0.5
1.0
0.6
0.8
1.0
D
tutor > BOS
BOS > tutor
cumulative % of cells
0
20
40
60
80
100
● LMAN
○ X
-2.50
-1.50
-0.50
0.50
1.50
2.50
d'BOS-TUTOR
E
LMAN mean RS, spikes/s
0
1
2
X mean RS, spikes/s
0
4
8
*
BOS
tutor

Figure 2-8

Figure 2-9 Clustering of BOS vs. tutor song preferences of LMAN neurons from individual birds and their correlations with measures of maturity. In each graph, the solid line marks the linear least squares fit of the mean data, and the dotted line marks the linear least squares fit of individual cell data (thus weighing each birds' contribution to the correlation by the number of cells recorded in each bird). A) Individual $d'_{\text{BOS-TUTOR}}$ values of LMAN neurons from each bird are plotted against the age of the bird; symbol types refer to neurons from the same bird. B) Each bird's mean $d'_{\text{BOS-TUTOR}}$ value from LMAN neurons is plotted against the bird's final similarity score; bars are SEM. Because individual scores were normalized by each observer's mean score, a value of 0.3 refers to a song with no similarity to the tutor song, and a 1.6 refers to a song with high similarity to the tutor song (see Methods). C) Each bird's mean $d'_{\text{BOS-TUTOR}}$ value from LMAN neurons is plotted against the bird's final stereotypy score; bars are SEM.. For the same reason as in B, a score of 0.3 represents low stereotypy, and a score of 1.6 corresponds to high stereotypy. D) Each bird's similarity score is plotted against its stereotypy score; bars are SEM.

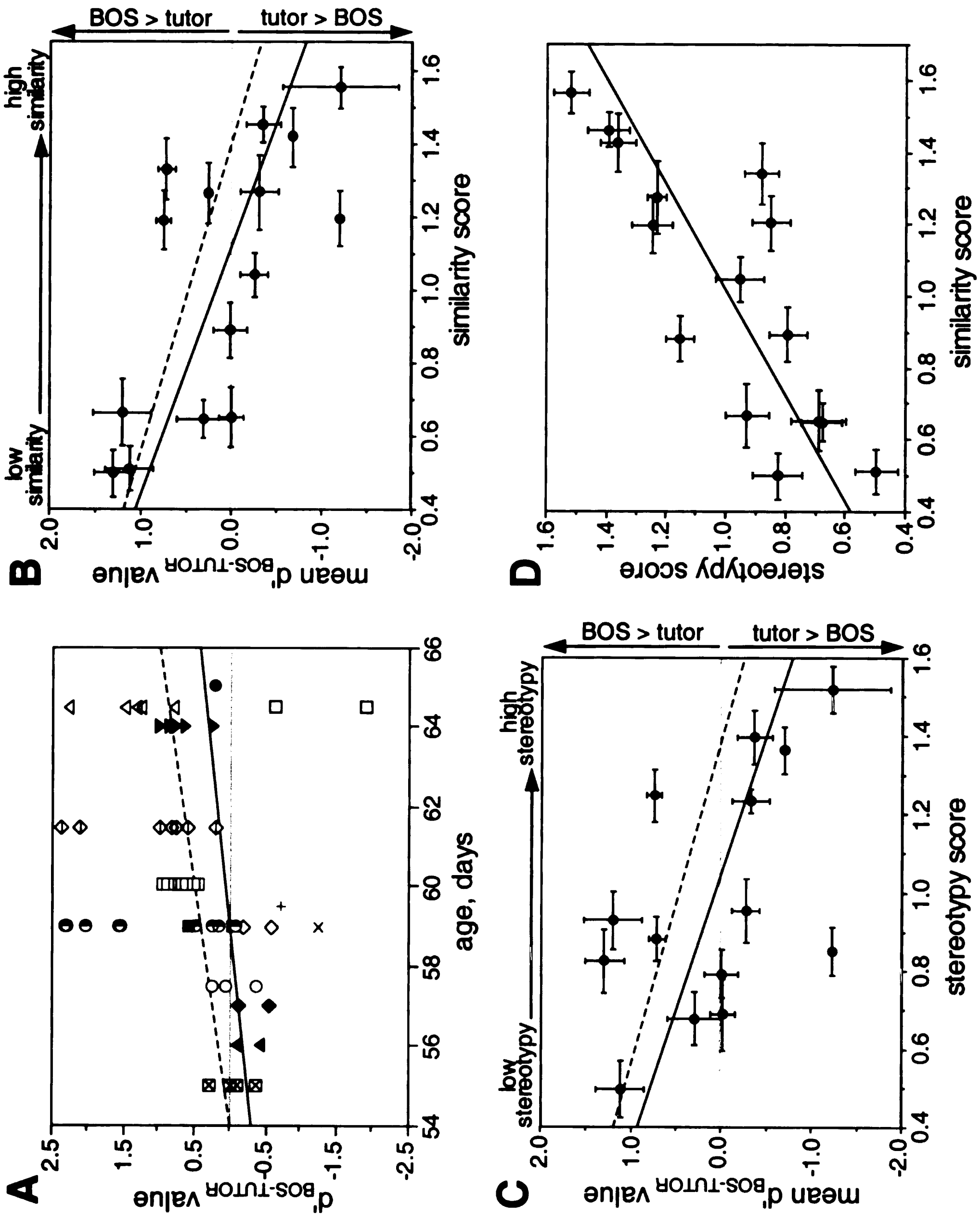

A
d'BOS-TUTOR value
2.5
1.5
0.5
-0.5
-1.5
-2.5
54
56
58
60
62
64
66
age, days
B
mean d'BOS-TUTOR value
2.0
1.0
0.0
-1.0
-2.0
0.4
0.6
0.8
1.0
1.2
1.4
1.6
similarity score
low similarity
high similarity
BOS > tutor
tutor > BOS
C
mean d'BOS-TUTOR value
2.0
1.0
0.0
-1.0
-2.0
0.4
0.6
0.8
1.0
1.2
1.4
1.6
stereotypy score
low stereotypy
high stereotypy
BOS > tutor
tutor > BOS
D
stereotypy score
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.4
0.6
0.8
1.0
1.2
1.4
1.6
similarity score

Figure 2-9

Figure 2-10 A comparison of selectivity at different ages. A) Cumulative distributions of d' values obtained from LMAN neurons at three stages of development are shown. For 30 day neurons, $d'_{\text{TUTOR-ADULT CON}}$ values are shown with open circles. For 60 day neurons, black triangles denote $d'_{\text{TUTOR-ADULT CON}}$ values; open black squares mark $d'_{\text{BOS-ADULT CON}}$ values. For adult neurons, $d'_{\text{BOS-ADULT CON}}$ values are shown as solid black circles. B) Cumulative distributions of d' values of X neurons from three stages of development are shown. Symbols are as in A. C) A comparison of LMAN responses to forward, reverse, and reverse order song at three stages of song development. Open circles mark 30 day neuron responses to order permutations of tutor song. 60 day responses to these variations of tutor song and BOS are marked with open squares and solid triangles, respectively. For adult neurons, black circles denote responses to permutations of BOS. Mean RS to each stimulus is shown; bars are SEM.

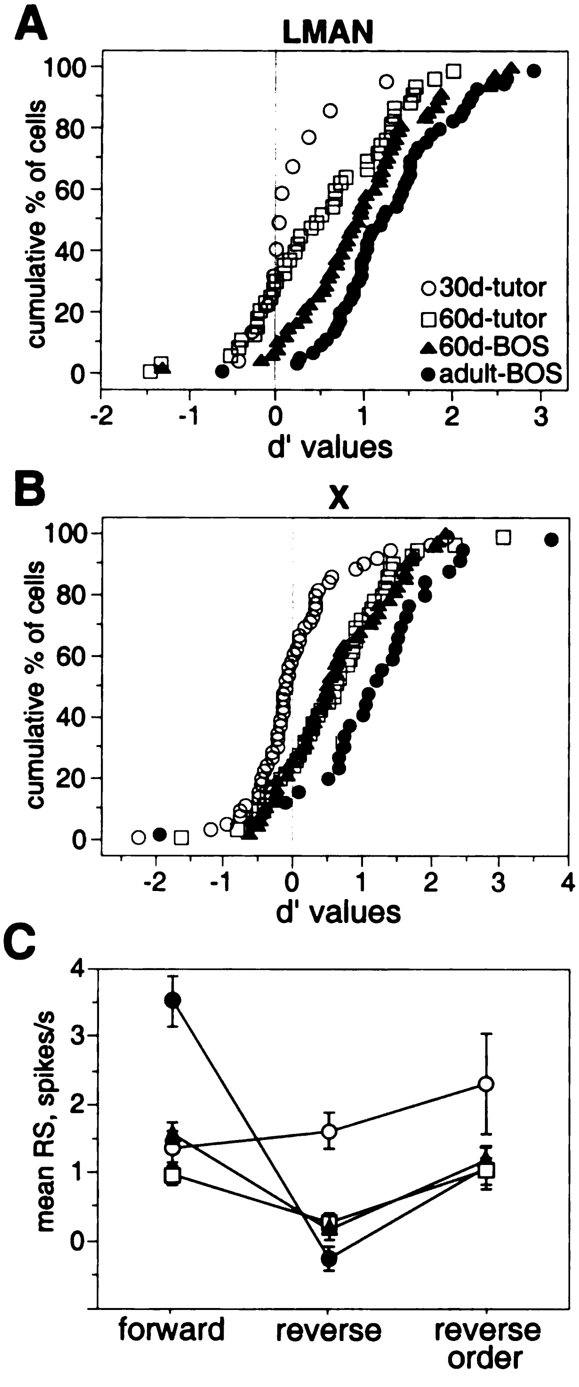

A
LMAN
cumulative % of cells
100
80
60
40
20
0
○30d-tutor
□60d-tutor
▲60d-BOS
●adult-BOS
-2
-1
0
1
2
3
d' values
B
X
cumulative % of cells
100
80
60
40
20
0
-2
-1
0
1
2
3
4
d' values
C
mean RS, spikes/s
4
3
2
1
0
forward
reverse
reverse order

Figure 2-10

Figure 2-11 Comparisons of RS at different ages. A) Histograms of mean RS to tutor, adult conspecific, and reverse tutor song stimuli from LMAN neurons at 30 days (white bars) and at 60 days (grey bars). B) Histograms of mean RS of LMAN neurons compare 60 day (grey bars) to adult (black bars) responses elicited by BOS, adult conspecific song, and BOS reverse. Bars are SEM, and asterisks identify significant differences between the ages ($p<0.05$, unpaired t-tests). C-D) Same as A-B respectively, but for X neurons. 60 day responses (grey bars) to tutor song were significantly greater than 30 day (white bars) responses (unpaired t-test, $p<0.0005$ for tutor; but $p<0.4765$ for tutor reverse and $p<0.1877$ for adult conspecific responses). 60 day responses to non-preferred stimuli were significantly greater than adult (black bars) responses (unpaired t-test, $p<0.0036$ for BOS reverse and $p<0.0030$ for adult conspecific responses; but $p<0.0547$ for responses to BOS).

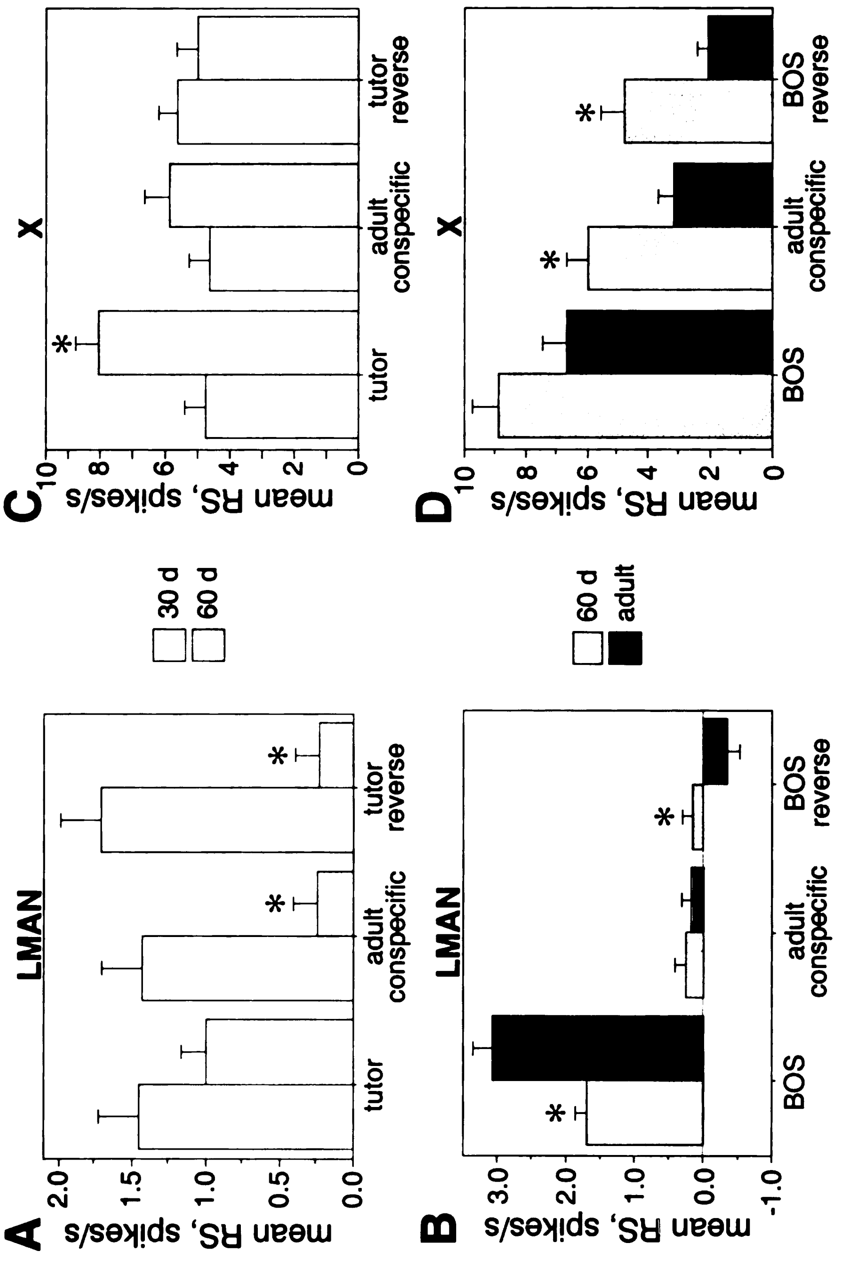
A
LMAN
mean RS, spikes/s
2.0
1.5
1.0
0.5
0.0
tutor
adult conspecific
tutor reverse
*
*
B
LMAN
mean RS, spikes/s
3.0
2.0
1.0
0.0
-1.0
BOS
adult conspecific
BOS reverse
*
*
30 d
60 d
60 d
adult
C
X
mean RS, spikes/s
10
8
6
4
2
0
tutor
adult conspecific
tutor reverse
*
D
X
mean RS, spikes/s
10
8
6
4
2
0
BOS
adult conspecific
BOS reverse
*
*

Figure 2-11

Table 2-1. Comparisons of selectivity in LMAN and X

selectivity category	LMAN	X
BOS > tutor	31/56 (55%)	19/49 (39%)
BOS > adult conspecific *	36/47 (77%)	24/47 (51%)
BOS > juvenile conspecific	17/30 (57%)	17/34 (50%)
BOS > heterospecific *	43/51 (84%)	31/47 (66%)
BOS > reverse BOS	34/47 (72%)	30/41 (73%)
BOS > reverse order BOS	15/37 (41%)	15/32 (47%)
tutor > BOS *	6/56 (11%)	14/49 (29%)
tutor > adult conspecific	20/41 (49%)	26/48 (54%)
tutor > heterospecific	24/42 (57%)	33/51 (65%)
tutor > reverse tutor	16/37 (43%)	25/45 (56%)
tutor > reverse order tutor*	5/21 (24%)	19/37 (51%)

The ratio of selective cells to the total tested in each category is shown for LMAN and X, followed by the percentages. Cells were considered selective if the d' value for the two stimuli compared was ≥ 0.5. Asterisks mark those comparisons for which the frequency of occurrence of selective cells was significantly different between LMAN and X neurons (chi-square tests; $\chi^2=8.84$ for BOS>adult conspecific, $p<0.0030$; $\chi^2=4.457$ for BOS>heterospecific, $p<0.0349$; $\chi^2=5.404$ for tutor>BOS, $p<0.0202$; $\chi^2=4.189$ for tutor>tutor reverse order, $p<0.0408$).

Chapter 3:

Contributions of Tutor and Bird's Own Song Experience to Neural Selectivity in the Songbird Anterior Forebrain

ABSTRACT

Auditory neurons of the anterior forebrain (AF) of zebra finches become selective for song during song learning. In adults, these neurons respond more to the bird's own song (BOS) than to the songs of other zebra finches (conspecifics) or BOS played in reverse. In contrast, AF neurons from young birds (30 d) respond equally well to all song stimuli. AF selectivity develops rapidly during song learning, appearing in 60 d old birds. At this age, many neurons also respond equally well to BOS and tutor song. These similar neural responses to BOS and tutor song might reflect contributions from both song experiences to selectivity, since auditory experience of both BOS and tutor song are essential for normal song learning. Alternatively, they may simply result from acoustic similarities between BOS and tutor song. Understanding which experience shapes selectivity could elucidate the function of song selective AF neurons.

To minimize acoustic similarity between BOS and tutor song, we induced juvenile birds to produce abnormal song by denervating the syrinx, the avian vocal organ, before song onset. We recorded single neurons extracellularly in the AF at 60 d, after birds had had substantial experience of both the abnormal BOS (tsBOS) and of tutor song. Some neurons preferred the unique tsBOS over the tutor song, clearly indicating a role for BOS experience in shaping neural selectivity. In addition, a sizable proportion of neurons responded equally well to tsBOS and tutor song, despite their acoustic dissimilarity. These neurons were not simply immature, because they were selective for tsBOS and tutor song relative to conspecific and reverse song. Furthermore, their similar responses to tsBOS and tutor song could not be attributed to residual acoustic similarities between the two stimuli, as measured by several song analyses. The neural sensitivity to two very different songs suggests that single AF neurons may be shaped by both BOS and tutor song experience.

INTRODUCTION

Songbirds, much like humans, depend on auditory experience during early life to learn their vocal behavior. This learning occurs in two stages, called the sensory and sensorimotor phases (Figure 3-1A). During the sensory phase, a young bird listens to and memorizes the song of its tutor; this memory is called the "template." The sensorimotor phase begins with the onset of singing: using auditory feedback, the juvenile compares its immature vocalizations to the tutor song template, and gradually modifies the plastic song until it produces a mature "crystallized song," which is highly stereotyped and resembles the tutor song. Thus, experience of both the tutor song and of the bird's own song (BOS) is required for normal song learning (Konishi, 1965; Price, 1979).

Likely candidates for circuits involved in processing BOS and tutor song experience during learning lie within the song system, a group of nuclei dedicated to song learning and production (Figure 3-1B). The motor pathway, which is necessary for normal song production throughout life, includes HVc, the robust nucleus of the archistriatum (RA), and the tracheosyringeal portion of the hypoglossal nucleus (nXIIts). The nXIIts contains the motor neurons innervating the muscles of the syrinx, the avian vocal organ. RA also projects to a group of nuclei associated with respiration, such as nucleus retroambigualis (RAm) and nucleus paraambigualis (PAm) (Wild, 1993; Reinke and Wild, 1998; Wild, 1997); these participate in vocalization by controlling the respiratory musculature involved in airflow through the syrinx. In contrast to the motor pathway, nuclei of the anterior forebrain (AF) pathway are not required for singing in adulthood, but play a critical, unknown role during song learning (Bottjer et al., 1984; Sohrabji et al., 1990; Scharff and Nottebohm, 1991; Basham et al, 1996). The AF pathway comprises Area X (X), the medial nucleus of the dorsolateral thalamus (DLM), and the lateral magnocellular nucleus of the anterior neostriatum (LMAN), and indirectly connects HVc to RA. Thus, the AF might process auditory information essential for learning, and use it to modulate motor pathway activity.

Consistent with an auditory role for the AF during learning, AF neurons in adult, anesthetized birds are auditory and respond selectively to the bird's own song (BOS) (Doupe and Konishi, 1991). Neurons selective for BOS prefer it to the songs of other zebra finches (conspecific song) and to BOS played in reverse. These song selective neurons resemble those found in HVc (Margoliash, 1983), as well as neurons tuned to species-specific vocalizations found in bats (Suga et al., 1978; Esser et al., 1997), rhesus monkeys (Rauschecker et al., 1995), and marmosets (Wang et al., 1995). AF neurons from young juvenile birds lack selectivity, however, responding equally well to all song stimuli at 30 d of age (Figure 3-1C). Song selectivity develops rapidly, since it is found in 60 d old juveniles (Solis and Doupe, 1997).

Determining the experience responsible for AF neuron selectivity could elucidate AF function during song learning. For example, neurons tuned by BOS experience could provide feedback about the current state of BOS, whereas those tuned by tutor song experience could store tutor song information. When neural responses to BOS and tutor song are compared at 60 d, a range of preferences for one song over another is evident (Figure 3-1D, adapted from Solis and Doupe, 1997). Many neurons prefer BOS over tutor song, suggesting a role for BOS experience in shaping selectivity. A few neurons prefer tutor song over BOS, suggesting that they were tuned by tutor song experience. Finally, many neurons respond equally well to both BOS and tutor song. These neurons are clearly selective, because they do not simply respond to any song stimulus. Such neurons could have been shaped by both BOS and tutor song experience. Alternatively, these neurons might indicate acoustic similarities between the two songs; by 60 d some juveniles' plastic songs clearly resemble their tutor song.

If neurons with similar responses to BOS and tutor song result from acoustic similarities between the two songs, then it is unclear which song experience is responsible for neural selectivity. Inducing a juvenile bird to produce an abnormal song could resolve this issue, because it would reduce similarity between BOS and tutor song (Figure 3-2A).

If neurons with equivalent responses to BOS and tutor song result from the similarities between these two songs, then such neurons should not exist in birds with songs very different from their tutor song (solid line, Figure 3-2B). Alternatively, if such neurons reflect the contributions of both song experiences, then neurons with similar responses to the abnormal song and tutor song should persist (dashed line, Figure 3-2B).

Birds producing abnormal songs could also clarify the experience responsible for neurons that prefer BOS over tutor song in normal 60 d birds. The simplest interpretation is that these neurons are shaped by BOS experience. If, however, a bird has poorly copied the tutor song during the sensory phase, then these neurons might instead represent the template. This possibility is schematized in Figure 3-2C: if a bird stores a poor copy of the tutor (A) as its template (a) and models its own song accurately after the template (a), then BOS itself is a better representation of the template than the tutor song. This issue could be resolved with birds induced to produce very abnormal songs: if neurons preferring BOS over tutor song persist in such birds, then it is likely that they result from experience of the song unique to that bird.

In this study, we minimized the similarity between the songs of juvenile birds and their tutors by transecting the tracheosyringeal portion of the hypoglossal nerve (NXIIts, or ts), which innervates the syringeal muscles, before song onset. Extracellular recordings of single LMAN and X neurons in these birds at 60 d showed that, although the BOS and tutor song were now acoustically very different, many neurons still responded equally well to both stimuli. This result is similar to that found in normal 60 d birds, and suggests a role for both song experiences in shaping AF selectivity.

MATERIALS AND METHODS

Experiments used male juvenile zebra finches (*Taeniopygia guttata*). The care and treatment of experimental animals was reviewed and approved by an university animal care and use committee at UCSF. Birds were raised in individual cages, with their parents and siblings from the same clutch. Opaque dividers between cages visually isolated birds from other conspecifics in the colony. Because juvenile birds shared a cage with a single adult male tutor and were visually isolated from other conspecifics within earshot, their learning should have been restricted to the tutor in their cage (Immelmann, 1969; Eales, 1987, 1989; Williams, 1990).

Surgery. When birds were 26-33 days old (mean±SD was 28±2days), the tracheosyringeal portion of the hypoglossal nerve (NXIIts) was transected bilaterally under isofluorane anesthesia (0.5-1.5%v/v; Abbott Laboratories, North Chicago, IL). The nerves were exposed by an incision along the skin of the neck, where lidocaine had been injected subcutaneously (2% solution, Elkins-Sinn, Cherry Hill, NJ). The NXIIts nerve was dissected away from the trachea at the proximal end of the incision and cut; dissection then continued along the length of the neck, and the nerve was pulled to remove the distal end. This removed ~1cm of nerve. Following bilateral transections, the skin was closed with skin adhesive (Krazyglue, Borden, Columbus, OH). The ts cut birds were returned to their home cages, until they were 60 d old.

Two days prior to the experiment, we prepared birds for recording by affixing a head post to the skull, and marking the location of the song nuclei on the skull (see Solis and Doupe, 1997 for details). On the day of the experiment, the bird was anesthetized with a 20% solution of urethane (5mL/kg, i.m.; Sigma, St. Louis, MO; delivered in 3 injections at 30 min intervals), placed in the stereotaxic apparatus, and immobilized via its head post. Body temperature was regulated with a temperature controller (FHC, Brunswick, ME). A craniotomy was performed above LMAN and X, the dura was opened, and the electrode was lowered into the brain with a microdrive (Fine Science Tools, Foster City, CA).

Stimuli. One to two days prior to the experiment, the songs of the ts cut bird and its tutor were recorded. Each bird was placed in a sound-attenuated chamber (Acoustic Systems, Austin, TX) connected to an automatically triggered audio system. Approximately 90 minutes of bird sounds were recorded and then scanned for song. A typical plastic song rendition was usually chosen after listening to at least 25 songs and looking at several song spectrograms; a typical song was considered to be the song most frequently sung. A typical tutor song was chosen after listening to 10 songs. Songs were digitized at 32 kHz and stored on a SPARC IPX computer (Sun Microsystems, Palo Alto, CA) at similar peak intensity levels (range was 64-73dB; software by Michael Lewicki and Larry Proctor, California Institute of Technology). In 15 experiments, 3 different plastic song renditions from a bird were stored for presentation during the experiment. The durations of tsBOS and tutor songs ranged from 602 ms to 2461 ms.

During electrophysiological recording, acoustic stimuli were presented by a speaker 25 cm away from the bird, inside a double-walled anechoic sound-attenuated chamber (Acoustic Systems, Austin, TX). The frequency response measured at the bird's location inside the chamber was flat (±5.0 dB) between 500Hz and 8kHz. The stimuli included songs of the ts cut juvenile (tsBOS), its tutor song, reverse manipulations of tsBOS and tutor song, the songs of other zebra finches (conspecific), the acoustically similar songs of other species of estrildid finches (heterospecific), broad band noise bursts and tone bursts. Stimuli were presented in a random, interleaved fashion. An effort was made to present each neuron with 15-20 trials of each stimulus type: tsBOS, reverse tsBOS, reverse order tsBOS, tutor, reverse tutor, reverse order tutor, at least 2 adult conspecific songs, at least 2 heterospecific songs, at least 2 juvenile conspecific songs, at least 2 ts cut juvenile conspecific songs, broad-band noise bursts and tone bursts; however, some neurons were lost before characterization was completed.

Electrophysiology. Extracellular neuronal signals were amplified and filtered between 300Hz and 10kHz (A-M Systems, Everett, WA). To locate auditory neurons, search stimuli included tsBOS, tutor song, adult conspecific song, heterospecific song, broad band noise bursts and tone bursts. Most neurons were isolated with a window discriminator (UCSF Physiology Shop). Twelve units were isolated offline, using spike sorting software (Lewicki, 1994). To do this, waveforms were recorded during stimulus presentation during the experiment. Later, spike models were constructed from waveforms recorded at an intermediate time during stimulus presentation. These spike models were then used to classify spikes within the rest of the waveforms. Both spike model construction and template matching algorithms were based on Bayesian probability theory. Neuronal responses were collected and analyzed by a SPARC IPX computer using software developed by Mike Lewicki and Larry Proctor (California Institute of Technology), and Frédéric Theunissen (UCSF). Electrolytic lesions were made at selected locations for reconstructing recording sites.

Anatomy. At the end of an experiment, the bird was deeply anesthetized with Metofane (Pitman-Moore, Mundelein, IL) and transcardially perfused with 0.9% saline, followed by 3.7% formalin in 0.025M phosphate buffer. Brains were postfixed and cut in 40μm sections with a freezing microtome. Sections were stained with cresyl violet, and electrode tracks and lesions were identified. Only neurons histologically confirmed to be in LMAN or X were used; their specific location within each nucleus was also documented.

RA volumes were measured for each ts cut bird and for normal 60 d birds, recorded in a previous study. Measurements were made blind to the experimental condition. The Nissl-defined boundaries of RA were traced at 80μm intervals and the resulting area calculated using an image analysis program (Image, NIH). The total area was multiplied by section thickness and then by the total number of sections to give a final volume. Because of individual differences in post-fixation time, each RA volume was normalized by the volume of the nucleus pretectalis (PT), which is unrelated to the song system. Final RA/PT ratios were compared between ts cut and normal birds. When measurements from both hemispheres were available, the mean RA volume and mean PT volume was used. For nine ts cut birds, PT volume was not available. Thus, RA volumes alone were also compared within all ts cut birds, for which post-fixation times were equivalent.

The syrinx of each ts cut bird was also dissected after perfusion. Each syrinx was cut 1 mm distal and 4 mm proximal of the bifurcation of the bronchi, and then weighed to assess relative muscle mass, a marker of denervation success.

Data analysis. We quantified responses to an acoustic stimulus during the period of stimulus presentation, offset by an estimate of the latency. The latency of each neuron was measured by examining its responses to a broad band or tone burst stimulus with a peristimulus time histogram (PSTH) divided into 5 or10 ms bins. The latency was defined as the onset of the first of two consecutive bins during the stimulus which had at least twice as many spikes as the mean number of spikes per bin during the background. LMAN neurons often did not respond to broad band noise or tone bursts. For these cases, the latency of another neuron from the same bird was used; if there was none, then the neuron was assigned a latency characteristic of neurons from normal 60 d birds (65 ms; from Solis and Doupe, 1997).

To be considered auditory and included for analysis, a neuron had to have an average firing rate during one of the stimuli that was significantly different from the background rate (two-tailed paired t-test, $p<0.05$). The firing rate during a stimulus was obtained by normalizing the number of spikes elicited during the stimulus by the duration of the stimulus. The background rate was calculated by averaging the firing rate of the neuron from two different time periods: two seconds preceding stimulus onset and 2-3 seconds beginning 1 sec after the end of the stimulus. The response strength (RS) of a neuron to a stimulus was the difference between the firing rate during the stimulus (offset by the latency) and the background rate. The RS was measured for each stimulus trial, and then averaged across trials to get the neuron's RS to that stimulus, expressed in spikes/s. Data for different stimuli, but of the same stimulus type, were also averaged in this way to get an RS for a stimulus type (e.g., to obtain the RS for adult conspecific song, the RS values for each trial of two different adult conspecific song stimuli were averaged together).

The selectivity of an individual neuron for one stimulus (A) over another (B) was quantified using the $d'_{A\text{-}B}$ measure (Green and Swets, 1966), where $d'_{A-B}=\frac{2(\overline{RS_A}-\overline{RS_B})}{\sqrt{\sigma_A{}^2+\sigma_B{}^2}}$. In this equation, $\overline{RS_A}$ and $\overline{RS_B}$ are the mean RS to stimulus A and B, respectively, and σ^2 is the variance of each RS. If $d'_{A\text{-}B}$ is positive, then stimulus A elicited a greater response; if it is negative, then stimulus B elicited a greater response. Values of $d'_{A\text{-}B}$ close to 0 indicate no difference in the RS elicited by the two stimuli. A particular d′ value was calculated only for neurons that had a significant response to at least one of the two stimuli compared. A neuron was considered selective for stimulus A over stimulus B if it had a $d'_{A\text{-}B}$ value ≥ 0.5. This criterion was based on the observation that neurons with $d'_{A\text{-}B}$ value ≥ 0.5 usually had a RS to stimulus A that was at least twice as great as that to stimulus B (Solis and Doupe, 1997). Also, a $d'_{A\text{-}B}$ value of 0.5 corresponds to a significantly greater response to stimulus A than to stimulus B, based on a paired t-test with 20 presentations of each stimulus ($p=0.031$).

To convey the magnitude of the difference between the RS elicited by two different stimuli, the selectivity index (SI) was also calculated (Volman, 1996; Doupe, 1997). The SI compared the mean RS to each stimulus in ratio form: $\frac{\overline{RS_A}}{(\overline{RS_A}+\overline{RS_B})}$.

When comparing RS to two stimuli with large differences in song duration, normalizing spike counts elicited by the two stimuli by stimulus duration may bias comparisons of the RS. For example, if two stimuli, one short and one long, elicit a similar response in which the neuron initially fires strongly and then fatigues, then normalizing by song duration will give a substantially decreased RS for the long stimulus relative to the shorter stimulus; this in turn will result in a d′ value that prefers short stimuli over long stimuli. Because large differences in song duration occurred in several experiments, a peak RS was also calculated to remove bias due to varying song durations in the comparisons of a neural response. First, a maximum firing rate during the stimulus was found using a 500 ms sliding window, which moved across a response in 1 ms increments. Second, the maximum background rate was also found using a 500 ms window. Third, the peak RS was calculated by taking the difference between the maximum firing rate during the stimulus and the maximum background rate; this peak measurement removes duration bias because it normalizes every spike count by 500 ms, regardless of the stimulus duration. Finally, peak d′ values were also calculated using the peak RS obtained from the 500 ms window. A 500 ms window was chosen for two reasons. First, it was shorter than the shortest song stimulus (602 ms). Second, for a subset of neurons (5 from LMAN and 5 from X), a series of sliding windows (10-2000ms) were used to calculate the peak RS and resulting peak d′ values. Among those windows shorter than 600 ms, the 500 ms window gave the largest peak d′ values between two stimuli of similar durations. For some cells, windows larger than 500 ms resulted in d′ values higher than those for short windows (see Chapter 5); this indicates that peak d′ measures can underestimate the selectivity of a cell.

Cluster analysis. We tested whether the $d'_{\text{tsBOS-tutor}}$ values of neurons recorded from each bird were more similar than expected by chance. To do this, the variance of the $d'_{\text{tsBOS-tutor}}$ values obtained experimentally from each bird was compared to a simulated distribution of variances created from the data from all birds. This distribution was determined from 1000 Monte Carlo simulations: each simulation randomly selected *n*

$d'_{\text{tsBOS-tutor}}$ values from the pool of all experimental $d'_{\text{tsBOS-tutor}}$ values (includes all cells from all birds), and calculated their variance (*n* equals the number of cells recorded in each bird). The median of the resulting distribution of simulated variances was compared to each bird's experimental variance. If the experimental variance was significantly less than the median of the simulated distribution (one sample sign test, $p<0.05$), the $d'_{\text{tsBOS-tutor}}$ values from that bird were considered clustered. A sign test determined whether the frequency of clustering in the group of birds studied was greater than expected by chance. This procedure was completed for $d'_{\text{tsBOS-tutor}}$ values from LMAN neurons alone, X neurons alone, and both neuron types together.

Song analysis: similarity. Once electrophysiology experiments were completed, we analyzed the tsBOS and tutor songs themselves using several methods. Song is composed of syllables, which are continuous acoustical signals, 10-200 ms in duration. Syllables are separated from other syllables by a sudden fall in amplitude to near zero or by brief silent intervals. Syllables are composed of smaller continuous signals called "notes." A repeated sequence of syllables is a "motif." A song "bout" consists of introductory notes followed by one or more motifs (for detailed song descriptions, see Price, 1979; Sossinka and Bohner, 1980).

The first song analysis was a matching task, completed by 9 human observers familiar with zebra finch song, but blind to the neural properties of each bird. Observers tried to match each experimental song with that of its tutor, which was present among a group of six potential tutors. The observers listened to and looked at sonograms and oscillograms of the songs before selecting the tutor song that best matched the experimental song. Thus, the percentage of observers that correctly matched the experimental song to its tutor song indicated the overall similarity between tsBOS and tutor song; this measure was called the "percent correctly matched". After selecting a "best match" tutor song, observers scored the song pair on spectral similarity and on temporal similarity, using a scale from 1-

5. For spectral similarity, observers only considered syllable morphology and sequence. A score of 1 referred to a song pair for which no elements in the experimental song resembled anything in the "best match" tutor song; 2 was given to a song pair when some notes in the experimental song resembled notes present in the "best match" song; 3 designated a song pair in which one or more syllables of the experimental song resembled distinctive syllables of the "best match" song; 4 referred to a song pair for which several experimental song syllables resembled those of the "best match" song, and the syllable sequences were somewhat similar; 5 was given to a song pair when the experimental song resembled the "best match" song in both syllable morphology and sequence, making it a good copy of the "best match" song.

To judge temporal similarity, observers disregarded the spectral features of song, and considered only the durations of syllables and intervals, and their patterns, or rhythm, within the songs. Each song pair was scored on a scale of 1-5. A score of 1 referred to a song pair for which a timing similarity between the experimental song and the "best match" song could not be detected; 2 indicated a song pair for which the relative durations of at least 2 syllables and the interval between them in the experimental song resembled timing in the "best match" song (e.g., doublets or triplets were heard in both songs); 3 was given to a song pair when combinations of doublets or triplets in the experimental song resembled the timing structures of the "best match" song; 4 was given to a song pair when many syllables and intervals of the experimental song had relatively similar duration and patterning as those in the "best match" song; 5 indicated a song pair for which the timing of the experimental song was highly similar to that of the "best match" song, although differences in speed may have been apparent.

Songs of non-ts cut birds were also included among the experimental songs for analysis, and their respective tutor songs were also present among the possible tutor choices; this provided references against which ts cut song similarity scores could be compared. Normal 60 d song (n=16), normal adult song (n=9), and randomly matched song (songs for which the correct tutor was not present among the possible tutor choices, n=6) were also matched to a tutor song, and scored for spectral and temporal similarity. Scores given to normal adult songs provided an upper bound of similarity between songs from normal adults and their tutors, whereas scores given to randomly matched songs provided a lower bound of similarity. Randomly matched songs included those from 2 normal adult, 2 normal 60 d, and 2 ts cut 60 d birds.

To control for slight scoring differences between observers, we normalized each observer's score for a song by the observer's mean score for all songs. Thus, if an observer scored the spectral similarity of a song pair as a 5, but the observer's mean score was a 3, then the score for this particular song pair was 5/3=1.7. The normalized scores for birds ranged from 0.30 to 2.43. The final score for each song was the average of each observer's normalized score. This final score included scores given to incorrect experimental-tutor song matches. Scores calculated with incorrect matches excluded were not significantly different (paired t-test, $p<0.05$); this indicates that incorrectly chosen tutor songs were as dissimilar from the experimental song as the tutor song itself. The mean score for song type (i.e., ts cut, normal 60 d, adult control and randomly matched) was calculated from the final scores for each song belonging to the song type.

The similarity between each experimental song (ts cut, normal 60 d, normal adult, and randomly matched songs) and its tutor song was also measured with a cross-correlation algorithm (Theunissen and Doupe, 1998). One song waveform was moved relative to another in 1 ms increments, and an r^2 value calculated for each time delay. The maximum was used as the "cross-correlation measure." Unlike the spectral and temporal similarity scoring in the matching task, cross-correlations were done between an experimental song and the correct tutor song (except for randomly matched songs; these were cross-correlated to the tutor song most often chosen by observers in the matching test).

To measure overall similarity, the entire spectrogram of an experimental song was cross-correlated to the entire spectrogram of the tutor song. To measure spectral similarity, the "syllables only" cross-correlation measure was calculated for each song pair. For this, each isolated syllable of the experimental song was compared to each isolated syllable of the tutor song. The cross-correlation measure was calculated for each comparison, and the maximum taken as the best match for the syllable. The resulting maxima were then averaged to produce the "syllables only" cross-correlation measure. To measure temporal similarity, each song waveform was rectified and lowpass filtered at 62.5Hz. The filtered versions of experimental song and tutor song were then cross-correlated to give the "temporal envelope" cross-correlation measure.

To further compare temporal features of song, overlap values were calculated between these song pairs (program by Michael Brainard, UCSF). For this, the syllables of each song were replaced with square pulses of equal amplitude. The resulting square pulse strings preserved syllable and interval durations, and their patterns found in the original songs. The square pulse string of an entire experimental song was then compared to that of the entire tutor song by calculating the percent overlap between syllables and intervals. The proportion of overlap between experimental syllables and tutor song syllables was calculated separately from the proportion of overlap between experimental intervals and tutor song intervals. The mean of the syllable and interval overlap values was the "song-song overlap" value.

In addition, a "motif-song overlap" value was calculated, which maximized the chance of overlap. The song-song overlap measure described above could miss timing similarities between motifs of two songs, if there were different intervals between multiple motifs within a song. To avoid this, the motif-song overlap value compared a string based on a single motif of the experimental song to a string based on the entire tutor song. In addition, the song-song overlap measure could miss timing similarities if there were differences in song speed; thus, the motif-song overlap calculations allowed the motif string to stretch proportionately 80-120% of its original length, in 2% increments. The percent overlap between each stretched version of the motif string and the tutor song string was calculated, and the maximum was taken as the "maximum overlap" value. Finally, overlap values are sensitive to the complexity of the motif string of the experimental bird. For example, a simple motif comprising only 2 syllables is likely to give a high maximum overlap value for both the tutor song and a random song. To correct for this, the maximum overlap value was normalized by how well the motif overlapped with random songs. To obtain a measure for random overlap, the maximum overlap value was determined between the motif string and 20 randomly chosen, normal adult song strings. The mean of the 20 maximum overlap values gave the "random overlap" value. This random overlap value was used to normalize the maximum overlap value obtained from the comparison of the motif and tutor song strings, such that: $\text{motif - song overlap} = \frac{(\text{maximum overlap} - \text{random overlap})}{(1 - \text{random overlap})}$.

Song analysis: stereotypy. We measured song stereotypy of each bird in three ways: human subjective scoring, syllables only cross-correlations, and motif-song overlap analysis. For reference, songs of normal adult and normal 60 d birds were included in the stereotypy test. For each bird, 10 song bouts were randomly selected for analysis (except in 4 cases: 2 normal 60 d birds had 2 songs each, 1 normal 60 d bird had 5 songs, and one ts cut 60 d bird had only 3 songs).

Three observers rated how consistently a particular motif was present in each song sample from a single bird on a scale from 1 to 5. They listened to each song sample and looked at their accompanying sonograms and oscillograms before deciding on the score. Both spectral and temporal pattern repeats contributed to the score. A score of 1 referred to a group of songs that were not at all stereotyped: short syllable sequences and small temporal patterns were rarely, if at all, repeated in the song samples. A score of 2 indicated that a particular syllable sequence or brief temporal pattern was repeated in half or fewer of the song samples. A 3 was given when a short syllable sequence or temporal pattern was repeated in almost all or all song samples. Alternatively, a 3 was given if an entire motif structure was repeated in only half of the song samples. The syllables outside of the repeated structures could vary in identity and ordering. A score of 4 was given when an entire motif structure was apparent in most or all of the song samples; however, some variability remained, with syllables added or dropped from the motif in different renditions. A score of 5 was given when identical motifs were found in every song sample. Each score was normalized by the observer's mean score, as described for the similarity scoring in the matching task. Normalized stereotypy scores ranged from 0.26 to 1.40.

To isolate spectral stereotypy, we employed cross-correlations to measure how consistently the syllables in one song were present in the other song samples. Spectral stereotypy was calculated in the same manner as the "syllables only" cross-correlation measure of similarity described above, except that the cross-correlations were done between syllables from songs of the same bird. The mean of the resulting "syllables only" cross-correlation measures (usually 9 coefficients) gave the spectral stereotypy measure for the bird.

Motif-song overlap analysis was used to measure temporal stereotypy. To measure how consistently temporal patterns were repeated in song samples from a bird, a motif-song overlap value was obtained for an experimental motif string and each song sample string (usually 9 sample strings), as described above. These were also normalized by a random overlap value, which was obtained by calculating the maximum overlap between the motif string and 9 randomly chosen songs from all the experimental groups (adult control, 60 d control, 60 d ts cut). The normalized motif-song overlap values for each comparison between songs from the same bird were then averaged to give an overlap stereotypy measure.

RESULTS

Songs of ts cut birds at 60 d

Bilateral NXIIts (ts) transections do not disturb the respiratory outputs involved in song production; thus, birds receiving ts cuts around 30 d of age readily sang, but because they could not control their syringeal musculature, they produced extremely abnormal songs by 60 d. These birds sang a series of simple syllables consisting of harmonically related notes. These "harmonic stack" syllables had little amplitude modulation (Figure 3-3A), and the frequencies of the stacks often fluctuated, giving the song a wavery quality. The song of this ts cut bird (tsBOS) was very different from its tutor song (Figure 3-3B). Although the syllables of the tsBOS shown were longer than normal, the average syllable and interval durations in ts cut song were not significantly different from those of normal adult or 60 d song ($p>0.635$ for all comparisons, unpaired t-tests). The song of a normal 60 d sibling of the bird in Figure 3-3A is shown for comparison (Figure 3-3C). Although this normal 60 d song had immature features such as noisier syllables and a longer song duration than the tutor, it had clear similarity in syllable morphology and timing to the tutor song. Thus, the ts cut manipulation produced songs that were considerably simpler than normal plastic song, and dramatically reduced the similarity between BOS and tutor song that can occur by 60 d.

We quantified the decrease in similarity between tsBOS and tutor songs using multiple methods of song analysis. In a matching task, observers tried to match an experimental song (ts cut 60 d, normal 60 d, normal adult, or random) with that of its tutor, which was present in a group of possible tutors (see Materials and Methods). Songs from ts cut birds were correctly matched to their tutor song significantly less frequently than were songs from normal 60 day birds (Figure 3-3D) (unpaired t-test, $p<0.002$). Because NXIIts transections in adult birds are known to preserve the overall timing of song while eliminating normal spectral features (Simpson and Vicario, 1990; Williams and McKibben, 1992), observers also scored separately the spectral and the temporal similarity between each experimental song and the chosen tutor song (see Materials and Methods). The resulting mean spectral similarity score and the mean temporal similarity score for ts cut songs were significantly lower than those for normal 60 day songs (Figure 3-3D) (unpaired t-test, $p<0.0001$ for spectral similarity and $p<0.001$ for temporal similarity). For reference, the mean spectral and temporal similarity scores for randomly matched songs (songs whose actual tutor song was not present among the choices; for details, see Materials and Methods) and for normal adult songs are also shown. Note that ts cut songs had significantly lower spectral similarity scores than did the randomly matched songs (unpaired t-test $p<0.002$).

Comparison of LMAN neural responses to tsBOS and tutor song

Extracellular recordings of 52 LMAN neurons from 16 ts cut birds revealed selectivity for tsBOS. Figure 3-4A shows a neuron which responded substantially more to tsBOS than to tutor song, adult conspecific song, and reverse tsBOS (a "mirror-image" reversed song in which both entire syllables and syllable sequence are reversed). Thus, this neuron was sensitive to the spectral and temporal properties of tsBOS, despite its simple structure. Many other neurons showed a strong preference for tsBOS over tutor song. We quantified the preference for tsBOS over tutor song for each neuron with a $d'_{\text{tsBOS-tutor}}$ value (see Materials and Methods); neurons with $d'_{\text{tsBOS-tutor}}$ values ≥ 0.5 were considered to prefer tsBOS over tutor song, and neurons with $d'_{\text{tsBOS-tutor}}$ values ≤ -0.5 were considered to prefer tutor over tsBOS. Classified in this way, 28% of LMAN neurons preferred tsBOS over tutor song and only 5% of neurons preferred tutor over tsBOS (Figure 3-4B). These strong preferences for the abnormal tsBOS over tutor song demonstrate the ability of BOS experience to shape LMAN neuron properties.

Unexpectedly, many LMAN neurons responded equally well to tsBOS and tutor song, despite the large acoustic differences between these two songs. Figure 3-5A shows an example of such a neuron, which came from a ts cut bird whose song was matched to the correct tutor song by only 1/9 observers. This type of neuron represented a substantial proportion of LMAN neurons recorded (Figure 3-4B): 67% of the neurons had $d'_{\text{tsBOS-tutor}}$ values between -0.5 and 0.5, thus classifying them as neurons with equivalent responses to both tsBOS and tutor song. Overall, the mean of $d'_{\text{tsBOS-tutor}}$ values of neurons from ts cut birds was not significantly different from that obtained from normal 60 d birds (Figure 3-4B) (unpaired t-test, $p=0.089$; normal 60 d data from Solis and Doupe, 1997). On average, tsBOS elicited a greater response than tutor song, as was true for LMAN neurons from normal 60 d birds (Figure 3-4B inset; paired t-test, $p<0.004$ for neurons from ts cut birds, $n=46$).

Neurons with equivalent responses to acoustically dissimilar tsBOS and tutor songs might indicate that both song experiences shape the selectivity of single neurons. There are alternative explanations for such neurons, however. First, these neurons might not have exhibited a stronger preference for tsBOS because they were tested with a version of tsBOS that was not optimal for eliciting responses; the variability of plastic song at 60 d makes this possible. Second, it is possible that neurons with similar responses to tsBOS and tutor song are simply immature: younger neurons from 30 d birds respond equally well to all song stimuli (Doupe, 1997). Third, the equivalent responses to tsBOS and tutor song could be due to residual similarities between the two songs. Although song analysis revealed that, on average, tsBOS songs share little similarity with tutor song, it is important to compare each bird's neural properties with the similarity between its tsBOS and its tutor song. The first two alternative explanations are discussed immediately below; the third possibility will be examined in the last section of the results, using detailed song analysis.

Plastic song renditions elicited equivalent neural responses

Because of plastic song variability normally present at 60 d, it seemed possible that neurons without a strong tsBOS preference had been presented with a version of plastic song to which neurons were less responsive. To assess this, LMAN neurons were presented with three different renditions of tsBOS in 8 experiments. Many neurons responded equally well to all three renditions, whereas others responded more to the tsBOS version most frequently produced by the bird. This version was always used as the primary tsBOS, to which all other songs were compared when measuring selectivity. Overall, there was no significant difference in the responses elicited by the three versions of tsBOS (ANOVA, $p=0.954$, $n=21$). Thus, it is unlikely that selectivity measurements were biased by inappropriate tsBOS presentation.

LMAN neurons with equivalent responses to tsBOS and tutor song were not simply immature

Because AF neuron selectivity increases between 30 d and adulthood (Doupe, 1997), selectivity can be used to assay neuronal maturity. Two types of selectivity were analyzed to determine whether neurons were immature. First, neural responses to tsBOS and tutor song were compared with those to adult conspecific songs. Second, neural responses to tsBOS and tutor song were compared with those to reversed versions of these songs; for such reversed stimuli, both the entire syllables and the sequence of syllables within the song were reversed. Immature neurons would respond equally well to all of these stimuli (Figure 3-1C). When we analyzed the selectivity of individual neurons with similar responses to tsBOS and tutor song, however, it was clear that these neurons were not simply immature. For example, although the neuron in Figure 3-5A responded strongly to both tsBOS and tutor song, it did not respond well to adult conspecific or reverse tutor song. Figure 3-5B further illustrates this selectivity by plotting the tsBOS versus tutor song preference of each neuron (indicated by its $d'_{\text{tsBOS-tutor}}$ value) against a measure of selectivity ($d'_{tsBOS-rev}$ and $d'_{tutor-rev}$). Many neurons responding equally well to tsBOS and tutor song had d′ values exceeding 0.5 for these measures of selectivity, indicating that they responded substantially more to tsBOS and tutor song than to reverse songs (points that lie within the gray zone and to the right of the dashed vertical line in Figure 3-5B). Similarly, neurons with equivalent responses to tsBOS and tutor songs still discriminated between these songs and adult conspecific song (data not shown). Figure 3-5C shows the result of classifying neurons as selective or unselective. We considered a neuron to be selective if it had a d′ value ≥ 0.5 for any one of four selectivity categories: tsBOS-adult conspecific, tutor-adult conspecific, tsBOS-reverse, and tutor-reverse. Classified in this way, 66% of neurons responding equally well to tsBOS and tutor song were selective. In comparison, 68% of this neuron type were classified as selective in normal 60 d birds (Solis and Doupe, 1997). Only 8 of 52 LMAN cells in the ts cut birds resembled 30 d neurons, with similar responses to every song stimulus, and seven of these came from the same animal.

Another measure of maturity is to consider the selectivity of a population of neurons by averaging their responses to different song stimuli. It is possible for individual neurons that do not themselves meet the d′ criterion for selectivity but whose responses are slightly biased toward selectivity to contribute to the selectivity of an entire population of cells. As a population, LMAN neurons with similar responses to tsBOS and tutor song had greater response strengths (RS) on average to tsBOS and tutor song than to adult conspecific (Figure 3-5D) and reverse songs (Figure 3-5E) (paired t-test, for tsBOS-adult conspecific $p<0.0001$, $n=27$; for tutor-adult conspecific $p<0.004$, $n=27$; for tsBOS-reverse $p<0.0001$, $n=26$; for tutor-reverse $p<0.011$, $n=21$). Thus, using both individual neuron and population measures, neurons with equivalent responses to tsBOS and tutor song exhibited selectivity, unlike immature neurons.

Alternative methods of measuring neural selectivity. In the previous analyses, comparisons of neural responses to different stimuli can be affected by stimulus duration. A neuron's RS to a stimulus was calculated by normalizing the number of spikes fired during the stimulus by the stimulus duration. If neural responses fatigue during presentation of a long stimulus, then this method will result in an RS which is less than the neuron's initial firing rate to the stimulus. This phenomenon can complicate comparisons between responses to two songs when the song durations differ substantially. For example, if two songs with large duration differences elicit the same number of spikes from a cell, then the RS to the longer song will be much less than that to the shorter song; d′ measures, which compare RS to two stimuli, would tend to favor the shorter of the two stimuli. In this study, 7/19 experiments had substantial differences between tsBOS and tutor song duration, where one song was at least twice as long as the other song. An example of the effect of normalizing by song duration is shown in Figure 3-6A: the $d'_{\text{tsBOS-tutor}}$ value obtained indicates a strong preference for the shorter tutor song, yet the PSTHs show qualitatively similar responses of an LMAN neuron to tsBOS and tutor song. When the $d'_{\text{tsBOS-tutor}}$ values of individual neurons were compared to the relative difference in duration between tsBOS and tutor song, as expressed by the ratio $\frac{(\text{duration}_{\text{tsBOS}} - \text{duration}_{\text{tutor}})}{(\text{duration}_{\text{tsBOS}} + \text{duration}_{\text{tutor}})}$, a strong correlation resulted ($r^2=0.584$, $p<0.0001$); $d'_{\text{tsBOS-tutor}}$ values reflected a preference for the shorter of the two songs.

To investigate the impact of this duration effect on the results so far described, data from experiments in which tsBOS and tutor song had similar durations (difference in duration was less than twice the shorter song) were analyzed separately (30 LMAN neurons, from 10 experiments). Within this data subset, the properties described for the whole population persisted: some LMAN neurons preferred tsBOS over tutor song, whereas others responded equally well to these two songs. Among LMAN neurons responding similarly to tsBOS and tutor song, 93% (13/14) were classified as selective, and. on average this type of neuron responded more to tsBOS and tutor song than to adult conspecific and reverse songs (data not shown) (paired t-test, for tsBOS-adult conspecific song, $p<0.001$, $n=14$; for tutor-adult conspecific song, $p<0.008$, $n=14$; for tsBOS-reverse $p<0.009$, $n=13$; for tutor-reverse $p<0.031$, $n=11$). Thus, the neuronal properties present for the whole dataset also described the subset of data collected from experiments with similar tsBOS and tutor song durations.

Another method of removing stimulus duration bias from selectivity measures is to obtain a peak firing rate for each stimulus. Peak firing rate assesses a neuron's maximum response during a stimulus, regardless of where it occurs in time. For each LMAN neuron, the maximum firing rate occurring within a sliding 500 ms window was used to calculate a peak RS to each stimulus (see Materials and Methods); thus, every response was normalized by 500 ms, regardless of stimulus duration. Peak d′ values were then calculated using the peak RS to different stimuli. The resulting peak $d'_{\text{tsBOS-tutor}}$ values indicated that there were still neurons that responded equally well to tsBOS and tutor song (53%), and neurons that preferred tsBOS over tutor song (47%) (Figure 3-6B). Of the neurons responding equally well to tsBOS and tutor song, 63% were selective, as determined from their peak d′ values in the 4 selectivity categories. In addition, the population of neurons with similar responses to tsBOS and tutor song were also selective when their responses were measured using peak RS: neurons responded on average significantly more to tsBOS and tutor song than to adult conspecific (Figure 3-6C) and reverse (data not shown) songs (paired t-tests, for tsBOS-adult conspecific $p<0.0001$, $n=19$; for tutor-adult conspecific $p<0.006$, $n=19$; for tsBOS-reverse $p<0.0004$, $n=18$; for tutor-reverse $p<0.017$, $n=16$). Thus, using peak RS and peak d′ values, neurons that responded similarly to tsBOS and tutor song were still selective. Although peak $d'_{tsBOS\text{-}tutor}$ values reclassified 39% of LMAN neurons in terms of their tsBOS and tutor song preferences, the overall distribution was only slightly shifted toward tsBOS preference relative to the original $d'_{tsBOS\text{-}tutor}$ values (mean difference in $d'_{tsBOS\text{-}tutor}=0.22$, paired t-test, $p<0.002$, $n=43$). Because there is no duration difference between forward and reverse versions of the same song, the maintenance of significant response differences between forward and reverse versions of song with peak RS also indicates that the 500 ms time window chosen was not too small to detect differences between responses to different stimuli.

Thus, LMAN properties in ts cut birds were the same when 1) the measurement of RS originally used in this and other studies was applied to the whole data set, 2) when the original RS was used for a data subset comprising neurons collected from experiments without large duration differences between tsBOS and tutor song, and 3) when peak RS was used to measure responses of the whole dataset. For all three analyses, neurons that preferred tsBOS over tutor song and neurons that responded equally well to tsBOS and tutor song were apparent. The latter neurons were also selective. The original measurement of RS will be used to describe further LMAN properties in this study because it has been employed in previous studies of AF neurons.

Song and order selectivity of the entire population of LMAN neurons

We also examined in detail the song and order selectivity of the entire population of LMAN neurons, regardless of their tsBOS versus tutor song preferences. By definition, song selective neurons respond more to tsBOS or tutor song than to other song stimuli, such as adult conspecific and heterospecific songs. For the entire population of LMAN cells recorded, song selectivity was apparent for both tsBOS and tutor song. On average, both tsBOS and tutor song produced significantly stronger responses than adult conspecific

(Figure 3-7A) and heterospecific songs (Figure 3-7B) (paired t-tests, p<0.0001 for tsBOS-adult conspecific, n=45, and tsBOS-heterospecific, n=47; p<0.004 for tutor-adult conspecific, n=43; p<0.010 for tutor-heterospecific, n=47). The song selectivity of individual LMAN neurons is illustrated with scatterplots comparing each neuron's RS to tsBOS (Figure 3-7D) or tutor song (Figure 3-7E) with its RS to adult conspecific song. In both plots, the majority of cells lie below the diagonal line, indicating their stronger responses to tsBOS or tutor song than to adult conspecific song. The percentages of selective LMAN cells in each song selectivity category are listed in Table 3-1.

To test whether neurons were tuned specifically to tsBOS, rather than to the noisy, immature features common to all plastic songs, other plastic songs of ts cut and normal 60 d birds were presented. On average, neurons responded more to tsBOS than to other plastic songs; however, this reached statistical significance for only the tsBOS-normal plastic song comparison (Figure 3-7C) (paired t-test, p<0.0001 for tsBOS-normal plastic, n=32; p=0.055 for tsBOS-ts cut plastic, n=28). Thus, LMAN neurons were tuned to features specific to tsBOS.

As a population, LMAN neurons from ts cut birds were also order selective. A neuron is considered to be order selective when it responds significantly more to forward song than to a song that is completely reversed (see labels in 8A). On average, LMAN neurons responded significantly more to tsBOS and tutor song than to reversed versions of these songs (Figure 3-8A) (paired t-test, p<0.002 for tsBOS-reverse, n=42; p<0.013 for tutor-reverse, n=30). The order selectivity of individual LMAN neurons is shown by plotting each neuron's RS to tsBOS (Figure 3-8D) or tutor song (Figure 3-8E) against its RS to the corresponding reverse song. In these scatterplots, many cells lie below the diagonal line, indicating their stronger responses to tsBOS or tutor song than to the corresponding reverse song stimuli.

Features important to order selectivity. To test the importance of syllable sequence within a song for order selectivity, "reverse order" stimuli were presented. Reverse order songs maintain the temporal order within individual syllables, but reverse the syllable sequence within a song (see labels in Figure 3-8B). On average, cells responded significantly more to forward tsBOS and tutor song than to reverse order versions of these songs (Figure 3-8B) (paired t-test, for tsBOS-reverse order $p<0.010$, $n=29$; for tutor-reverse order $p<0.050$, $n=33$). Thus, cells were sensitive to the syllable sequences within tsBOS and tutor song.

Because of the simple harmonic stack structure of syllables in many tsBOS, it seemed possible that neurons would be insensitive to reversal of the temporal structure within syllables from tsBOS. To test the contribution of individual syllable structure to order selectivity for tsBOS, we also presented "syllable reverse" stimuli. Syllable reverse stimuli maintain the correct syllable sequence within a song, but reverse the individual syllables (see labels in Figure 3-8C). On average, cells responded significantly more to forward tsBOS than to syllable reverse tsBOS (Figure 3-8C) (paired t-test; $p<0.003$, $n=13$). Thus, cells were also sensitive to the temporal structure within the simpler tsBOS syllables. The percentage of selective neurons in each order selectivity category is listed in Table 3-1.

Comparison of X neural responses to tsBOS and tutor song

X is the first nucleus in the AF pathway: it receives inputs from HVc, and itself projects to DLM, which in turn goes to LMAN. In addition, X receives feedback via projections from LMAN. To understand the circuitry underlying AF selectivity, and potential interactions between LMAN and X, 64 single X neurons were also recorded from 19 ts cut birds.

As in LMAN, some X neurons responded more to tsBOS than to tutor song. The neuron in Figure 3-9A not only strongly preferred tsBOS over tutor song, but it also preferred tsBOS over adult conspecific song and reverse tsBOS. In addition, many X neurons responded equally well to tsBOS and tutor song, despite the acoustic dissimilarity of these songs; an example of such a neuron is illustrated in Figure 3-10A. The distribution of $d'_{\text{tsBOS-tutor}}$ values from individual X neurons is shown in Figure 3-9B: 37% of X neurons recorded preferred tsBOS over tutor song, 35% responded equally well to tsBOS and tutor song, and 28% preferred tutor song over tsBOS. This distribution did not differ significantly from that obtained from X neurons in normal 60 d birds (unpaired t-test, $p=0.711$; normal 60 d data from Solis and Doupe, 1997). On average, in ts cut birds, neural responses to tsBOS were not significantly different from those to tutor song (inset, Figure 3-9B) (paired t-test, $p=0.862$, $n=63$).

Plastic song renditions and neuronal maturity. Neurons which did not strongly prefer tsBOS were unlikely to have resulted from inappropriate tsBOS choice: X neurons responded equally well to three different renditions of tsBOS (ANOVA, $p=0.079$, $n=38$). Furthermore, neurons with similar responses to tsBOS and tutor song were also selective, indicating that they were not immature. For example, the neuron in Figure 3-10A responded strongly to both tsBOS and tutor song, but substantially less to conspecific song and reverse tsBOS. The song selectivity of neurons that responded equivalently to tsBOS and tutor song was examined by plotting the $d'_{\text{tsBOS-tutor}}$ value of each neuron against its corresponding $d'_{\text{tsBOS-adult con}}$ value and $d'_{\text{tutor-adult con}}$ value (Figure 3-10B). In this scatterplot, many neurons responding equally well to tsBOS and tutor song discriminated between these songs and adult conspecific song, as demonstrated by their $d'_{\text{tsBOS-adult con}}$ or $d'_{\text{tutor-adult con}}$ values of at least 0.5. Similar order selectivity was apparent for these neurons when $d'_{\text{tsBOS-tutor}}$ values were plotted against $d'_{\text{tsBOS-rev}}$ and $d'_{\text{tutor-rev}}$ values (data not shown). Figure 3-10C shows that 86% of X neurons responding equally well to tsBOS and tutor song were classified as selective; in comparison, 63% of this neuron type were considered selective in normal 60 d birds (Solis and Doupe, 1997). In ts cut birds, only 2 X cells (each from a different animal) resembled those from 30 d birds. When neurons with equivalent responses to tsBOS and tutor song were analyzed as a population, they were also selective. On average, these X neurons responded significantly more to tsBOS than to adult conspecific (Figure 3-10D) and reverse songs (Figure 3-10E) (paired t-test, for tsBOS-adult conspecific $p<0.001$, $n=21$; for tsBOS-reverse $p<0.0004$, $n=18$). They also responded more on average to tutor song than to adult conspecific and reverse songs, but this was only significant for the tutor-adult conspecific comparison (paired t-test, $p<0.002$, $n=21$; for tutor-reverse $p=0.124$, $n=18$). Thus, X neurons with similar responses to tsBOS and tutor song were not immature: their selectivity was apparent both in individual neurons and in most population measures.

Alternative methods of measuring neural selectivity. The effect of differences between tsBOS and tutor song duration was also analyzed for X neurons; as for LMAN cells, the basic selectivity described above persisted. When RS was calculated by normalizing by song duration, the resulting $d'_{\text{tsBOS-tutor}}$ values of individual X neurons correlated well with the relative difference in duration between tsBOS and tutor song ($r^2=0.639$, $p<0.0003$). When data from experiments with similar tsBOS and tutor song durations were analyzed separately (33 X neurons, from 12 experiments), only 4 neurons (13%) preferring tutor song over tsBOS remained (compare to 28% in the entire data set). Also, in this data subset, 40% of the neurons preferred tsBOS over tutor song, and 47% responded equally well to both. For the latter type of neuron, 79% (11/14) were classified as selective and this population responded on average more to tsBOS and tutor song than to adult conspecific or reverse songs (data not shown) (paired t-test, for tsBOS-adult conspecific $p<0.005$, $n=14$; for tsBOS-reverse $p<0.013$, $n=11$; for tutor-adult conspecific $p<0.018$, $n=14$); however, the response to tutor song was also not significantly different from that to reverse (paired t-test, $p=0.078$, $n=11$).

Peak RS and peak $d'_{\text{tsBOS-tutor}}$ values were also calculated for all X neurons. When classifying preferences using peak $d'_{\text{tsBOS-tutor}}$ values, only 3% of neurons preferred tutor song over tsBOS. In addition, 32% of X neurons preferred tsBOS over tutor song, and 65% responded equally well to both (Figure 3-6B). Importantly, 64% of this latter type of neuron were considered selective given their peak d′ values in the four selectivity categories. These neurons were also selective when analyzed as a population: their averaged peak RS to tsBOS and tutor song were greater than those to adult conspecific (Figure 3-6C) and reverse songs (data not shown) (paired t-tests, for tsBOS-adult conspecific $p<0.0001$, n=39; for tutor-adult conspecific $p<0.0002$, n=39; for tsBOS-reverse $p<0.0001$, n=31). As with the original RS measurement, the greater peak response to tutor song relative to reverse was not statistically significant (paired t-test, p=0.097, n=31). Thus, peak measurements found that neurons with similar responses to tsBOS and tutor song were still selective according to individual neuron and most population measurements of selectivity. Although peak $d'_{\text{tsBOS-tutor}}$ values reclassified 50% of X neurons in terms of their tsBOS versus tutor song preference, the resulting distribution of peak $d'_{\text{tsBOS-tutor}}$ values was similar to that of the original $d'_{\text{tsBOS-tutor}}$ values (paired t-test, p=0.114, n=60).

Song and order selectivity of the entire population of X neurons

When the entire population of X neurons recorded was considered, regardless of their tsBOS versus tutor song preferences, they were song selective for both tsBOS and tutor song. Using the original RS values normalized by stimulus duration, X neurons responded significantly more to tsBOS and tutor song than to adult conspecific (Figure 3-11A) and heterospecific song (Figure 3-11B) (paired t-test, $p<0.0001$ for tsBOS-conspecific, n=63; tsBOS-heterospecific, n=64; and tutor-heterospecific, n=63; $p<0.0004$ for tutor-conspecific, n=62). The song selectivity of individual X neurons is illustrated with scatterplots which compare the RS to adult conspecific song of each neuron with its RS to tsBOS (Figure 3-11D) and to tutor song (Figure 3-11E).

Further tests of tsBOS song selectivity indicated that neurons were tuned to tsBOS specifically, rather than to features common to plastic songs. Responses to tsBOS were significantly greater than responses to plastic songs of ts cut and normal 60 d birds (Figure 3-11C) (paired t-test, $p<0.0005$ for tsBOS-ts cut, $n=49$; $p<0.0002$ for tsBOS-normal, $n=51$). The percentages of selective X cells in each song selectivity category are listed in Table 3-1.

The X neurons recorded were also order selective for tsBOS (Figure 3-12A). On average, X neurons responded significantly more to tsBOS than to reverse tsBOS (paired t-test, $p<0.0001$, $n=54$). Although the average response to tutor song was slightly more than to that to reverse tutor song, this difference was not statistically significant (paired t-test, $p=0.144$, $n=45$). The order selectivity of individual neurons is shown by plotting each neuron's mean RS to tsBOS (Figure 3-12D) and to tutor song (Figure 3-12E) against its mean RS to the corresponding reverse stimulus.

To test the importance of syllable sequence on X neuron order selectivity, reverse order stimuli were presented (Figure 3-12B). In contrast to LMAN, X neurons did not discriminate between tsBOS and reverse order tsBOS (paired t-test, $p=0.411$, $n=48$). This suggests that these neurons become selective for syllable identity first, and then later for the syllable sequence within song. However, X neurons did respond significantly more to forward than to reverse order tutor song ($p<0.001$, $n=45$), indicating that they were sensitive to the syllable sequences within tutor song. This difference between forward and reverse order tutor song was small, however, and similar to that between forward and reverse tutor song. This suggests that discrimination between the two reverse manipulation types was not really that different for X cells; consistent with this, the percentage of selective neurons in the tutor-reverse category was similar to neurons in the tutor-reverse order category (Table 3-1). Finally, to assay the contribution of temporal features within tsBOS syllables to X responses, syllable reverse stimuli were presented. Neurons responded significantly more to forward tsBOS than to syllable reverse tsBOS, indicating a sensitivity to the temporal structure within a syllable (Figure 3-12C) (paired t-test, $p<0.029$, $n=17$). The percentages of selective X cells in each order selectivity category are listed in Table 3-1.

Comparisons of LMAN and X neurons

X neurons were more broadly responsive than LMAN neurons: they responded readily to broad band noise bursts, tone bursts, and "non-preferred" song stimuli (i.e., those not eliciting the largest RS). In X, 53% (34/64) of cells recorded responded significantly to all non-preferred stimuli presented, whereas in LMAN, only 4% (2/52) of neurons did. When both nuclei were sampled in an individual bird (13 experiments), mean selectivity values for each nucleus were calculated. When selectivity was measured by the ratio of response magnitudes to two stimuli ("SI"; see Materials and Methods), LMAN was significantly more selective than X for only two categories of selectivity: tsBOS-tutor song and tsBOS-adult conspecific song comparisons (data not shown; paired t-test, $p<0.006$ and $p<0.002$, respectively). In contrast, comparisons of mean d′ values across nuclei yielded no significant differences in selectivity. Thus, in general, LMAN and X shared similar degrees of selectivity to song stimuli.

Comparisons between neural properties of ts cut birds and those of normal juvenile birds

Although the distribution of their responses to tsBOS and tutor song were similar to those obtained from normal 60d birds (Figures 3-4B and 3-9B), AF neurons from ts cut birds were less selective than neurons from normal 60 d birds. The mean d′ values for different selectivity categories are compared between ts cut and normal 60 d birds in Figure 3-13 (normal 60 d data from Solis and Doupe, 1997). The mean d′ values of LMAN neurons from ts cut birds were significantly lower than those from normal 60 d birds when tsBOS selectivity was analyzed (unpaired t-tests; $p<0.0001$ for tsBOS-reverse and $p<0.001$ for tsBOS-adult conspecific). The mean d′ values of X neurons from ts cut birds were significantly lower than those from normal 60 d birds when order selectivity was examined (unpaired t-test, $p<0.009$ for tsBOS-reverse and $p<0.002$ for tutor-reverse). This lower selectivity relative to normal 60 d birds was also maintained when only data from ts cut and normal birds with the same tutor (different clutches) were compared (data not shown). Thus, the lower selectivity is probably not due to differences in tutor bird efficacy between the normal and ts cut studies. Furthermore, this lower selectivity was apparent when the percentage of selective neurons in ts cut birds was compared to that found in normal 60 d birds. In LMAN, the percentages of selective neurons in the tsBOS>tutor and the tsBOS>reverse tsBOS categories were significantly lower in ts cut birds. In X, the percentages of selective neurons in the tsBOS>reverse tsBOS, tutor>reverse tutor, and tsBOS>reverse order tsBOS categories were significantly lower in ts cut birds (Table 3-1).

Despite this lower selectivity, neurons from ts cut birds were clearly selective relative to neurons from normal 30 d birds: as seen in Figures 3-7, 3-8,3-11 and 3-12, neurons from ts cut 60 d birds on average discriminated tutor song from adult conspecific song and reverse tutor song (except in X), which is not true for 30 d neurons. In addition, when classified according to tutor song categories of selectivity only (i.e., $d'_{\text{tutor-adult con}}$ and $d'_{\text{tutor-rev}}$), ts cut birds had significantly more selective LMAN and X neurons than did 30 d birds (30 d data from Doupe, 1997; χ^2 tests, $p<0.0004$ for both comparisons).

RA volumes were not affected by NXIIts transections

Transecting the NXIIts nerve might have caused neuronal atrophy or death in upstream nuclei, which could lead to non-specific changes in LMAN or X selectivity. To estimate potential retrograde effects of the nerve transections, RA volumes were measured. In ts cut birds, the mean RA volume (0.244±0.053 mm^3, ± SD) was within the range of RA volumes previously reported for normal adults (0.220-0.372 mm^3; Gurney, 1981). Also, although syrinx weight of ts cut birds was significantly less than that of normal 60 d birds (unpaired t-test, $p<0.0001$), the normalized RA volume was not significantly different between the same two groups of birds (unpaired t-test, $p=0.300$); this indicates that nerve transection did not affect RA volume. Finally, the mean RA volume of a ts cut bird did not correlate with its mean $d'_{tsBOS-tutor}$ value from LMAN or X (Table 3-2). Thus, possible retrograde effects of nerve transection, as measured here with RA volume, do not account for the difference in selectivity between ts cut and normal 60 d birds, nor for the range of tsBOS versus tutor song preferences.

Differences between individual ts cut birds

Neurons recorded from the same bird had similar tsBOS versus tutor song preferences (Figure 3-14). Experimental $d'_{tsBOS-tutor}$ values of LMAN neurons clustered in 9 of 9 birds in which multiple cells were recorded (see Materials and Methods). This frequency of clustering was greater than expected by chance (sign test, $p<0.002$). Similarly, the $d'_{tsBOS-tutor}$ values of X neurons within a bird were also clustered more frequently than expected by chance (12/14 birds; sign test, $p<0.006$). The $d'_{tsBOS-tutor}$ values obtained from both LMAN and X cells in a single bird were also more similar than expected (15/17 birds were clustered, $p<0.001$). The similarity of $d'_{tsBOS-tutor}$ values for neurons from the same bird suggests that factors specific to the experiment or to the bird could account for the responses to these two songs.

A bird's neural preference for tsBOS versus tutor song was not readily explained by conditions that varied between experiments (Table 3-2). Potential measures of anesthesia depth, such as the spontaneous rate, maximum RS of a neuron, or time at which each neuron was recorded relative to anesthesia administration, were not well correlated to the neuron's $d'_{\text{tsBOS-tutor}}$ value. Anatomical location within the nucleus did not predict the $d'_{\text{tsBOS-tutor}}$ value of a neuron in either the anteroposterior, mediolateral, or dorsoventral dimension. Neither slight intensity differences between tsBOS and tutor song stimuli nor the ages of individual birds correlated well with the mean $d'_{\text{tsBOS-tutor}}$ value from each bird. To weigh the contribution that each bird made to these correlations by the number of cells recorded from it, these last two comparisons were also made using the d' values of individual neurons; this did not improve their correlations (Table 3-2). Furthermore, no significant correlations were found among these variables when only data from experiments with equivalent tsBOS and tutor song durations were considered, or when peak $d'_{\text{tsBOS-tutor}}$ values were used (Table 3-2). The inclusion of immature neurons in these correlations could obscure a relation between the variables tested and $d'_{\text{tsBOS-tutor}}$ values, because these cells would have equivalent responses regardless of any experimental condition tested here. Thus, these correlations were recalculated with the 10 unselective neurons excluded from the dataset; this still did not reveal any significant correlations (data not shown). Thus, a bird's neural preference for tsBOS versus tutor song did not seem to depend on conditions that varied between experiments.

Acoustic similarity between the tsBOS and tutor song does not correlate with neural responses to these two song stimuli

Because the equivalent responses to tsBOS and tutor song were not the result of inappropriate tsBOS stimulus choice nor of neuronal immaturity, we considered another possible explanation for such responses. In principle, residual acoustic similarities between tsBOS and tutor song might have produced similar responses to tsBOS and tutor song. Although the average similarity between tsBOS and tutor songs was lower than normal (Figure 3-3D), it remained possible that this similarity for an individual ts cut bird predicted the song preference of that bird's neurons. If the $d'_{tsBOS\text{-}tutor}$ values obtained from a bird reflect residual acoustic similarity between tsBOS and tutor songs, then neurons with equivalent responses to tsBOS and tutor song should come from birds with tsBOS similar to tutor song. As similarity decreases, neurons would have strong preferences for either tsBOS or tutor song, depending on the experience shaping selectivity (Figure 3-15A, upper panel). This "similarity hypothesis" predicts a negative correlation between the absolute value of $d'_{tsBOS\text{-}tutor}$ values ($|d'_{tsBOS\text{-}tutor}|$) and the measured similarity between tsBOS and tutor song (Figure 3-15A, lower panel).

Matching task. To assess whether such a correlation existed in the data, we analyzed pairs of tsBOS and tutor songs in order to compare their acoustic similarity to the corresponding neural data. Similarity between tsBOS and tutor song was analyzed in several ways. In the matching task described earlier, the percentage of observers correctly matching tsBOS to tutor song was used as a measure of overall similarity between the two songs. When this percentage was compared to the mean $|d'_{tsBOS\text{-}tutor}|$ value of LMAN neurons obtained from each bird, no correlation was evident (Figure 3-15B) (r^2=0.023). Birds whose songs were infrequently matched to the correct tutor song, a sign of dissimilarity, still had neurons that responded equally well to tsBOS and tutor song.

Since the measure of percent correctly matched combines both spectral and temporal features of song, these features were also scored separately to control for the possibility that ts cut birds could imitate the timing of the tutor song without mimicking its spectral content. When the mean spectral similarity score for each bird was compared to the mean $|d'_{tsBOS\text{-}tutor}|$ value from its LMAN neurons, no correlation resulted (Figure 3-15C)

(r^2=0.034). Comparing temporal similarity scores to the mean $|d'_{tsBOS\text{-}tutor}|$ values from LMAN neurons in each bird also failed to yield a correlation (Figure 3-15D) (r^2=0.046). Furthermore, when mean peak $|d'_{tsBOS\text{-}tutor}|$ values were used, regression lines with slopes of the same sign were obtained, but the correlations were also weak (for percent of correct observers, r^2=0.0003, for mean similarity score r^2=0.008, for mean timing score r^2=0.132).

Cross-correlations. Because some similarities may have been too subtle for detection by human observers, we also employed cross-correlation methods to analyze song pairs. Three types of cross-correlation measures were calculated between each tsBOS and its tutor song. First, each tsBOS spectrogram was cross-correlated with the corresponding tutor song spectrogram. Second, in order to analyze spectral similarity alone, all syllables from each tsBOS were isolated from the song and individually cross-correlated with the isolated syllables of the tutor song. Third, to compare temporal similarity, the amplitude envelopes of each song were cross-correlated. Figure 3-16A shows the mean cross-correlation measures obtained from tsBOS and tutor song comparisons. For reference, mean cross-correlation measures were also calculated between tutor song and other song types, including normal adult, normal 60 d, and randomly matched songs. Of these measures, only the "syllables only" cross-correlation measures distinguished differences between these song types. Songs from ts cut 60 d birds had less similarity with their tutor songs than did normal 60 d songs (unpaired t-test, $p<0.004$); however, the similarity between normal 60 d songs and tutor song was not significantly different from that between normal adult-tutor song pairs. In contrast, cross-correlation measures for the entire spectrogram and temporal envelope comparisons were uniformly low for all song types; no song type's mean cross-correlation measure was significantly different from that obtained from randomly matched song pairs. Thus, the entire spectrogram and temporal envelope cross-correlations were not sensitive enough to detect similarities that were apparent to humans (Figure 3-3D). When the most sensitive "syllables only" cross-correlation measure of similarity was compared to the mean $|d'_{tsBOS-tutor}|$ value from LMAN neurons, no strong correlation resulted (Figure 3-16B, $r^2=0.128$). This was also true when mean peak $|d'_{tsBOS-tutor}|$ values were used ($r^2=0.021$).

Overlap analysis. To further investigate temporal similarity between tsBOS and tutor song pairs, we used an overlap analysis (see Materials and Methods). The proportion of overlap between syllables and of intervals of an entire tsBOS string and entire tutor song string was calculated to give a song-song overlap value. To maximize the possibility of detecting temporal similarity, a motif-song overlap value was calculated between a single tsBOS motif and the entire tutor song (see Materials and Methods). Whereas the song-song overlap values did not distinguish between different song types, the motif-song overlap values were slightly sensitive to differences in temporal similarity between them (Figure 3-16A). Motif-song overlap values for ts cut-tutor song pairs were significantly less than those for normal adult-tutor song pairs (unpaired t-test, $p<0.014$); this was the only significant difference between song types. When the motif-song overlap values were plotted against each bird's mean $|d'_{tsBOS-tutor}|$ value, a weak correlation resulted (Figure 3-16C) ($r^2=0.341$, $p<0.022$); however, this positive correlation was in the opposite direction of that predicted by the similarity hypothesis. With increasing temporal similarity between tsBOS and tutor song, neurons tended to prefer tsBOS over tutor song. This correlation decreased when mean peak $|d'_{tsBOS-tutor}|$ values were used instead ($r^2=0.042$).

For the X data, comparisons of mean $|d'_{tsBOS-tutor}|$ values and song similarity resulted in one weak negative correlation for the temporal similarity scores obtained from the matching task (data not shown, $r^2=0.286$, $p<0.018$). Although this was predicted by the similarity hypothesis, it was not corroborated by the motif-song overlap measure of temporal similarity (r^2=0.015). No other substantial correlations resulted for the other similarity measures (for percent of correct observers r^2=0.163, p=0.087, for spectral similarity r^2=0.002, for syllables only cross-correlation, r^2=0.011). When mean peak $|d'_{tsBOS\text{-}tutor}|$ values were used, no strong correlations resulted, not even for temporal similarity scores (for percent of correct observers, r^2=0.006, for mean similarity score r^2=0.002, for mean timing score r^2=0.130, p=0.129, for syllables only cross-correlation r^2=0.182, p=0.069, for motif-song overlap, r^2=0.025).

To weigh each bird's contribution to the correlation by the number of neurons recorded from the bird, the $|d'_{tsBOS\text{-}tutor}|$ value or peak $|d'_{tsBOS\text{-}tutor}|$ value of each cell was also compared to each measure of similarity. This did not reveal any substantial correlations (Table 3-3). Thus, the relative responses to tsBOS and tutor song were not strongly dependent on the similarity between tsBOS and tutor song as measured in this study.

Because correlations between $d'_{tsBOS\text{-}tutor}$ values and measures of song similarity could be weakened by the presence of unselective neurons, the above correlations were recalculated excluding data from the bird which had contributed most of the unselective neurons (7/8 unselective LMAN neurons came from this animal). The lack of correlation persisted for these comparisons; the strongest trend was a positive correlation between mean $|d'_{tsBOS\text{-}tutor}|$ values from LMAN and motif-song overlap (r^2=0.316, p<0.037; data not shown).

Song stereotypy shows little correlation with neuronal song preference

Rather than reflecting the acoustic properties of songs, the tsBOS versus tutor song preferences found in each bird might instead reflect its stage of song maturity. Although a weak correlation existed between mean $d'_{\text{tsBOS-tutor}}$ values and age, the variability in rate of song development between birds makes age a less reliable indicator of song maturity. Because song stereotypy also increases with song development, we used it as a more direct measure of song maturity. Song stereotypy was estimated by analyzing the similarity between multiple (usually 10) song samples from each bird. Stereotypy was measured using human subjective scoring, "syllables only" cross-correlations, and motif-song overlap analyses (see Materials and Methods). Only the human scores and the overlap measures distinguished between all three song types (Figure 3-17A). Songs from ts cut birds were significantly less stereotyped than normal 60 d songs (unpaired t-test, $p<0.005$ for human scores, $p<0.003$ for overlap values). As expected, normal 60 d songs were less stereotyped than normal adult songs (unpaired t-test, $p<0.001$ for human scores and $p<0.024$ for overlap values). The syllables only cross-correlation measures did not find significant differences between adult and juvenile song stereotypy, regardless of whether adult song was compared tots cut or normal 60 d songs (unpaired t-tests, $p=0.148$ and $p=0.062$, respectively).

When we compared the mean $d'_{\text{tsBOS-tutor}}$ values obtained from LMAN of each bird to the two sensitive measures of song stereotypy, no strong correlations were found (Figure 3-17B and C) ($r^2=0.147$ for human scores, $r^2=0.019$ for motif-song overlap). These stereotypy measures also failed to predict the mean $d'_{\text{tsBOS-tutor}}$ values obtained from X (data not shown) ($r^2=0.027$ for human scores, $r^2=0.012$ for motif-song overlap). When mean peak $d'_{\text{tsBOS-tutor}}$ values of LMAN cells were compared to the human stereotypy scores, a weak positive correlation resulted ($r^2=0.206$, $p=0.089$), suggesting that an increase in stereotypy was related to an increased neural preference for tsBOS. Otherwise, no strong correlations occurred using mean peak $d'_{\text{tsBOS-tutor}}$ values in either LMAN or X (in

LMAN, r^2=0.012 for motif-song overlap; in X, r^2=0.014 for human scores, r^2=0.017 for motif-song overlap). In addition, comparing the individual $d'_{tsBOS\text{-}tutor}$ values of each cell to these stereotypy measures did not improve these correlations (Table 3-3), nor did excluding data from the bird contributing the majority of unselective neurons (data not shown).

DISCUSSION

This study addressed the relative contributions of BOS and tutor song to AF selectivity. NXIIts transections caused juvenile birds to produce songs that were acoustically different from tutor song. Some neurons preferred the tsBOS over the tutor song, demonstrating that BOS experience can shape AF selectivity. Other neurons responded equally well to tsBOS and tutor song, despite their acoustic differences. Many of these neurons were also selective, and several methods of song analysis did not find residual similarities between tsBOS and tutor songs that could account for these responses. These results strengthen the idea that both BOS and tutor song can contribute to the selectivity of single AF neurons.

NXIIts cut song shows evidence of poor temporal and spectral learning

The effects of NXIIts transections on song in this study indicate the importance of respiratory and vocal muscle interactions during song development. The timing of syllable occurrence within song depends on the patterns of airflow through the syrinx, which are controlled by both syringeal and respiratory musculature (Hartley and Suthers, 1989; Goller and Suthers, 1996b; Vicario, 1991; Suthers, 1997). The song system coordinates these muscle groups during song through RA, which projects both to motor neurons of syringeal muscles contained in nXIIts, and to respiratory premotor nuclei such as RAm and PAm (Wild, 1993; Reinke and Wild, 1998; Wild, 1997). In adults, NXIIts transections transform syllables into harmonic stacks, but maintain the timing of syllable occurrence within song (Simpson and Vicario, 1990; Williams and McKibben, 1992); thus, in NXIIts transected adults, the respiratory pathway alone can maintain the temporal structure of song. In contrast, NXIIts transections made in juveniles resulted in songs that consisted mainly of harmonic stacks, but which shared little spectral or temporal similarity with the tutor song. Thus, for these juveniles, the connection from RA to respiratory centers was not sufficient to produce timing similar to the tutor song. Because RA volume was unaffected by these transections, it seems unlikely that retrograde effects of NXIIts transection on RA resulted in improper signalling to the respiratory centers. Instead, syringeal control through NXIIts appears to participate in learning song timing.

Of the song analysis methods used here, the human matching task was the most sensitive to similarities between songs from different birds. For example, humans readily detected similarities between normal 60 d songs and tutor songs that the cross-correlation and overlap measures did not. The insensitivity of some of the automated methods was surprising: some cross-correlation analyses and overlap values measured the similarity apparent to humans between normal adult song and their tutor songs to be as low as that between randomly matched songs. These automated measures may be more suitable for quantifying similarity between songs from the same bird; indeed, in the stereotypy analysis, the motif-song overlap measure was as sensitive as human scoring. Despite the subjectivity inherent in human judgement of song qualities, these methods are currently the most sensitive and remain the standard for song analysis (Scharff and Nottebohm, 1991; Nordeen and Nordeen, 1992; Williams, 1990; Eales, 1985). An important step in the birdsong field will be the development of algorithms capable of detecting and quantifying similarities between songs from different birds at least as well as humans.

Contributions of two song experiences to AF selectivity

Many AF neurons responded equally well to tsBOS and tutor song, despite the acoustic differences between these songs. These neurons were also song and order selective, which demonstrates that they were not indiscriminately responding to any stimulus. Thus, such neurons support the idea that both tsBOS and tutor song can shape the selectivity of single AF neurons. These neurons might be useful for comparisons of tsBOS and tutor song, which must occur behaviorally during song learning (Konishi, 1965; Price, 1979). The AF could be involved in calculating an error signal that measures the difference between tsBOS and the tutor song template; this error signal could then guide the modification of plastic song during learning.

Examples of single neuron selectivity for two different stimuli have been found in other neural systems. Some neurons in the bat auditory cortex are tuned to two different durations, depending on whether neurons are responding to biosonar pulses or communication calls (Ohlemiller et al, 1996). Dual selectivity for more complex stimuli has been found in the inferior temporal visual cortex. There, neurons selectively respond to dissimilar fractal patterns that had been consecutively presented during training (Miyashita, 1988). This dual selectivity may reflect an association made between two stimuli consistently experienced together. Similarly, the selectivity for both tsBOS and tutor song could reflect experience of both songs during learning.

Other types of neurons in this study also supported roles for tsBOS and tutor song experience in shaping AF selectivity. Neurons with strong preferences for the abnormal tsBOS over tutor song clearly indicate that, rather than reflecting poor tutor song copying, BOS experience itself can shape AF selectivity. The sensitivity of these neurons to tsBOS makes them well-suited to provide information about the state of plastic song during sensorimotor learning, perhaps participating in the evaluation of BOS. Neurons that preferred tutor song over tsBOS, although rare, are also consistent with an influence of tutor song experience on AF selectivity. Such neurons could represent the stored memory of tutor song. Their rarity, however, combined with the sizable population of neurons responding to both tsBOS and tutor, raises the possibility that tutor song information is primarily found in neurons that also respond to BOS, rather than residing in neurons dedicated solely to encoding tutor song. Alternatively, the rarity of neurons with strong tutor song preferences might indicate that tutor song information is distributed sparsely, or is encoded differently or elsewhere in the brain (Doupe and Solis, 1997).

Recordings from normal 60 d birds had already suggested a contribution of both BOS and tutor song experience to AF selectivity (Solis and Doupe, 1997). Thus, the tuning of these neurons to two stimuli may be a normal feature of learning in songbirds. As birds mature, tutor song responses may eventually be lost, leaving selectivity only for BOS. Consistent with this idea, the average response of LMAN neurons to tutor song was less than that to tsBOS, and in general, selectivity for tutor song over other stimuli was weaker than that for tsBOS. A transition in selectivity also occurs in the barn owl optic tectum, where tuning to two different auditory cues precedes the establishment of selectivity for only one cue during the recalibration of auditory-visual maps (Brainard and Knudsen, 1995). Thus, raising ts cut birds to adulthood might reveal a strong preference for BOS over tutor song. Furthermore, the lower selectivity of ts cut birds relative to normal birds could reflect the delayed song development in ts cut birds, which was evident in their low song stereotypy. Again, ts cut birds raised to adulthood may show an increase in AF selectivity.

Measuring neural responses in LMAN and X

The differences in duration between tsBOS and tutor song stimuli in some experiments compelled us to evaluate different methods of measuring neural responses. The appropriate way to measure a neural response depends on how neurons downstream from LMAN and X respond to their inputs. If downstream neurons integrate afferent activity only for a short time, then the peak RS is more appropriate. However, if they integrate incoming signals for the duration of each song stimulus, then the original RS is appropriate. Longer integration times seem more suitable for analyzing these neurons: for a sample of AF neurons, the best discrimination between two stimuli occurred for window sizes greater than 500 ms (see Chapter 5). Other clues may also come from behavioral studies. If song duration has behavioral significance in zebra finches, then it is reasonable to measure responses over an entire song stimulus, whatever its duration. Some species respond differently to a song when its duration is varied by altering the number of repeated motifs (Kroodsma, 1976; Becker, 1982). Nevertheless, the peak RS and peak d′ values gave results similar to the original measures of neural response, indicating the robust nature of the selectivity found in this study.

Song experience shaping HVc, the source of AF input

Neurons in HVc also become selective during song learning (Volman, 1993). Studies in both adult (Margoliash, 1986) and juvenile (Volman, 1993) white-crowned sparrows have shown that HVc neurons are selective for BOS. In both studies a subset of birds had songs different from the tutor song, and their neurons showed strong preferences for BOS over tutor song. The weak tutor song responses in these birds might seem inconsistent with the significant tutor song responses found in the AF in this study, since HVc is the source of auditory inputs to the AF. It is possible, however, for AF neurons to derive their selectivity differently from their HVc inputs. HVc comprises two populations of neurons, one projecting to the AF, the other to RA (Sohrabji et al, 1989). X-projecting neurons could be shaped by both tutor song and BOS, whereas RA-projecting neurons could be shaped by BOS alone. Alternatively, tutor song responses may have been overlooked in these HVc studies. First, both employed multiunit recordings, which could miss other kinds of selective neurons if they are few. Second, the strong BOS preferences of HVc neurons in this subset of birds could reflect poor copying of the tutor song model. In one study, the birds with songs unlike the tutor song had been tutored with an abnormal tutor song model, unlike natural white-crowned sparrow song (Margoliash, 1986). As juveniles, the birds may have had difficulty memorizing such an abnormal song, potentially leading to storage of something other than the tutor song model as the template. In contrast, the NXIIts transections employed in this study reduced the similarity between BOS and tutor song by manipulating the juvenile song itself, rather than the tutor song model. Thus, the stronger tutor song responses found here may reflect greater agreement between what was presented as the tutor song and what was stored as the template.

Range of tsBOS versus tutor song preferences among AF neurons

It remains intriguing that there is a variety of tsBOS versus tutor song preferences in ts cut birds, ranging mainly from neurons that strongly prefer tsBOS over tutor song to neurons that respond equally well to both songs. Although not found here, it is theoretically possible that a different method of song analysis, perhaps one that exclusively measures those elements salient to the birds themselves, could find residual similarities between tsBOS and tutor song that were responsible for the equivalent responses to these songs. Alternatively, the state-dependence of auditory responses in neurons of the song system might have influenced the preferences recorded (Hessler and Doupe, 1997; Schmidt and Konishi, 1998; Dave et al, 1998). Although measures of anesthesia depth did not correlate with $d'_{\mathrm{tsBOS\text{-}tutor}}$ values, it remains possible that subtle differences in arousal state may have differentially emphasized BOS or tutor song responses in neurons actually capable of responding equally well to both stimuli. A third possibility is that the $d'_{\mathrm{tsBOS\text{-}tutor}}$ values from a bird could reflect its maturity. One measure of song similarity weakly supported this idea: as motif-song overlap values increased (which presumably happens as a bird matures), neural preference for BOS also increased. In contrast, neither the age nor song stereotypy of a bird predicted the $d'_{\mathrm{tsBOS\text{-}tutor}}$ values obtained from its neurons.

Finally, differences in the accuracy of learning from a tutor bird may have contributed to the range of $d'_{\text{tsBOS-tutor}}$ values. In another system, differences between individual animals in neural processing of a task resulted from slightly different behavioral strategies for solving that task (Seidemann et al, 1998). In our study, it is difficult to know whether the tutor song presented during the experiment was similar to what the bird had memorized. To favor similarity between the tutor song and the template, juveniles were housed in conditions that maximize copying from the tutor bird (see Materials and Methods). Also, the great degree of similarity between songs from normal adults and their tutor songs shows that there is a high incidence of tutor song copying in the colony used in this study. Nonetheless, because there is currently no direct way to assay what has been stored as the template, it remains possible that all neurons are actually equally shaped by tsBOS and tutor song: neurons with strong preferences for BOS may have been presented with a tutor song that did not match the template.

Possible mechanisms underlying dual selectivity

Although the site of plasticity initially giving rise to selectivity is not addressed in this study, possible mechanisms for mediating selectivity to two different songs exist within the AF. Both LMAN and X have complex intrinsic circuitry that could form multiple, differentiated populations of synapses onto single neurons, enabling them to process tsBOS and tutor song separately (Sohrabji et al., 1993; Vates and Nottebohm, 1995; Luo and Perkel, 1999; Farries and Perkel, 1998). Indeed, synapses onto single LMAN neurons differ markedly in their pharmacological and temporal properties (Boettiger and Doupe, 1998a). Moreover, in addition to their auditory responses, LMAN and X neurons are strongly active during singing (Hessler and Doupe, 1999; Dave et al, 1997). This raises the possibility that tuning to different stimuli occurs in different behavioral states, such as singing or listening. The motor-related activity in AF neurons, which could represent efference copy signals (Troyer et al, 1996), might actually contribute to the auditory tuning of these cells. Finally, the acoustical basis of the dual responses to tsBOS and tutor song could be determined in experiments that systematically decompose tsBOS and tutor song stimuli, thus revealing the song components essential for a neural response. Such experiments would contribute to our understanding of a single neuron's selectivity for two different stimuli.

Figure 3-1 A) Zebra finches learn to sing in two overlapping phases. The sensory phase ends at ~60 d; the sensorimotor phase begins at ~30 d and continues until 90 d+. B) The anatomy of the song system is diagrammed. Motor pathway nuclei are gray, and the AF nuclei are black. C) AF neurons develop selectivity for song during development. At 30 d, LMAN neurons have equal response strengths (RS) to tutor song (TUT), conspecific song (CON), and reverse tutor song (REV). At 60 d, these neurons respond significantly more to TUT than to CON or to REV. In addition, BOS elicits a stronger RS than CON or reverse BOS (REV). In adults, LMAN neurons are extremely selective for BOS. D) At 60 d, there is a range of BOS versus tutor song preferences among LMAN neurons. The cumulative distribution of preferences is shown, as quantified with a $d'_{\text{BOS-tutor}}$ value for each neuron (see Materials and Methods). Neurons with values ≥ 0.5 are considered to prefer BOS over tutor song, and neurons with values ≤ -0.5 are considered to prefer tutor over BOS. Gray shading highlights those values for which there was no strong preference for one song over the other ($-0.5 < d'_{\text{BOS-tutor}} < 0.5$).

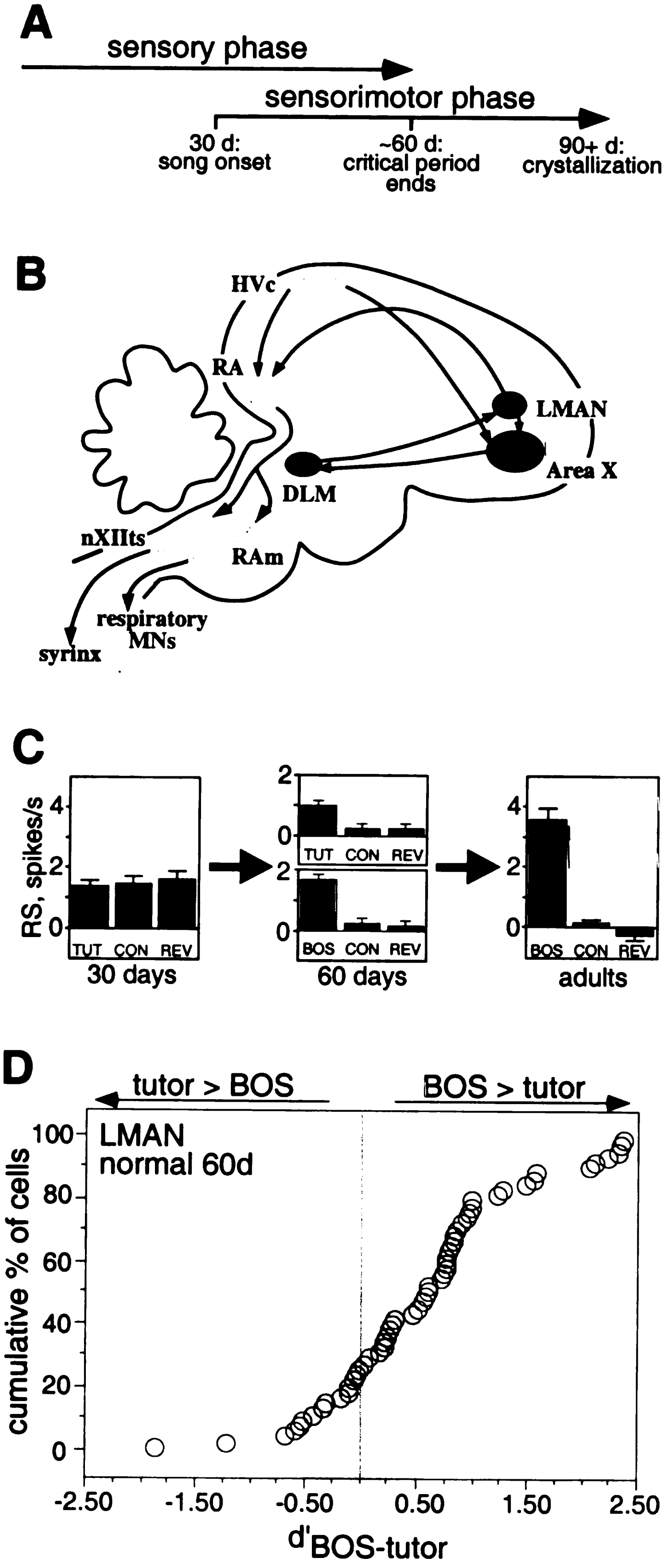

A
sensory phase
sensorimotor phase
30 d:
song onset
~60 d:
critical period
ends
90+ d:
crystallization
B
HVc
RA
LMAN
Area X
DLM
nXIIts
RAm
respiratory
MNs
syrinx
C
RS, spikes/s
4
2
0
TUT
CON
REV
30 days
BOS
60 days
adults
D
tutor > BOS
BOS > tutor
LMAN
normal 60d
cumulative % of cells
100
80
60
40
20
0
-2.50
-1.50
-0.50
0.50
1.50
2.50
$d'_{\mathrm{BOS\text{-}tutor}}$

Figure 3-1

Figure 3-2 Consequences of decreasing similarity between BOS and tutor song. A) When a juvenile stores a good copy of the tutor song (A) as its template (A), and accurately models its own song after the template, the resulting BOS (A) will highly resemble the tutor song. Thus, if a neuron is tuned by BOS experience only, it could also respond well to tutor song when the two songs are similar enough. This ambiguity could be resolved by making the BOS very different (B) from the tutor song. B) Decreasing the similarity between BOS and tutor song has two predicted outcomes on the distribution of $d'_{BOS\text{-}tutor}$ values. If BOS experience shapes some neurons, and tutor song experience shapes others, then the distribution should be split in two, with some neurons preferring tsBOS over tutor song, and others preferring tutor over tsBOS, but none responding equally well to both (solid line). Alternatively, if both tsBOS and tutor song influence the neural properties of single neurons, then neurons with equivalent responses should persist (dotted line). C) If a poor copy of the tutor song (A) is stored as the template (a), and then a good copy of the template is produced, then the resulting BOS (a) is a better model of the template than the tutor song itself. In this case, neurons preferring BOS would nonetheless reflect tutor song experience. Inducing an abnormal BOS by disrupting sensorimotor learning (B) should decrease the similarity between BOS and a song resulting from poor memorization of the tutor song.

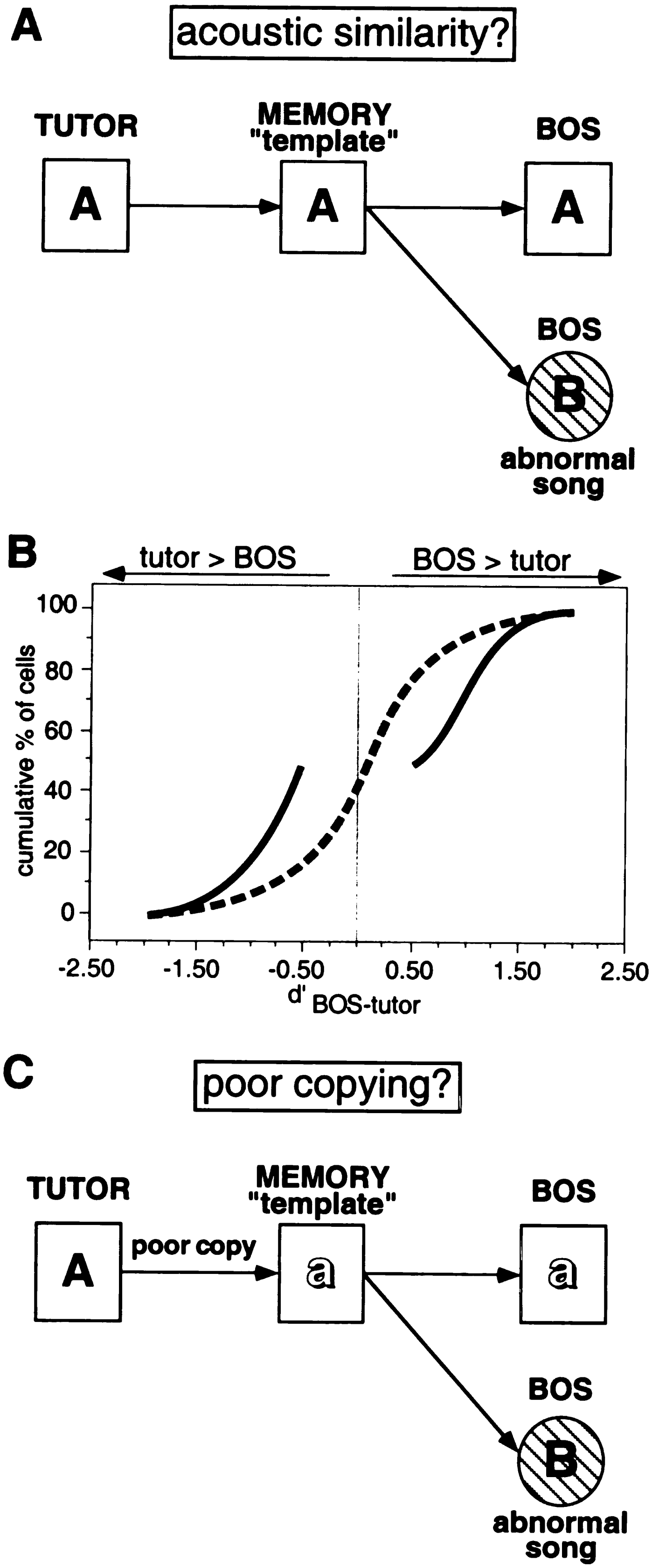

A
acoustic similarity?
TUTOR
MEMORY
"template"
BOS
A
A
A
BOS
B
abnormal
song
B
tutor > BOS
BOS > tutor
100
80
60
40
20
0
cumulative % of cells
-2.50
-1.50
-0.50
0.50
1.50
2.50
d' BOS-tutor
C
poor copying?
TUTOR
MEMORY
"template"
BOS
A
poor copy
a
a
BOS
B
abnormal
song

Figure 3-2

Figure 3-3 NXIIts nerve transections minimized the similarity between BOS and tutor song at 60 d. A) Sonogram and oscillogram of the song of a ts cut bird at 60 d, which underwent nerve transections at 29 d. Sonograms plot frequency versus time, and the energy of each frequency band is indicated by its darkness; oscillograms plot the amplitude of the song waveform versus time. The scale bar represents 500 ms, and also applies to the songs in B and C. B) Tutor song of the ts cut bird in A. Introductory notes (I) and syllables (A, B) are labelled. C) Song of a 60 d juvenile whose tutor song is also shown in B. Syllables that resemble those in the tutor song are labelled (i.e., syllable b in the juvenile song is similar to syllable B in the tutor song). D) Measures of similarity to tutor song from the matching task are shown for different bird groups. Black circles show the mean percentage of observers that matched a song to the correct tutor song (left-hand ordinate). This mean averages the frequency of matching across all songs in each song type. There is no percentage for random matches because their correct tutor was never present among the tutor song choices. The mean spectral (open circles) and temporal (open triangles) similarity scores are plotted along the right-hand ordinate. Error bars are SEM.

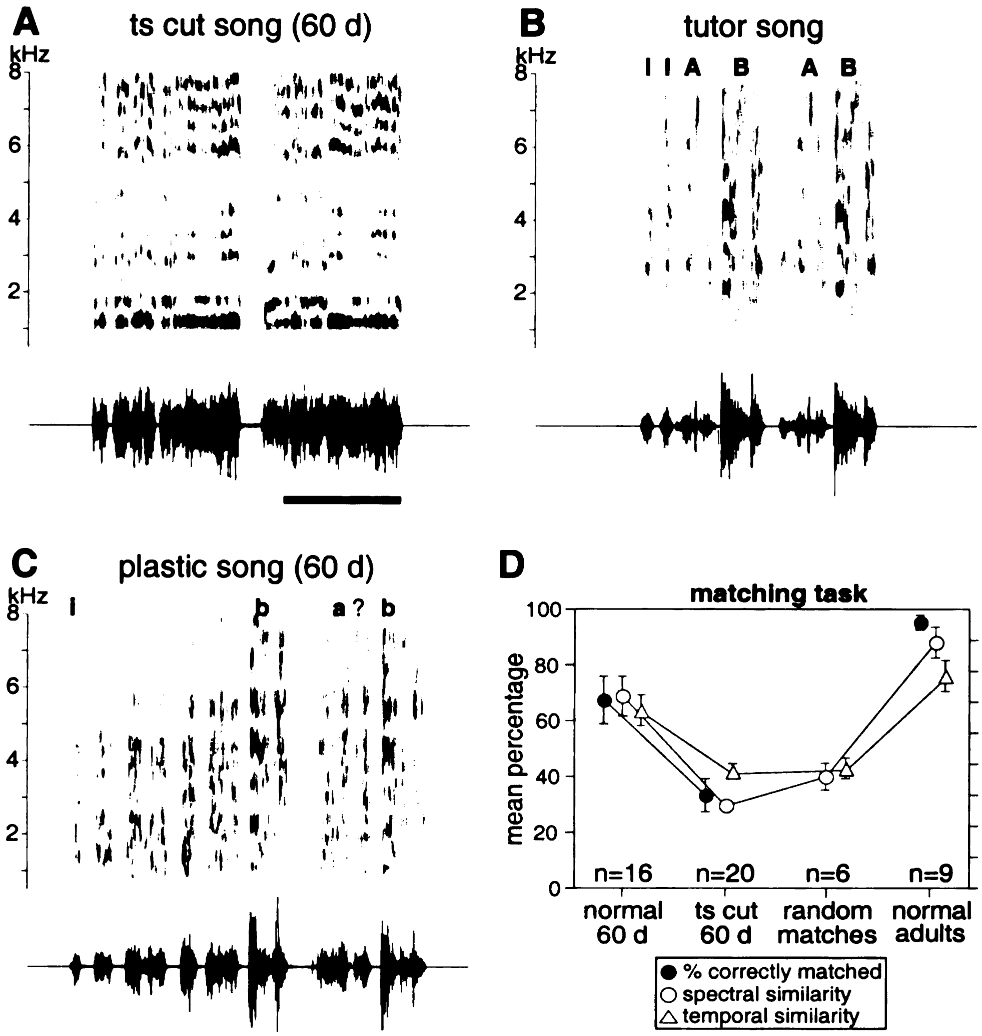

A
ts cut song (60 d)
kHz
8
6
4
2
B
tutor song
kHz
I I A B A B
8
6
4
2
C
plastic song (60 d)
kHz
I b a ? b
8
6
4
2
D
matching task
100
80
60
40
20
0
mean percentage
1.6
1.2
0.8
0.4
0.0
mean score
n=16
n=20
n=6
n=9
normal 60 d
ts cut 60 d
random matches
normal adults
● % correctly matched
○ spectral similarity
△ temporal similarity

Figure 3-3

Figure 3-4 LMAN selectivity for tsBOS at 60 d. A) Peristimulus time histograms (PSTHs) show the greater response of a single LMAN neuron to tsBOS than to tutor song, reverse tsBOS, and adult conspecific song; 20 trials of each song were presented. For this neuron, $d'_{\text{tsBOS-tutor}}=1.50$, $d'_{\text{tsBOS-rev}}=1.11$, and $d'_{\text{tsBOS-adult con}}=1.41$. B) The cumulative distribution of tsBOS versus tutor song preferences for all LMAN neurons recorded, as quantified with $d'_{\text{tsBOS-tutor}}$ values, is shown with white circles. For comparison, the distribution of $d'_{\text{BOS-tutor}}$ values from normal 60 d birds is shown with black circles. Gray shading highlights those cells considered to respond equally well to both songs. The inset shows the mean RS of all LMAN neurons recorded to BOS and tutor song for both ts cut (white circles) and normal (black circles) 60 d birds. Error bars are SEM.

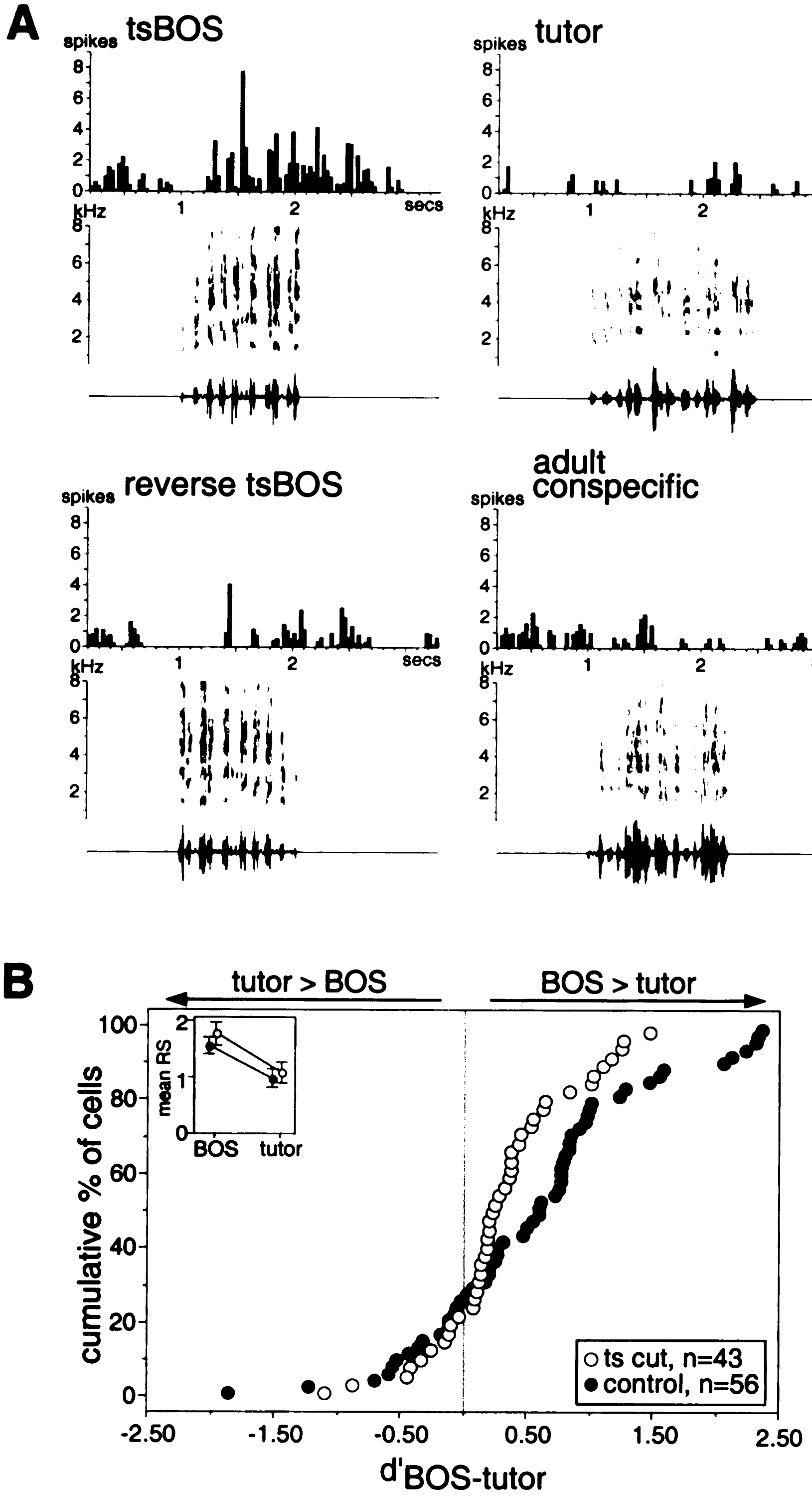

A
spikes
tsBOS
tutor
reverse tsBOS
adult conspecific
8
6
4
2
0
kHz
1
2
secs
B
tutor > BOS
BOS > tutor
cumulative % of cells
100
80
60
40
20
0
mean RS
BOS
tutor
○ ts cut, n=43
● control, n=56
-2.50
-1.50
-0.50
0.50
1.50
2.50
d'BOS-tutor

Figure 3-4

Figure 3-5 Equivalent responses to tsBOS and tutor song. A) PSTHs show the responses of a single LMAN neuron to 13 presentations of each song. While this neuron responded equally well to tsBOS and tutor song ($d'_{tsBOS\text{-}tutor}=0.14$), it did not respond well to adult conspecific song ($d'_{tsBOS\text{-}adult\ con}=1.13$, $d'_{tutor\text{-}adult\ con}=1.08$) nor to reverse tutor song ($d'_{tutor\text{-}rev}=1.08$). B) The tsBOS versus tutor song preference of each LMAN neuron is compared to its selectivity by plotting $d'_{tsBOS\text{-}tutor}$ values against $d'_{tsBOS\text{-}rev}$ (black circles) and $d'_{tutor\text{-}rev}$ (open circles) values. Gray shading indicates those neurons that responded equally well to tsBOS and tutor song. The dashed vertical line marks the criterion for selectivity ($d'=0.5$) C) This histogram shows the number of LMAN neurons classified as selective (solid) and unselective (hatched) in the three different tsBOS versus tutor song preference categories. D) For those neurons responding equally well to both tsBOS and tutor song, histograms show paired comparisons between the mean RS to tsBOS (black bars) or tutor song (white bars) and the mean RS to adult conspecific song. E) For those neurons responding equally well to tsBOS and tutor song, histograms show paired comparisons between the mean RS to tsBOS (black bars) or tutor song (white bars) and the mean RS to their corresponding reverse songs. In D and E, error bars are SEMs, and asterisks denote significant differences between each pair of stimuli.

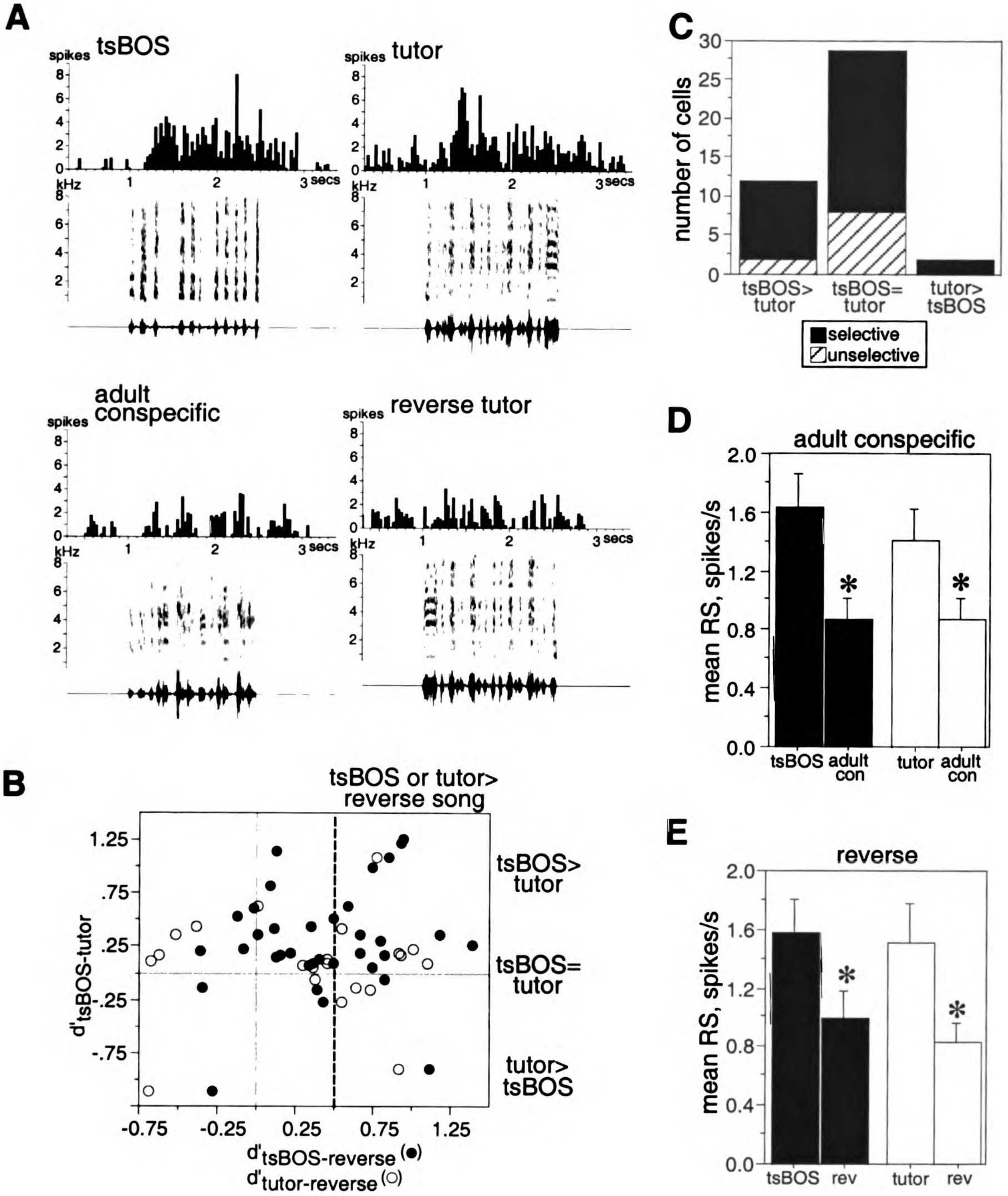

A
spikes tsBOS
8
6
4
2
0
kHz
1
2
3 secs
8
6
4
2
spikes tutor
adult conspecific
spikes reverse tutor
B
tsBOS or tutor> reverse song
1.25
.75
.25
-.25
-.75
d'tsBOS-tutor
-0.75
-0.25
0.25
0.75
1.25
d'tsBOS-reverse (●)
d'tutor-reverse (○)
tsBOS> tutor
tsBOS= tutor
tutor> tsBOS
C
number of cells
30
25
20
15
10
5
0
tsBOS> tutor
tsBOS= tutor
tutor> tsBOS
■selective
▨unselective
D
adult conspecific
mean RS, spikes/s
2.0
1.6
1.2
0.8
0.4
0.0
*
*
tsBOS
adult con
tutor
adult con
E
reverse
mean RS, spikes/s
2.0
1.6
1.2
0.8
0.4
0.0
*
*
tsBOS
rev
tutor
rev

Figure 3-5

Figure 3-6 Stimulus duration can influence the quantification of tsBOS versus tutor song preference. A) PSTHs show the responses of a single LMAN neuron to 12 presentations of tsBOS and a short tutor song (602 ms). Although the responses appear equivalent, the $d'_{\text{tsBOS-tutor}}$ value indicates a preference for the shorter tutor song. B) The distributions of peak $d'_{\text{tsBOS-tutor}}$ values are shown for all LMAN (black) and X (white) cells, regardless of stimulus duration. C) For those cells responding equally well to tsBOS and tutor song (according to their peak $d'_{\text{tsBOS-tutor}}$ values), histograms show the mean peak RS to different stimuli for LMAN (left panel) and X (right panel) cells. Paired comparisons show that tsBOS (black bars) and tutor song (white bars) elicited greater average responses than did adult conspecific song; asterisks denote significant differences, and error bars are SEM.

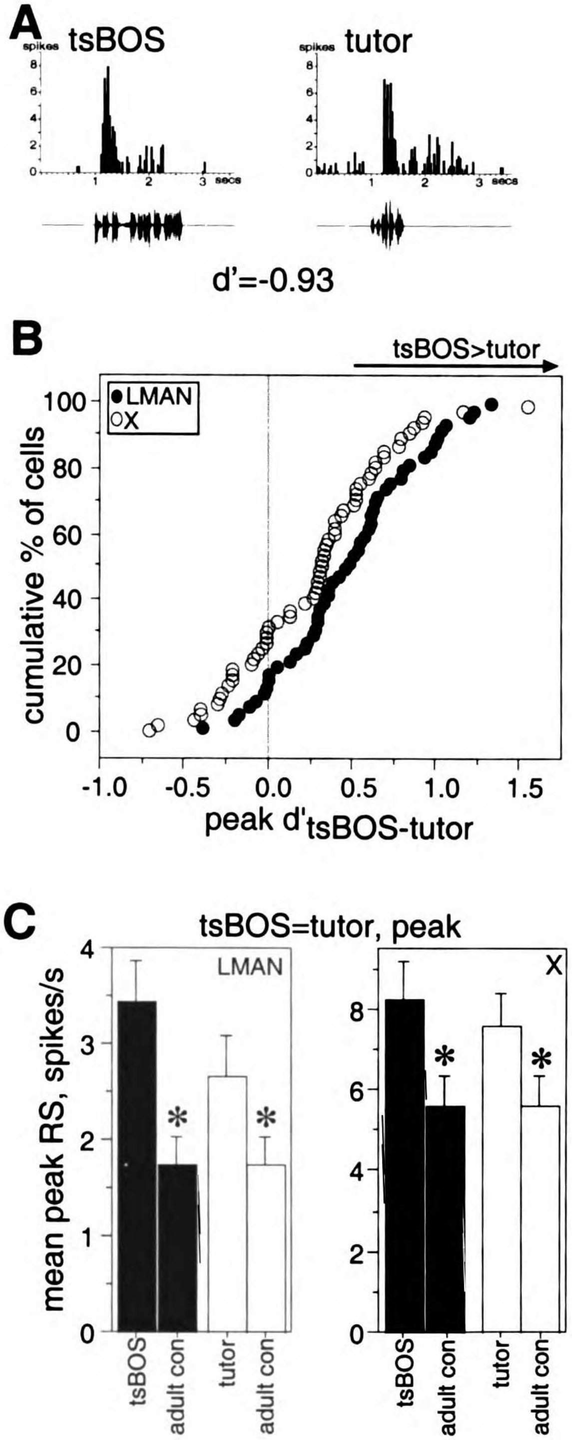

A
tsBOS
tutor
spikes
8
6
4
2
0
1
2
3
secs
d'=-0.93
B
tsBOS>tutor
LMAN
X
cumulative % of cells
100
80
60
40
20
0
-1.0
-0.5
0.0
0.5
1.0
1.5
peak d'tsBOS-tutor
C
tsBOS=tutor, peak
mean peak RS, spikes/s
LMAN
X
4
3
2
1
0
8
6
4
2
0
*
*
*
*
tsBOS
adult con
tutor
adult con
tsBOS
adult con
tutor
adult con

Figure 3-6

Figure 3-7 Song selectivity of the entire population of LMAN neurons recorded in ts cut birds. Paired comparisons of mean RS show that neurons responded more to tsBOS and tutor song than to adult conspecific (A) and heterospecific song (B). C) Paired comparisons also show greater responses to tsBOS than to ts cut and normal 60 d songs. In A,B, and C, error bars are SEM, and asterisks mark significant differences between song pairs. D) The mean RS to tsBOS of each neuron is plotted against its mean RS to adult conspecific song ("adult con"). The diagonal line marks where cells lie if the RS to the two stimuli were equal. Black circles indicate those neurons with significantly greater responses to the stimulus on the abscissa ($p<0.05$, unpaired t-test between abscissa stimulus trials and all adult conspecific trials). E) The mean RS to tutor song of each neuron is plotted against the mean RS to adult conspecific song. Conventions are as in D.

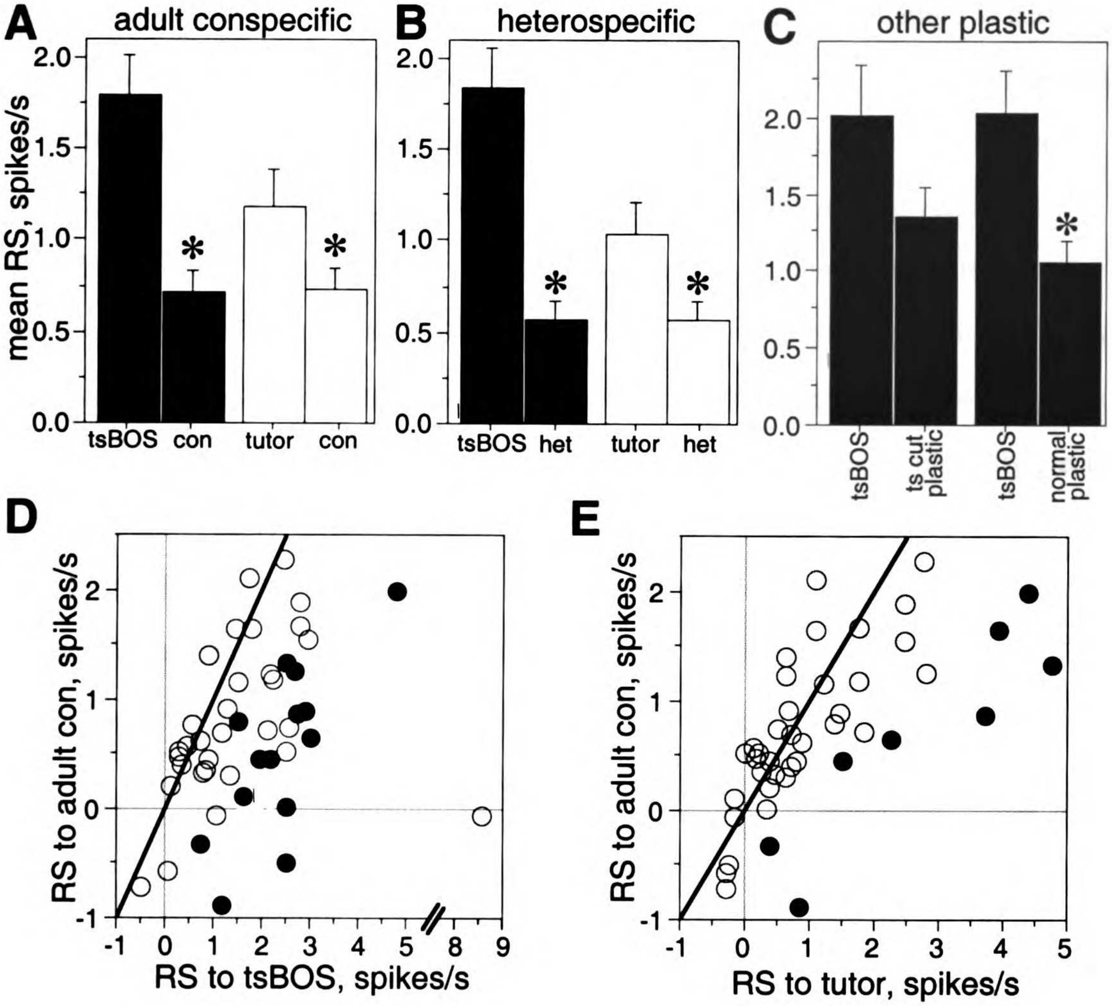

A
adult conspecific
mean RS, spikes/s
2.0
1.5
1.0
0.5
0.0
tsBOS
con
tutor
con
*
*
B
heterospecific
2.0
1.5
1.0
0.5
0.0
tsBOS
het
tutor
het
*
*
C
other plastic
2.0
1.5
1.0
0.5
0.0
tsBOS
ts cut plastic
tsBOS
normal plastic
*
D
RS to adult con, spikes/s
RS to tsBOS, spikes/s
-1
0
1
2
3
4
5
8
9
E
RS to adult con, spikes/s
RS to tutor, spikes/s
-1
0
1
2
3
4
5

Figure 3-7

Figure 3-8 Order selectivity of the population of LMAN neurons recorded from ts cut birds. A) Paired comparisons of mean RS show that neurons responded more to tsBOS and tutor song in the forward direction than to their respective reverse songs. B) The mean RS to tsBOS and tutor song were greater than those to reverse order versions of these songs. C) The mean RS to tsBOS was greater than to the syllable reverse version of tsBOS. In A, B, and C, error bars are SEM and asterisks mark significant differences between song pairs. D) The mean RS to tsBOS of each neuron is plotted against its mean RS to reverse tsBOS. The diagonal line shows where cells lie when they respond equally to the two stimuli compared. Black circles indicate those cells that had significantly greater RS to the stimulus on the abscissa than to the reverse manipulation ($p<0.05$, unpaired t-test between forward song trials and corresponding reverse song trials). E) The mean RS to tutor song of each neuron is plotted against its mean RS to reverse tutor song. Conventions are as in D.

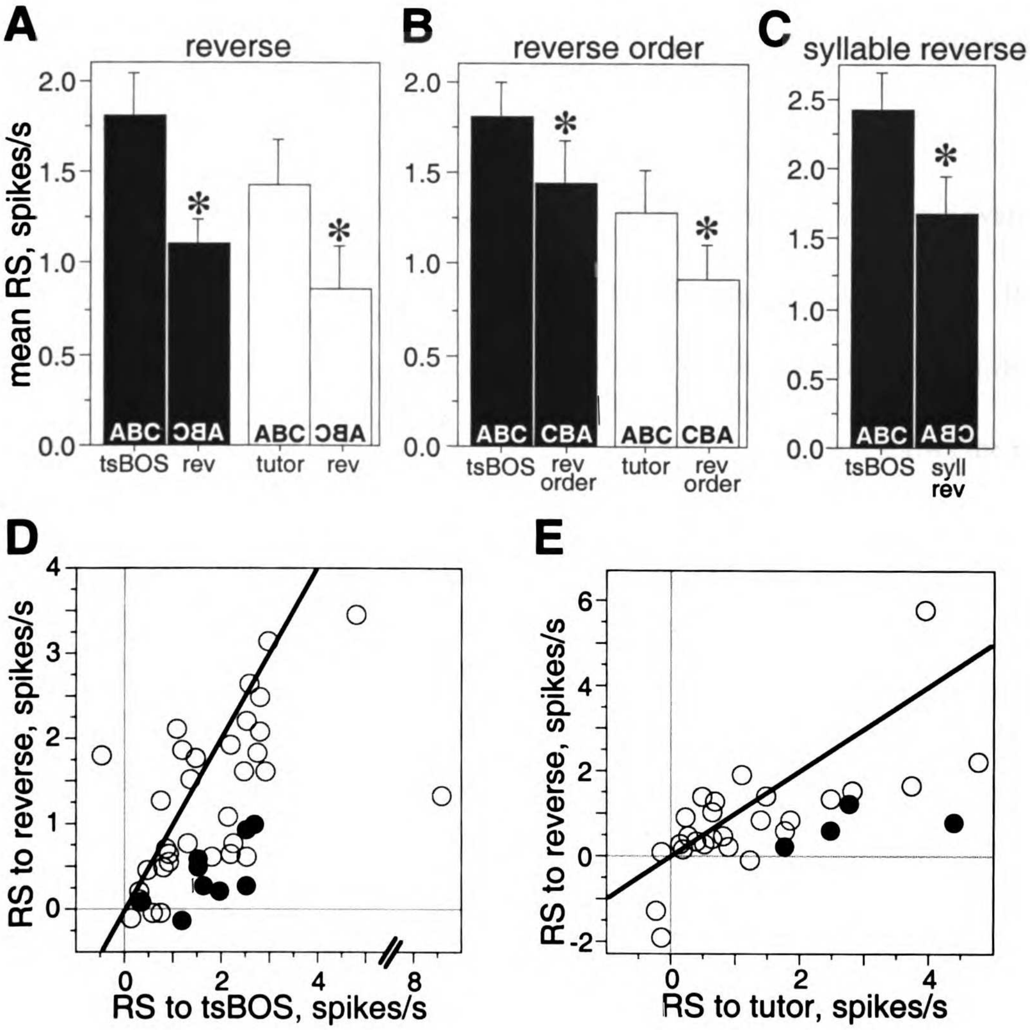

A
reverse
mean RS, spikes/s
2.0
1.5
1.0
0.5
0.0
ABC
ƆBA
ABC
ƆBA
tsBOS
rev
tutor
rev
*
*
B
reverse order
2.0
1.5
1.0
0.5
0.0
ABC
CBA
ABC
CBA
tsBOS
rev order
tutor
rev order
*
*
C
syllable reverse
2.5
2.0
1.5
1.0
0.5
0.0
ABC
AB Ɔ
tsBOS
syll rev
*
D
RS to reverse, spikes/s
4
3
2
1
0
0
2
4
8
RS to tsBOS, spikes/s
E
RS to reverse, spikes/s
6
4
2
0
-2
0
2
4
RS to tutor, spikes/s

Figure 3-8

Figure 3-9 Selectivity for tsBOS in X. A) PSTHs show the responses of a single X neuron to 20 presentations of each stimulus. This neuron responded more to tsBOS than to tutor song, reverse tsBOS, and an adult conspecific song ($d'_{\text{tsBOS-tutor}}$=1.52, $d'_{\text{tsBOS-rev}}$=0.80, and $d'_{\text{tsBOS-adult con}}$=2.35). The dashed white line indicates the neuron's average spontaneous firing rate. Note that the ordinate of the PSTHs begins at 10 spikes/s. B) The cumulative distributions of $d'_{\text{tsBOS-tutor}}$ values of individual X neurons from ts cut birds (white circles) and normal 60 d birds (black circles) are shown. The inset shows the mean RS to BOS and tutor song of the population of X neurons recorded from ts cut (white circles) and normal (black circles) 60 d birds. Error bars are SEM.

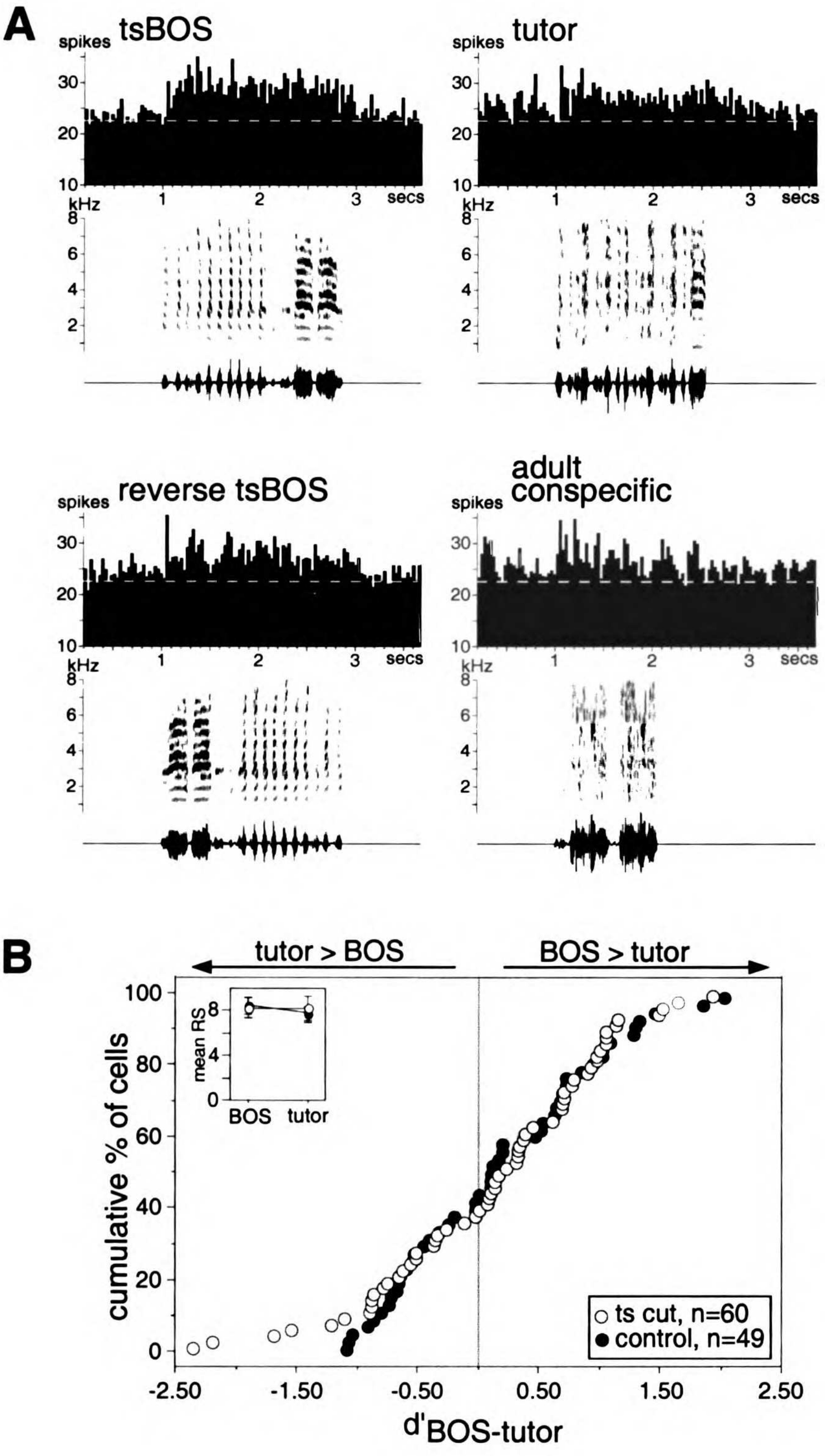

A
spikes tsBOS
spikes tutor
spikes reverse tsBOS
spikes adult conspecific
30
20
10
1
2
3
secs
kHz
8
6
4
2
B
tutor > BOS
BOS > tutor
cumulative % of cells
100
80
60
40
20
0
mean RS
8
4
0
BOS
tutor
○ ts cut, n=60
● control, n=49
-2.50
-1.50
-0.50
0.50
1.50
2.50
d'BOS-tutor

Figure 3-9

Figure 3-10 Some X neurons responded equally well to tsBOS and tutor song. A) PSTHs made from the responses of a single X neuron to 10 presentations of each stimulus are shown. This neuron responded more to tsBOS and tutor song than to reverse tsBOS or adult conspecific. For the responses shown, $d'_{\text{tsBOS-tutor}}=0.43$, $d'_{\text{tsBOS-rev}}=1.43$, $d'_{\text{tsBOS-adult con}}=1.06$, and $d'_{\text{tutor-adult con}}=0.96$. The white dashed line indicates the neuron's average spontaneous firing rate. Note that the ordinate of the PSTHs begins at 10 spikes/s. This particular tsBOS was matched to the correct tutor song by only 1 out of 9 observers. B) The $d'_{\text{tsBOS-tutor}}$ value of each X neuron is plotted against two measures of selectivity: $d'_{\text{tsBOS-adult con}}$ (black circles) and $d'_{\text{tutor-adult con}}$ (white circles). The gray region highlights those neurons considered to have responded equally well to tsBOS and tutor song. The dashed vertical line marks the criterion for selectivity ($d'=0.5$). C) The number of X neurons classified as selective (solid) and unselective (hatched) in the three different tsBOS versus tutor song preference categories is shown. D) For those neurons responding equally well to both tsBOS and tutor song, histograms show paired comparisons of the mean RS to tsBOS (black bars) or tutor song (white bars) to adult conspecific song. E) For those neurons responding equally well to tsBOS and tutor song, histograms show paired comparisons of the mean RS to tsBOS (black bars) or tutor song (white bars) and their corresponding reverse songs. In D and E, error bars are SEMs, and asterisks denote significant differences between each pair of stimuli.

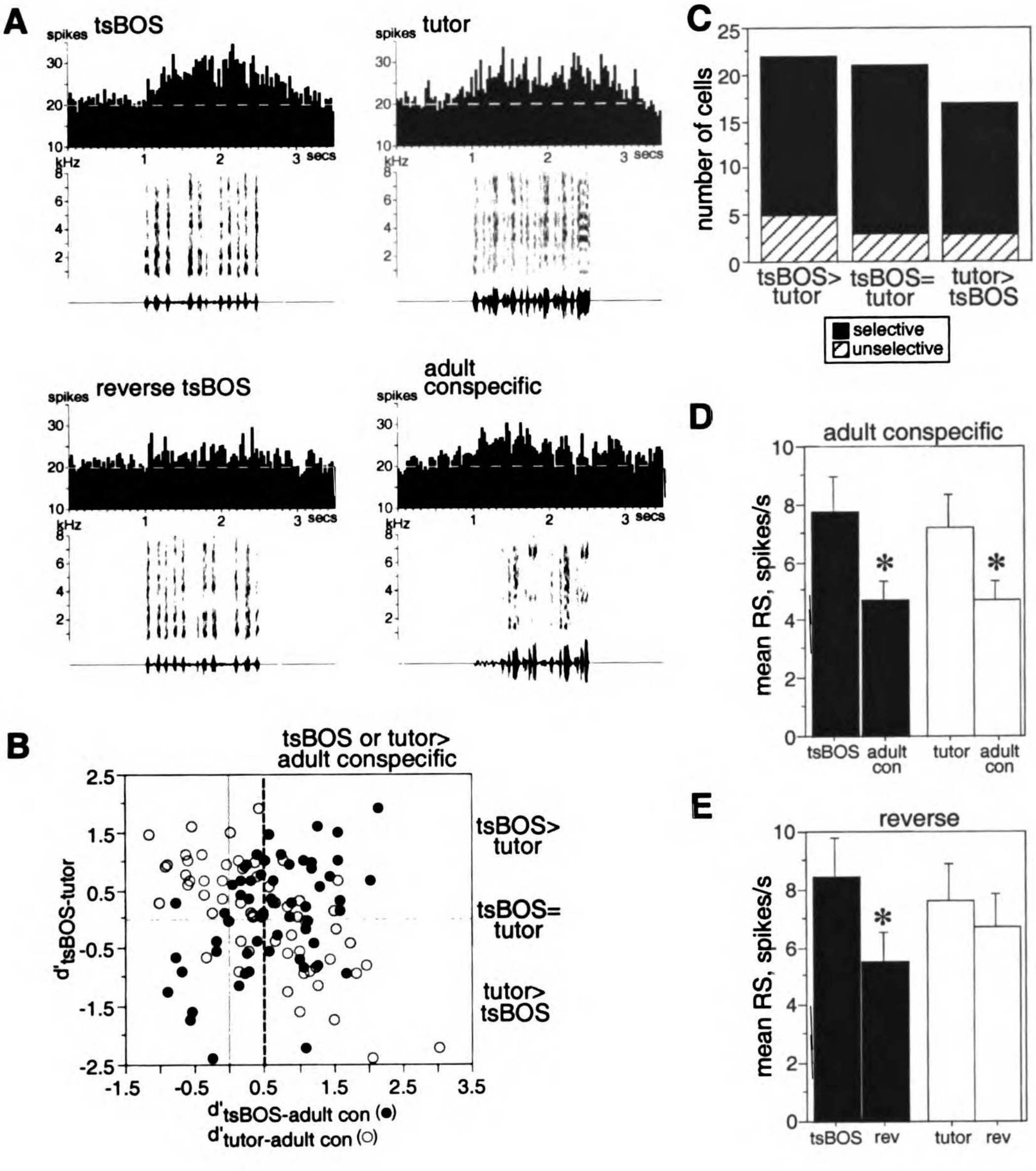

A
spikes tsBOS
spikes tutor
spikes reverse tsBOS
spikes adult conspecific
30
20
10
1
2
3 secs
kHz
8
6
4
2
B
tsBOS or tutor> adult conspecific
tsBOS> tutor
tsBOS= tutor
tutor> tsBOS
d'tsBOS-tutor
2.5
1.5
0.5
-0.5
-1.5
-2.5
-1.5
-0.5
0.5
1.5
2.5
3.5
d'tsBOS-adult con (●)
d'tutor-adult con (○)
C
number of cells
25
20
15
10
5
0
tsBOS> tutor
tsBOS= tutor
tutor> tsBOS
■ selective
▨ unselective
D
adult conspecific
mean RS, spikes/s
10
8
6
4
2
0
*
*
tsBOS
adult con
tutor
adult con
E
reverse
mean RS, spikes/s
10
8
6
4
2
0
*
tsBOS
rev
tutor
rev

Figure 3-10

Figure 3-11 Song selectivity of the entire population of X neurons recorded in ts cut birds. Paired comparisons of mean RS show that neurons responded more to tsBOS and tutor song than to A) adult conspecific and B) heterospecific song. C) Paired comparisons also show greater responses to tsBOS than to ts cut and normal 60 d songs. In A, B, and C, error bars are SEMs and asterisks indicate significant differences between song pairs. D) The mean RS to tsBOS of each neuron is plotted against its mean RS to adult conspecific song (adult con). The diagonal line marks where cells lie if the RS to each stimulus were equal. Black circles indicate those neurons with significantly greater responses to stimulus on the abscissa ($p<0.05$, unpaired t-test between abscissa stimulus trials and all adult conspecific trials). E) The mean RS to tutor song of each neuron is plotted against the mean RS to adult conspecific song. Conventions are as in D.

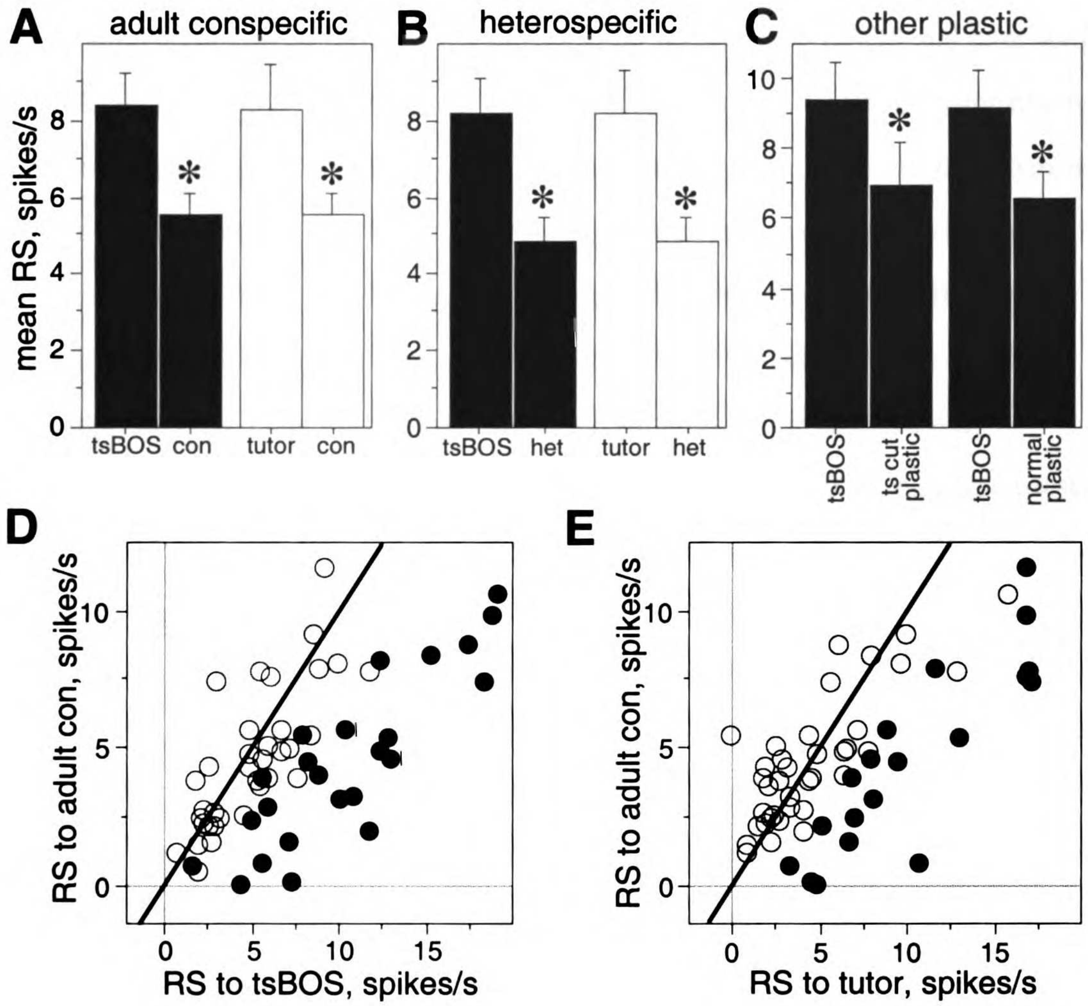
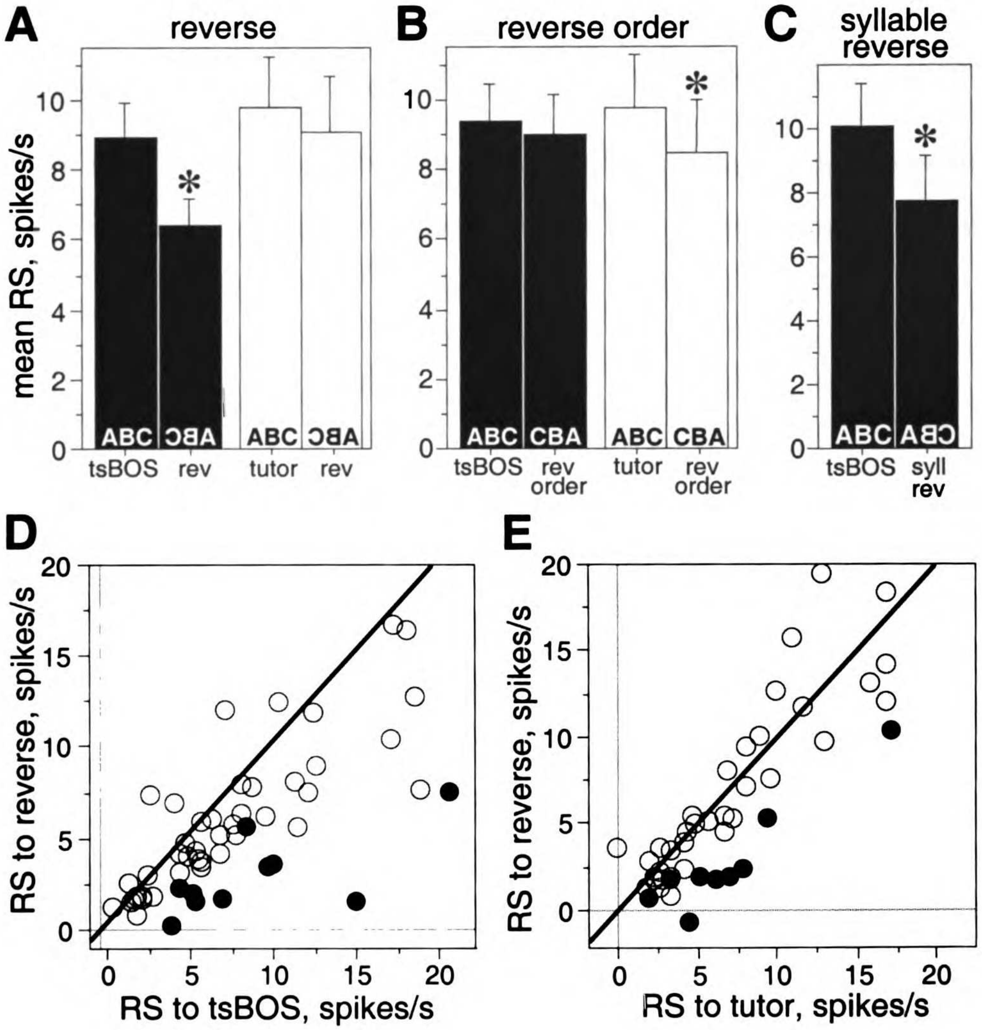

A
adult conspecific
B
heterospecific
C
other plastic
mean RS, spikes/s
0
2
4
6
8
10
*
tsBOS
con
tutor
con
tsBOS
het
tutor
het
tsBOS
ts cut plastic
tsBOS
normal plastic
D
E
RS to adult con, spikes/s
RS to tsBOS, spikes/s
RS to tutor, spikes/s
0
5
10
15

Figure 3-11

Figure 3-12 Order selectivity of the entire population of X neurons recorded in ts cut birds. Paired comparisons of mean RS show greater responses to tsBOS and tutor song than to A) reverse and B) reverse order versions of these songs. C) Paired comparisons show greater RS to tsBOS than to syllable reverse tsBOS. In A, B, and C, error bars are SEMs and asterisks mark significant differences between song pairs. D) The mean RS of each X neuron to tsBOS is plotted against its mean RS to reverse tsBOS. The diagonal line indicates where cells would lie if their responses to the two stimuli were equal. Black circles indicate those cells whose responses to the stimulus on the abscissa were significantly greater than its responses to the ordinate stimulus ($p<0.05$, unpaired t-test). E) The mean RS of each X neuron to tutor song is plotted against its mean RS to reverse tutor song. Conventions are as in D.



A
reverse
B
reverse order
C
syllable reverse
mean RS, spikes/s
0
2
4
6
8
10
*
ABC
ƆBA
ABC
ƆBA
tsBOS
rev
tutor
rev
ABC
CBA
ABC
CBA
tsBOS
rev order
tutor
rev order
ABC
ABƆ
tsBOS
syll rev
D
E
RS to reverse, spikes/s
RS to tsBOS, spikes/s
RS to tutor, spikes/s
0
5
10
15
20

Figure 3-12

Figure 3-13 The degree of selectivity of AF neurons from ts cut 60 d birds (white circles) is less than that observedin normal 60 d neurons (black circles). The mean d′ values for each selectivity category (listed on the ordinate) are plotted along the abscissas; error bars are SEM. Asterisks denote significant differences between ts cut and normal 60 day birds.

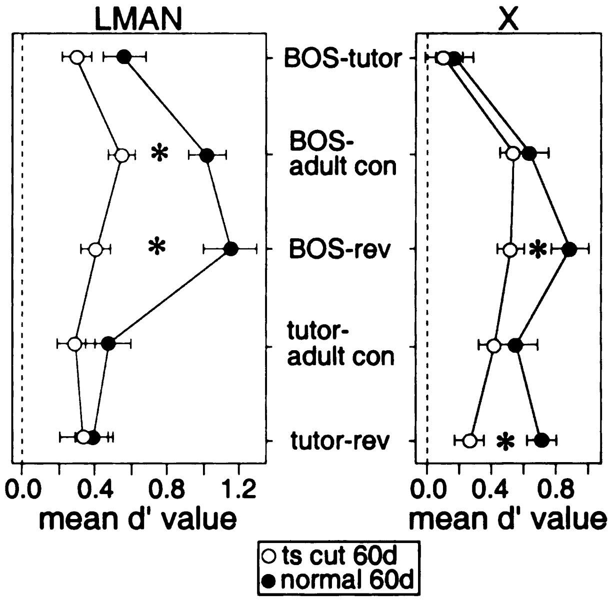

LMAN
X
BOS-tutor
BOS-
adult con
BOS-rev
tutor-
adult con
tutor-rev
*
*
*
*
0.0
0.4
0.8
1.2
mean d' value
0.0
0.4
0.8
mean d' value
○ ts cut 60d
● normal 60d

Figure 3-13

Figure 3-14 Clustering of $d'_{\text{tsBOS-tutor}}$ values within each bird. The individual $d'_{\text{tsBOS-tutor}}$ values obtained from each LMAN (open circles) and X (gray circles) cell recorded from the same bird are shown; each number on the ordinate refers to an individual bird.

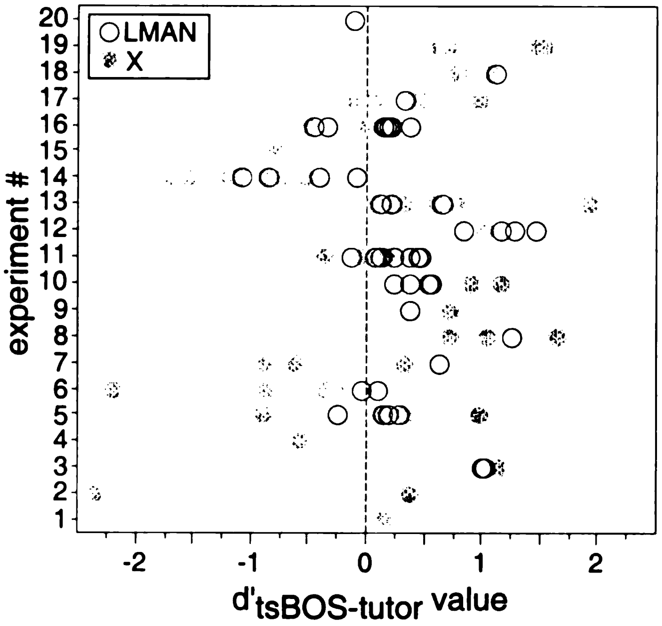

○ LMAN
● X
experiment #
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
-2
-1
0
1
2
d'tsBOS-tutor value

Figure 3-14

Figure 3-15 A) If residual acoustic similarity between tsBOS and tutor song accounts for the neural tsBOS versus tutor song preferences, then the trends shown with the gray dashed lines in the upper panel are expected. For both scenarios, high similarity between tsBOS and tutor song is associated with low $d'_{\text{tsBOS-tutor}}$ values. If tsBOS shapes neural selectivity, then low similarity should be associated with strong tsBOS preference over tutor song; conversely, if tutor song shapes selectivity, then low similarity should be associated with strong tutor song preference. The lower panel combines these two trends by plotting the absolute values of $d'_{\text{tsBOS-tutor}}$ against similarity. According to this similarity hypothesis, large mean $|d'_{\text{tsBOS-tutor}}|$ values should occur in birds with low similarity to tutor song, and smaller mean $|d'_{\text{tsBOS-tutor}}|$ values should occur in those with higher similarity to tutor song. B) The percent of observers who correctly matched the tsBOS song to the tutor song is plotted against the mean $|d'_{\text{tsBOS-tutor}}|$ values obtained for each bird. The regression coefficient (slope) is -0.219±0.853 (±95% confidence intervals). Points to the right of the dashed line refer to birds whose frequency of correct matches by 9 observers was greater than expected by chance ($p<0.05$, sign test). In B, C, and D, error bars are SEMs, and the thick black line is the linear least squares fit of the data. C) The mean spectral similarity score for each ts cut bird is plotted against the mean $|d'_{\text{tsBOS-tutor}}|$ value for neurons recorded from that bird; the regression coefficient is 0.869±2.781. The dashed line marks the mean spectral similarity score given to randomly matched songs. For the abscissa, the error bars refer to the variance in observer scoring; because of normalization, a value of 0.4 refers to low similarity and a value of 2 refers to high similarity. D) The mean temporal similarity score for each ts cut bird is compared to the mean $|d'_{\text{tsBOS-tutor}}|$ value for neurons recorded from that bird. The regression coefficient is -0.388±1.062. The dashed line marks the

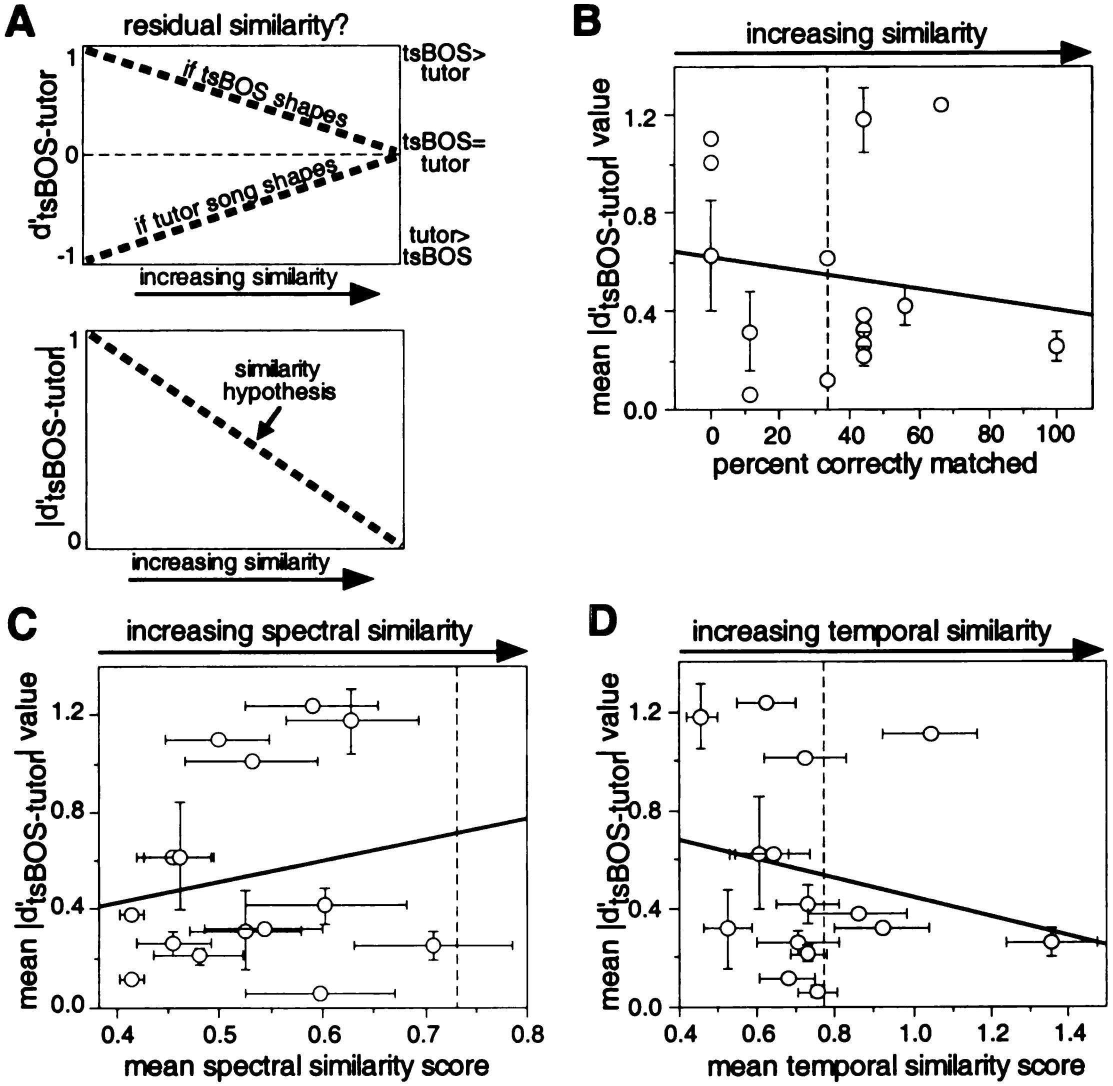

A
residual similarity?
1
0
-1
d'tsBOS-tutor
if tsBOS shapes
if tutor song shapes
tsBOS> tutor
tsBOS= tutor
tutor> tsBOS
increasing similarity
|d'tsBOS-tutor|
1
0
similarity hypothesis
increasing similarity
B
increasing similarity
mean |d'tsBOS-tutor| value
1.2
0.8
0.4
0.0
0
20
40
60
80
100
percent correctly matched
C
increasing spectral similarity
mean |d'tsBOS-tutor| value
1.2
0.8
0.4
0.0
0.4
0.5
0.6
0.7
0.8
mean spectral similarity score
D
increasing temporal similarity
mean |d'tsBOS-tutor| value
1.2
0.8
0.4
0.0
0.4
0.6
0.8
1.0
1.2
1.4
mean temporal similarity score

Figure 3-15

Figure 3-16 Automated measures of similarity between experimental and tutor song pairs. A) Mean cross-correlation measures (left ordinate) for each song group are shown, obtained from entire spectrogram (open circles), syllables only (black squares), and temporal envelope (open triangles) comparisons. In addition, mean overlap values (right ordinate) are plotted for song-song overlap (open squares) and motif-song overlap (black triangles) values. Error bars are SEM. B) Each bird's mean $|d'_{tsBOS\text{-}tutor}|$ value of LMAN neurons is plotted against the syllable only cross-correlation measures obtained from comparisons of tsBOS to tutor song. The thick black line is the least squares fit of the data, the vertical dashed line indicates the mean obtained for randomly matched songs, and error bars are SEM. The regression coefficient (slope) is -2.437±3.816 (±95% confidence intervals). C) Each bird's mean $|d'_{tsBOS\text{-}tutor}|$ value of LMAN neurons is plotted against the motif-song overlap values, which measure temporal similarity between tsBOS and tutor song. Conventions as in B. The regression coefficient is 0.714±0.595.

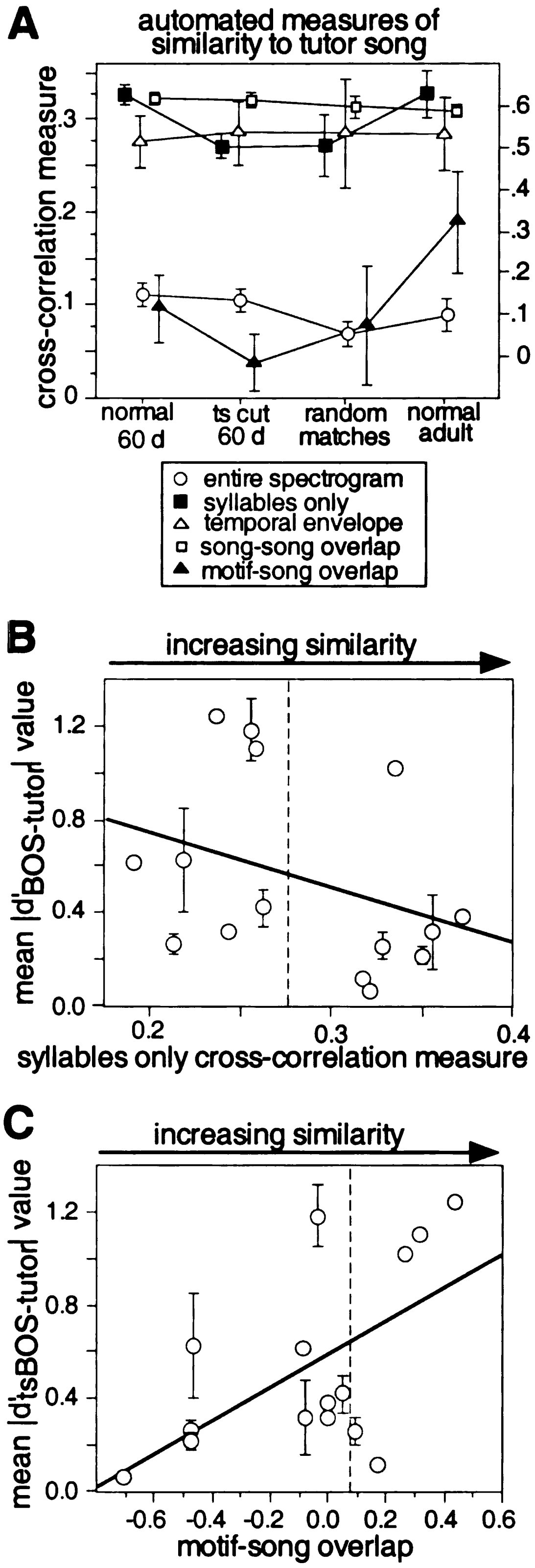

A
automated measures of similarity to tutor song
cross-correlation measure
0
.1
.2
.3
overlap value
0
.1
.2
.3
.4
.5
.6
normal 60 d
ts cut 60 d
random matches
normal adult
○ entire spectrogram
■ syllables only
△ temporal envelope
□ song-song overlap
▲ motif-song overlap
B
increasing similarity
mean |d'BOS-tutor| value
0.0
0.4
0.8
1.2
0.2
0.3
0.4
syllables only cross-correlation measure
C
increasing similarity
mean |d'tsBOS-tutor| value
0.0
0.4
0.8
1.2
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
motif-song overlap

Figure 3-16

Figure 3-17 Song stereotypy analysis. A) Three measures of stereotypy are compared for different song groups. The mean syllables only cross-correlation measures (open circles) and motif-song overlap values (solid triangles) are shown on the left-hand ordinate, and the mean human stereotypy scores are shown on the right-hand ordinate. Error bars are SEM, and in one case are smaller than the symbol itself. B) The mean $d'_{\text{tsBOS-tutor}}$ value of LMAN neurons from each ts cut bird are compared to the human scores of stereotypy. Because individual scores were normalized by the observer's mean score, a value of 0.3 refers to low stereotypy, and a value of 1.4 refers to high stereotypy. The thick black lines are least squares fit of the data, and error bars are SEMs. The regression coefficient is 1.133±1.64 (±95% confidence intervals). C) The mean $d'_{\text{tsBOS-tutor}}$ values of LMAN neurons from each ts cut bird are compared to the motif-song overlap measure of stereotypy. The regression coefficient is -0.328±1.42. Conventions are as in B.

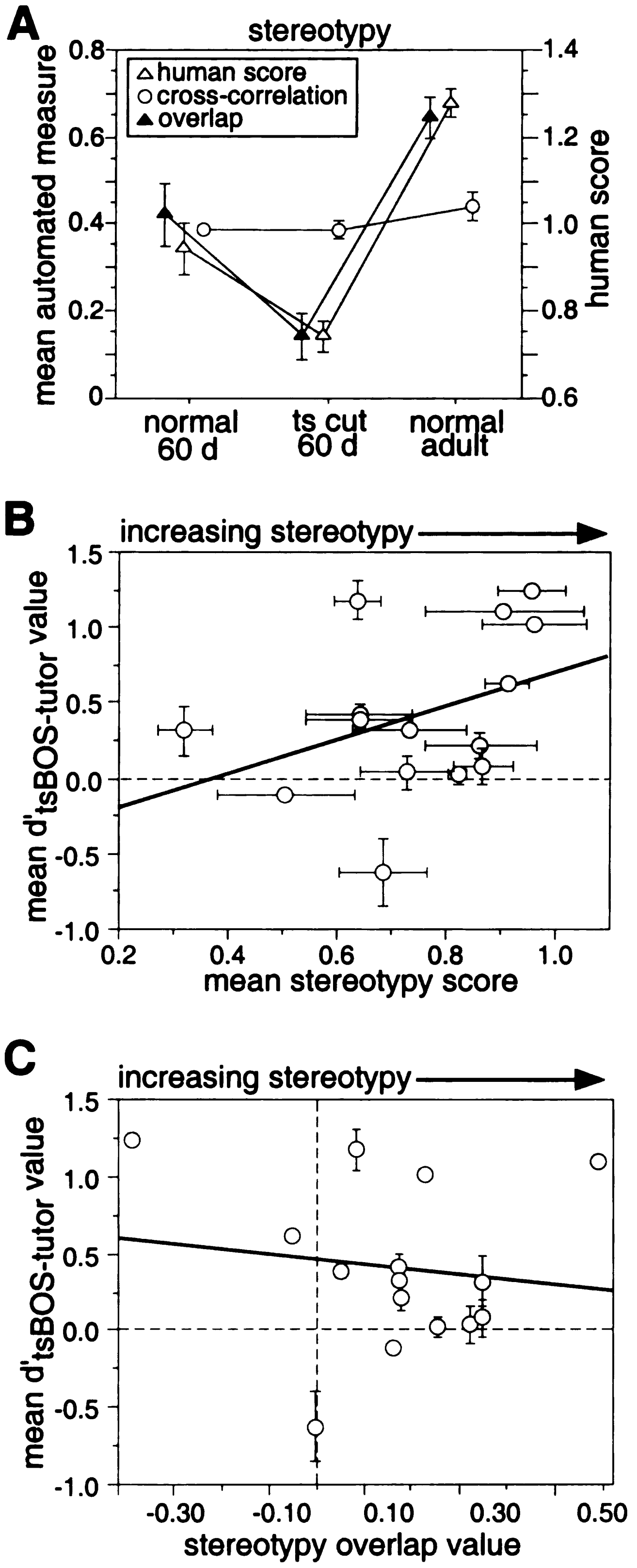

A
stereotypy
△human score
○cross-correlation
▲overlap
mean automated measure
0.8
0.6
0.4
0.2
0
1.4
1.2
1.0
0.8
0.6
human score
normal 60 d
ts cut 60 d
normal adult
B
increasing stereotypy
mean d'tsBOS-tutor value
1.5
1.0
0.5
0.0
-0.5
-1.0
0.2
0.4
0.6
0.8
1.0
mean stereotypy score
C
increasing stereotypy
mean d'tsBOS-tutor value
1.5
1.0
0.5
0.0
-0.5
-1.0
-0.30
-0.10
0.10
0.30
0.50
stereotypy overlap value

Figure 3-17

Table 3-1: Frequency of selective cells

selectivity category	LMAN	X
tsBOS > tutor	12/43 (28%)**	22/60 (37%)
tsBOS > adult conspecific	22/39 (56%)	31/63 (49%)
tsBOS > juvenile conspecific	14/30 (47%)	28/51 (55%)
tsBOS > ts cut juvenile*	7/28 (25%)	28/48 (58%)
tsBOS > heterospecific	27/38 (71%)	36/62 (58%)
tsBOS > reverse tsBOS	15/37 (41%)**	27/54 (50%)**
tsBOS > reverse order tsBOS	4/27 (15%)	10/48 (21%)**
tsBOS > syllable reverse tsBOS	4/15 (27%)	6/15 (40%)
tutor > tsBOS*	2/43 (5%)	17/60 (28%)
tutor > adult conspecific	7/29 (24%)	27/61 (44%)
tutor > heterospecific	16/28 (57%)	31/60 (52%)
tutor > reverse tutor	10/21 (48%)	12/44 (27%)**
tutor > reverse order tutor	6/22 (27%)	13/44 (30%)

The ratio of selective cells over the total tested in each category is shown for LMAN and X cells, followed by the percentages. A cell was considered selective if it had a d′ value of at least 0.5 for the two stimuli compared.

*Comparisons for which the frequency of selective cells was significantly different between LMAN and X (χ^2 tests; $p<0.05$).

**Comparisons for which the frequency of selective cells was significantly lower in ts cut than in normal 60 d birds (χ^2 tests; $p<0.05$; normal 60 d data from Solis and Doupe, 1997).

Table 3-2: Correlations of d′ values

	LMAN, r^2 values		X, r^2 values	
variable vs.$d'_{\text{tsBOS-tutor}}$ value	original d′	peak d′	original d′	peak d′
background firing rate	0.210	0.018	0.036	0.075
maximum RS	0	0.022	0.061	0.058
experiment duration	0.036	0.040	0	0.001
anteroposterior location within nucleus	0.056	0.045	0.117	0.007
mediolateral location within nucleus	0.120	0.202	0.044	0.001
dorsoventral location within nucleus	0	0.001	0.007	0.006
relative intensity of stimuli				
mean:	0.031	0	0.002	0.027
individual:	0	0.002	0.001	0.028
RA volume				
mean:	0.106	0.010	0.192	0.025
individual:	0.043	0	0.105	0.009
age				
mean:	0.209	0.010	0.002	0.107
individual:	0.271	0.006	0.053	0.042

The coefficient of determination (r^2) is shown for the linear least squares fits of the data, which compare each independent variable with the $d'_{\text{tsBOS-tutor}}$ value of a cell or group of cells (mean values). Entries of 0 refer to r^2 values less than 0.001.

Table 3-3: Correlations between d′ values and song characteristics

	LMAN, r^2 value		X, r^2 value	
similarity measure vs. individual $\lvert d'_{tsBOS\text{-}tutor} \rvert$ values	original $\lvert d' \rvert$	peak $\lvert d' \rvert$	original $\lvert d' \rvert$	peak $\lvert d' \rvert$
percent correctly matched	0.059	0.019	0.093	0.001
spectral similarity score	0.007	0.002	0.009	0.007
temporal similarity score	0.120	0.131	0.090	0.050
syllables only cross-correlation	0.063	0	0.007	0.085
motif-song overlap	0.161	0.006	0	0.027
stereotypy measure vs. individual $d'_{tsBOS\text{-}tutor}$ values	original d'	peak d'	original d'	peak d'
human stereotypy score	0.010	0.041	0.034	0.017
motif-song overlap	0.007	0.002	0.001	0.031

The coefficients of determination (r^2) result from the linear least squares fit of the data; each similarity or stereotypy measure is compared to the $d'_{tsBOS\text{-}tutor}$ value of each cell recorded. Entries of 0 refer to r^2 values less than 0.001.

Chapter 4:

Abnormal Neural Selectivity in Adult Songbirds Prevented from Matching their Tutor Song

ABSTRACT

During song learning, auditory neurons of the anterior forebrain (AF) of zebra finches become extremely selective for song stimuli. In adults, these neurons respond more to the bird's own song (BOS) than to the songs of other zebra finches (conspecifics) or BOS played in reverse. In contrast, AF neurons in young birds that have not begun singing (30 d) respond equally well to all song stimuli. Selectivity develops rapidly, however, as it is present in birds at an intermediate stage of song learning (60 d). At this stage, there is selectivity for both BOS and tutor song, indicating that both experiences shape selectivity. To test whether neural correlates of both song experiences persist in adulthood, we assessed the independent contributions of BOS and tutor song to AF selectivity in adult birds. Because adult song closely resembles tutor song, however, it was necessary to minimize acoustic similarities between the two songs. We accomplished this by denervating the syrinx of birds before the onset of singing. These birds usually produced abnormal songs as adults, which were dissimilar from the tutor song. Despite this, they had clearly attained normal adult levels of stereotypy in song production.

The abnormal BOS and the tutor song were used in characterizing AF selectivity with extracellular recordings in anesthetized zebra finches. In Area X, a range of preferences for BOS versus tutor song were found: many neurons preferred tsBOS over tutor song, a few neurons preferred tutor song over BOS, and an unexpected number of neurons responded equally well to BOS and tutor song. The neural responses to BOS and tutor song observed for each cell were not due to acoustic similarity between the two stimuli, and they did not depend on song maturity. Furthermore, although these birds produced crystallized song, their X neurons did not exhibit normal adult levels of selectivity: instead, the degree of selectivity of their X neurons was similar to that observed in 60 d juveniles. In addition, auditory responses from LMAN cells in these adults were rare. The impoverished selectivity in X and the lack of auditory responses in LMAN might

INTRODUCTION

To learn to sing, songbirds depend on auditory experience of both their tutor song and their own vocalizations (Figure 4-1A) (Konishi, 1965; Price, 1979). During the first, or sensory, phase of learning, a young bird listens to and memorizes the song of its tutor; this memory is often called the template. In the second, or sensorimotor, phase of learning the juvenile uses auditory feedback to compare its immature vocalizations to the tutor song template, and gradually modifies its song until it produces a mature "crystallized" song. This song is highly stereotyped and often very closely resembles the tutor song. Thus, neural circuits mediating song learning must process auditory experiences of the bird's own song (BOS) and its tutor song.

Neurons of the songbird anterior forebrain (AF) could process BOS and tutor song experience during learning. The AF belongs to a group of nuclei dedicated to song learning and production (Figure 4-1B), and it plays an essential, but poorly understood role during learning (Bottjer et al, 1984; Sohrabji et al, 1990; Scharff and Nottebohm, 1991; Basham et al, 1996). The AF is also in a position to guide vocal learning via its projection to the nuclei involved in song production. Furthermore, AF neurons seem specialized for processing song. In adult, anesthetized birds, AF neurons are auditory and respond selectively to BOS, strongly preferring it to songs of other zebra finches (conspecific song), or to BOS played in reverse (Doupe and Konishi, 1991). Song selectivity emerges during development: although AF neurons in 30 d birds are auditory, they are not selective as they respond equally well to any song stimulus (Doupe, 1997). By an intermediate stage of song learning (60 d), however, selectivity is apparent in two nuclei of the AF, Area X (X) and the lateral portion of the magnocellular nucleus of the neostriatum (LMAN) (Solis and Doupe, 1997). At this age, selectivity appears to have been shaped by experience of both BOS and tutor song experience, since many AF neurons respond equivalently to BOS and tutor song even in birds with songs that do not resemble the tutor song (Figure 4-1C) (Solis and Doupe, 1999).

Selectivity for both BOS and tutor song could be useful during song learning. Such selective neurons could provide feedback about the current state of BOS, store tutor song information, or compare BOS to tutor song during song learning. Once song learning is complete, however, tutor song information may lose its importance. This could be indicated by a loss of tutor song responses in AF neurons from adult birds, leaving selectivity only for BOS. Alternatively, tutor song responses may persist throughout life, perhaps providing a song reference for the bird. To investigate whether tutor song selectivity in the AF is eventually overwritten by BOS selectivity, or is instead maintained into adulthood, we examined the responses of AF neurons to both songs in adulthood.

Because adult song often resembles the tutor song, interpreting the responses to these two stimuli in adulthood is complicated by their acoustic similarities. Figure 4-1D shows that half of the AF cells recorded in normal adults respond equally well to BOS and tutor song. Similarities between these two songs might actually mask a preference for one song over another that is apparent for other cells. We have previously shown that denervating the syrinx, the avian vocal organ, in young birds prior to song onset reliably prevents birds from producing a song that resembles their tutor song at 60 d (Solis and Doupe, 1999). For the present study, we again performed bilateral transections of the tracheosyringeal portion of the hypoglossal nerve (NXIIts, or ts) before song onset, and found that, even in adulthood, "ts cut" birds produced songs that were unlike the tutor song. Extracellular recordings in these ts cut adults revealed a population of neurons in X that responded equivalently to tsBOS and tutor song, despite the dramatic acoustic differences between them. Furthermore, these X neurons, although selective, were significantly less selective than X neurons found in normal adults. In LMAN, auditory responses were unusually rare. The maintenance of a population of neurons that respond equally well to tsBOS and tutor song in X, the intermediate level of selectivity in X, and the rare auditory responses in LMAN were unexpected qualities of adult neurons. These

MATERIALS AND METHODS

Experiments used male zebra finches (*Taeniopygia guttata*). The care and treatment of experimental animals was reviewed and approved by an university animal care and use committee at UCSF. Birds were raised in individual cages, with their parents and siblings. Opaque dividers between cages visually isolated birds from other conspecfiics in the colony. Because juvenile birds shared a cage with a single adult male tutor and were visually isolated from other conspecifics within earshot, their learning should have been restricted to the tutor in their cage (Immelmann, 1969; Eales, 1987, 1989; Williams, 1990).

Surgery. When birds were approximately 30 d old, the tracheosyringeal portion of the hypoglossal nerve (NXIIts) was transected bilaterally under isofluorane anesthesia (0.5-1.5%v/v; Abbott Laboratories, North Chicago, IL). The nerves were exposed by an incision along the skin of the neck, where lidocaine had been injected subcutaneously (2% solution, Elkins-Sinn, Cherry Hill, NJ). The NXIIts nerve was dissected away from the trachea at the proximal end of the incision and cut; dissection then continued along the length of the neck, and the nerve was pulled to remove the distal end. This removed ~1cm of nerve. Following bilateral transections, the skin was closed with cyanoacrylate skin adhesive. The ts cut birds were returned to their home cages where they remained until they were adults.

Both ts cut and normal adult birds were used in this study. The mean age for ts cut birds was 200 d (range 92-625), and 241 d for normal adults (range 105-336). Two days prior to the experiment, we prepared birds for neurophysiological recording by affixing a head post to the skull, and marking the location of the song nuclei on the skull (see Solis and Doupe, 1997, for details). On the day of the experiment, the bird was anesthetized with a 20% solution of urethane (5mL/kg i.m.; Sigma, St. Louis, MO; delivered in 3 injections at 30 min intervals), placed in the stereotaxic apparatus, and immobilized via its head post. Body temperature was regulated with a temperature controller (FHC, Brunswick, ME). A craniotomy was performed above LMAN and X, the dura was opened, and the electrode was lowered into the brain with a microdrive (Fine Science Tools, Foster City, CA).

Stimuli. One to two days prior to the experiment, the songs of the adult bird and its tutor were recorded. Each bird was placed in a sound-attenuated chamber (Acoustic Systems, Austin, TX) that was connected to an automatically triggered audio system. Approximately 90 minutes of bird sounds were recorded and then scanned for song. A typical song was chosen after listening to at least 25 songs and looking at several song spectrograms. Songs were digitized at 32kHz and stored on a SPARC IPX computer (Sun Microsystems, Palo Alto, CA) at similar peak intensity levels (range was 64-73dB; software by Michael Lewicki and Larry Proctor, California Institute of Technology).

During electrophysiological recording, acoustic stimuli were presented by a speaker 25 cm away from the bird, inside a double-walled anechoic sound-attenuated chamber (Acoustic Systems, Austin, TX). The frequency response measured at the bird's location inside the chamber was flat (±5.0 dB) between 500Hz and 8 kHz. The stimuli included the bird's own song ("BOS" for normal birds; "tsBOS" for ts cut adult birds), tutor song, reversed versions of tsBOS or BOS and tutor song, at least two different songs of other zebra finches (conspecifics), broad band noise bursts and tone bursts. Stimuli were presented in a random, interleaved fashion. An effort was made to present each neuron with 15-20 trials of each stimulus type; however, some neurons were lost before characterization was completed.

Electrophysiology. Extracellular neuronal signals were amplified and filtered between 300Hz and 10kHz (A-M Systems, Everett, WA). To locate auditory neurons, search stimuli included tsBOS or BOS, tutor song, adult conspecific song, heterospecific song, broad band noise bursts and tone bursts. Neurons were isolated with a window discriminator (UCSF Physiology Shop), and their responses were collected and analyzed by a SPARC IPX computer using software developed by Mike Lewicki and Larry Proctor

(California Institute of Technology), and Frédéric Theunissen (UCSF). Electrolytic lesions were made at selected locations for reconstructing recording sites.

Anatomy. At the end of an experiment, the bird was deeply anesthetized with Metofane (Pitman-Moore, Mundelein, IL) and transcardially perfused with 0.9% saline, followed by 3.7% formalin in 0.025M phosphate buffer. Brains were postfixed and cut in 40μm sections with a freezing microtome. Sections were stained with cresyl violet, and electrode tracks and lesions were identified. Only neurons histologically confirmed to be in LMAN or X were used. The syrinx of each ts cut or normal adult bird was also dissected after perfusion. Afterwards, each syrinx was cut 1 mm distal and 4 mm proximal of the bifurcation of the bronchi, and then weighed to assess relative muscle mass, a marker of denervation success.

Data analysis. Neural responses to song were measured as described in detail in Solis and Doupe, 1997. In brief, we quantified responses to an acoustic stimulus during the period of stimulus presentation, offset by an estimate of the latency. To be considered auditory and included for analysis, a neuron had to have an average firing rate during one of the stimuli that was significantly different from the background rate (two-tailed paired t-test, $p<0.05$). The response strength (RS) of a neuron to a stimulus was calculated as the difference between the firing rate during the stimulus (offset by the latency) and the background rate. The selectivity of an individual neuron for one stimulus (A) over another (B) was quantified using the d'_{A-B} measure (Green and Swets, 1966), where

$d'_{A-B} = \frac{2(\overline{RS_A} - \overline{RS_B})}{\sqrt{\sigma_A^2 + \sigma_B^2}}$. In this equation, $\overline{RS_A}$ and $\overline{RS_B}$ are the mean RS to stimulus A and B, respectively, and σ^2 is the variance of each RS. A neuron was considered selective for stimulus A over stimulus B if it had a d'_{A-B} value ≥ 0.5. Neural selectivity data from previous studies were also used here for comparison (30 d data from Doupe, 1997; normal 60 d data from Solis and Doupe, 1997; ts cut 60 d data from Solis and Doupe, 1999).

Cluster analysis. We tested whether the $d'_{\text{BOS-tutor}}$ values of neurons recorded from each bird were more similar than expected by chance. To do this, the variance of the $d'_{\text{BOS-tutor}}$ values obtained experimentally from each bird was compared to a simulated distribution of variances created from the data from all birds. This distribution was determined from 1000 Monte Carlo simulations; this procedure is described in detail in Solis and Doupe, 1997. If the experimental variance was less than the median of the simulated distribution, the $d'_{\text{BOS-tutor}}$ values from that bird were considered clustered.

Song analysis: similarity. Once electrophysiology experiments were completed, we analyzed the acoustic similarity between tutor song and BOS from normal adults and tsBOS from ts cut adults. Song itself is composed of syllables, which are continuous acoustical signals, 10-200 ms in duration. Syllables are separated from other syllables by a sudden fall in amplitude to near zero or by brief silent intervals. Syllables are composed of smaller continuous signals called "notes." A repeated sequence of syllables is a "motif." A song "bout" consists of introductory notes followed by one or more motifs (for detailed song descriptions, see Price, 1979; Sossinka and Böhner, 1980).

The methods of song similarity analysis used here have been described in detail elsewhere (Solis and Doupe, 1999). Briefly, song similarity was judged in a matching task, completed by human observers familiar with zebra finch song, but blind to the neural properties of each bird. Observers tried to match each experimental song with that of its tutor, which was present among a group of six potential tutors, by listening and looking at sonograms and oscillograms of the songs. Thus, the percentage of observers that correctly matched the experimental song to its tutor song indicated the overall similarity between BOS and tutor song. After selecting a "best match" tutor song, observers also scored the song pair on spectral similarity and on temporal similarity, using a scale from 1-5. To judge temporal similarity, observers disregarded the spectral features of song, and considered only the durations of syllables and intervals, and their patterns, or rhythm, within the songs. To control for slight scoring differences between observers, we normalized each observer's score for a song by the observer's mean score for all songs. The normalized scores for ts cut and normal birds ranged from 0.41 to 2.07. The final score for each song was the average of each observer's normalized score. The mean score for song type (i.e., ts cut adult, normal adult, and randomly matched) was calculated from the final scores for each song belonging to the song type.

Songs of other birds were also included among the experimental songs for analysis, and their respective tutor songs were also present among the possible tutor choices; this provided references against which ts cut and normal adult song similarity scores could be compared. Thus, songs of juvenile ts cut (n=20) and randomly matched song (songs for which the correct tutor was not present among the possible tutor choices, n=6) were also matched to a tutor song, and scored for spectral and temporal similarity. Randomly matched songs included those from 2 normal adult, 2 normal 60 d, and 2 ts cut 60 d birds. Song analysis data for randomly matched songs and ts cut 60 d birds were also used in a previous study (Solis and Doupe, 1999). All but 8 songs were scored by the same 9 observers; the remaining 8 songs were scored by 4 of the same observers. These last 8 songs were from additional experiments that were done after the bulk of song analysis was finished for most experimental birds. This second song analysis included songs previously scored in the first analysis, which confirmed that the four observers were scoring songs according to the same scale.

Song analysis: stereotypy. We measured song stereotypy of each bird using human subjective scoring and an automated overlap stereotypy analysis, as described in Solis and Doupe, 1999. Briefly, 10 song bouts from each bird were randomly selected for analysis. Observers rated on a scale from 1 to 5 how consistently a particular motif was present in each song sample after they listened to and looked at sonograms and oscillograms of each song sample. Normalized stereotypy scores ranged from 0.32 to 1.37. For comparison, songs of ts cut 60 d birds were included in the stereotypy test, and some of these results have been used in a previous study (Solis and Doupe, 1999). Three observers scored all songs but eight; the remaining 8 songs were scored by four observers.

An automated overlap analysis was employed to measure how consistently temporal patterns were repeated in song samples from a bird. For each song, the syllables were replaced with square pulses of equal amplitude. The resulting square pulse strings preserved syllable and interval durations, and their patterns found in the original songs. The square pulse string of a single motif was then compared to that of each song sample by calculating the percent overlap between syllables and intervals; high proportions of overlap indicated high levels of stereotypy. This measure is the same as the motif-song overlap value explained in Solis and Doupe, 1999.

RESULTS

Many songs of ts cut adults remained acoustically different from tutor song

Because NXIIts (ts) transections do not interfere with the respiratory pathways involved in song production, ts cut birds were able to sing throughout song development. Due to the loss of control over their syringeal musculature, however, these ts cut adults had songs that were usually extremely abnormal. For many birds, ts transections at 30 d resulted in adult song that consisted of simple syllables, which were either noisy, broadband signals or harmonic stacks. These ts cut adult songs did not clearly resemble their tutor song. For example, Figure 4-2A shows the tutor song of two birds that received ts transections as juveniles. The resulting ts cut adult songs for each bird are shown in Figure 4-2B and 4-2C, which did not resemble their tutor song. To quantify the similarity between the ts cut songs and tutor song, observers tried to match each ts cut song to its tutor song, which was present among 6 potential tutor songs. Thus, the number of correct matches indicates the level of similarity between the two songs. The ts cut adult songs in Figure 4-2B and 4-2C was matched to their correct tutor song by 0/9 and 3/9 observers, respectively. Thus, despite the long time after nerve transection (mean=160 d, range 60-260 d), these ts cut songs remained acoustically different from the tutor song.

In contrast, some songs from ts cut adults were highly similar to the tutor song. Of all birds receiving ts cuts as juveniles, 5/17 of them produced songs which were correctly matched to the tutor song with the same frequency as normal adult songs (7/9 or more observers correctly matched the two songs; this is ≥78%). For example, the ts cut adult song in Figure 4-2E shared many of the syllables found in its tutor song (Figure 4-2D), with the exception of a high, tonal note (circled). The absence of this note could reflect the weakened syringeal musculature, as notes with high fundamentals require strong contraction of the syringeal musculature (Goller and Suthers, 1996a; Vicario, 1991). For comparison, the song of a normal adult which had the same tutor is also shown in Figure 4-2F. The ts cut adult song in Figure 4-2E was correctly matched to its tutor song by 8/9 observers, whereas the normal adult song in Figure 4-2F was correctly matched by 9/9 observers. The ability of some ts cut birds to produce a song resembling their tutor song is likely due to reinnervation of the syrinx: consistent with this, there was a weak positive correlation between the syrinx weight of each ts cut bird and the percent of correct matches between the ts cut song and the tutor song ($r^2=0.24$, $p<0.032$).

Overall, ts cut adult songs were less similar to the tutor than normal adult songs. The percentage of correct matches between the ts cut adult songs and tutor songs was significantly less than that between normal adults and their tutor songs (Figure 4-3A; triangles) (unpaired t-test, $p<0.0001$). In addition, the frequency of correct matches for ts cut adult songs was not significantly increased relative to that for songs from ts cut juveniles which had received transections at 30 d, and produced abnormal songs at 60 d (data from Solis and Doupe, 1999). In addition to matching each song with a tutor song, each pair was scored for similarity. Because of the possibility that ts cut birds might imitate the timing of the tutor song without mimicking its spectral content, spectral and temporal similarity were scored separately. Yet, both the mean spectral similarity score and the mean temporal similarity score for ts cut adult songs were significantly lower than those for normal adult songs (Figure 4-3A; circles) (unpaired t-test, $p<0.0001$ for both spectral and temporal similarity scores). The spectral similarity to tutor song was significantly increased in ts cut adults relative to the ts cut juveniles (unpaired t-test, $p<0.0013$); however, there was no difference in temporal similarity scores. Furthermore, ts cut adult songs had spectral and temporal similarity scores similar to those given to randomly matched songs (see Materials and Methods).

Despite their overall low similarity to tutor song, songs of ts cut adults had attained adult levels of stereotypy. Song stereotypy refers to the reproducibility with which the same song motif is repeated in different song bouts. For both human and automated stereotypy measures (see Methods), the stereotypy of ts cut adults was not significantly different from that found in normal adult birds (Figure 4-3B). In addition, ts cut adult song was significantly more stereotyped than ts cut juvenile song (unpaired t-test, $p<0.0001$ for both human scores and for overlap values). Because there were cases in which ts cut birds had high similarity with the tutor song, it was possible that they inflated the stereotypy measured for the whole population of birds. When these birds were eliminated from the analysis (those with frequency of matching ≥78%), however, it was clear that the remaining ts adult songs with low similarity to the tutor were still highly stereotyped (open symbols, Figure 4-3B), and this stereotypy was not significantly different from that measured in normal adults (unpaired t-test, $p=0.235$ for human score and $p=0.302$ for overlap analysis). Thus, although they were unable to match their tutor song, ts cut adults had developed normal adult levels of song stereotypy.

Comparisons of AF responses to tsBOS and tutor song

Having obtained adult songs that did not resemble the tutor song, we were able to examine whether AF responses to tutor song were lost in adulthood, while maintaining strong responses to tsBOS. For clarity, data from the five ts cut adults with high similarity to the tutor song were excluded. Among the 12 ts cut adults with low similarity to the tutor song, responses to tutor song were observed in X, the first nucleus in the AF pathway. Extracellular recordings of 51 single units in X revealed a range of tsBOS and tutor song preferences: whereas some neurons preferred tsBOS over tutor song, a few preferred tutor song over tsBOS, and many responded similarly to these two songs, despite their acoustic differences (Figure 4-4A). The responses of each neuron to tsBOS and tutor song was quantified with a $d'_{\text{tsBOS-tutor}}$ value; the range and distribution of these values is shown in Figure 4-4B (open circles). Although these were adult birds, 27/51 (53%) of the X cells recorded had equivalent responses to tsBOS and tutor song ($-0.5<d'_{\text{tsBOS-tutor}}<0.5$, gray region in Figure 4-4B), similar to neural responses found in ts cut and normal 60 d birds.

For comparison, the individual $d'_{tsBOS-tutor}$ values from X cells recorded in ts cut juveniles are also shown in Figure 4-4B (solid circles; n=41); the mean $d'_{tsBOS-tutor}$ values were not significantly different between ts cut juvenile and ts cut adults (unpaired t-test, p=0.283). Thus, a population of neurons with equivalent responses to tsBOS and tutor song was maintained in ts cut adults, despite their maturity and song crystallization; this suggests that tutor song responses are not lost in adulthood.

In LMAN, another nucleus in the AF pathway, auditory responses were unusually rare: out of eight ts cut adult experiments with passes through LMAN, data was obtained for only three LMAN neurons (2 birds). The non-auditory cells in LMAN appeared to be normal based on their spike shapes and spontaneous firing rates. The frequency of obtaining auditory data from experiments with passes through LMAN in ts cut birds (2 out of 8 birds, or 25%) was less than that for normal adults, where auditory data was obtained from 6 out of 9 experiments with passes through LMAN (67%). The difference in these frequencies was not significantly different, however (χ^2test, p=0.081). The eight experiments with passes through LMAN were not completely devoid of auditory responses, however: in contrast to the 3 LMAN neurons with auditory responses, 34 X neurons yielded auditory data. In normal adult birds, the experiments yielding auditory data from 15 LMAN cells also had auditory data from 13 X cells. The difference in the frequency of auditory LMAN cells relative to X cells was significantly different between ts cut and normal adults (χ^2test, p<0.0001).

All three LMAN cells responded more to tsBOS than to tutor song (Figure 4-4C, open circles). The mean $d'_{tsBOS-tutor}$ value calculated from the three LMAN neurons from ts cut adults was also significantly greater than that from ts cut juveniles (compare to black circles, Figure 4-4C; unpaired t-test, p<0.029). Although this shift toward tsBOS tuning supports the idea that BOS responses overwrite tutor song responses in adulthood, the small sample of LMAN neurons precludes any clear interpretations.

Neurons with similar responses to tsBOS and tutor song were selective

X neurons with equivalent responses to tsBOS and tutor song in ts cut adults did not reflect an absence of selectivity, as seen in 30 d birds (Doupe, 1997). As assayed by their responses to other song stimuli, these neurons clearly exhibited selectivity. For example, the cell shown in Figure 4-5A responded strongly to both tsBOS and tutor song, despite their marked acoustic differences (2/9 correct matches between tsBOS and tutor song). Yet, this neuron did not respond well to either reverse BOS or reverse tutor song, which reversed both the temporal structure of notes within a syllable as well as the sequence of syllable order within the song. Furthermore, this X neuron did not respond to two different conspecific songs. Thus, the ability of this cell to discriminate tsBOS and tutor song from other song stimuli indicates that the cell was indeed selective.

As for the neuron in Figure 4-5A, selectivity was a common feature among X neurons with similar responses to tsBOS and tutor song. This is shown by plotting the $d'_{\text{tsBOS-tutor}}$ of each neuron against its degree of selectivity, measured by $d'_{\text{tsBOS-reverse}}$ or $d'_{\text{tutor-reverse}}$ values (Figure 4-5B). Note that neurons with equivalent responses to tsBOS and tutor song (gray region) often exceeded the d′ criterion values of 0.5 in these selectivity measures. These neurons were also selective when $d'_{\text{tsBOS-con}}$ or $d'_{\text{tutor-con}}$ measures of selectivity were considered (data not shown). Neurons were subsequently categorized as selective if they had a d′ value that was equal to or greater than 0.5 for at least one of four selectivity categories: tsBOS-reverse, tutor-reverse, tsBOS-conspecific, tutor-conspecific). Classified in this way, 72% of neurons with equivalent responses to tsBOS and tutor song were selective. This percentage was similar to that found in normal and ts cut 60 d birds (68% and 66%, respectively; from Solis and Doupe, 1997, and Solis and Doupe, 1999).

Thus, when based on individual d′ measures of selectivity, X neurons with similar responses to tsBOS and tutor song were commonly selective.

This selectivity was also confirmed in a more rigorous analysis. Because 30 d birds had not yet developed their own song, the selectivity of their neurons was characterized relative to their responses to tutor song only (Doupe, 1997). When X neurons from ts cut adults were reclassified as selective based only on their $d'_{\text{tutor-con}}$ or $d'_{\text{tutor-reverse}}$ values, their prevalence of selective neurons was still significantly greater than that in 30 d birds (data from Doupe, 1997; compare 58% in ts cut adults to 21% in 30 d juveniles; χ^2 test, $p<0.002$). Furthermore, population measures of selectivity also confirmed the selectivity of ts cut birds. Whereas 30 d neurons respond equally well to tutor song, reverse tutor song and conspecific, X neurons from ts cut birds responded significantly more to tutor song than to reverse tutor song or conspecific (Figure 4-5C). ($p<0.010$ for tutor-reverse comparisons, $n=16$; $p<0.050$ for tutor-conspecific, $n=23$). This selectivity distinguishes X neurons with similar responses to tsBOS and tutor song in ts cut adults from the unselective neurons found in 30 d juveniles.

Neural responses to tsBOS and tutor song were not related to song properties

The neural preferences for tsBOS over tutor song may reflect something specific to a bird's experience. This was suggested by the observation that X cells recorded from the same bird often shared similar preferences for tsBOS over tutor song. The $d'_{\text{tsBOS-tutor}}$ values obtained from each bird were considered to be clustered in 10/15 birds; however, this frequency of clustering was not greater than expected by chance ($p=0.092$, sign test). Nonetheless, the prevalence of clustering suggests that factors particular to a bird, perhaps its song experience, influence its neural responses to tsBOS and tutor song.

Although the neurons examined here came from birds whose songs bore little resemblance to their tutor song, it was theoretically possible that residual acoustic similarities between these songs produced the similar neural responses to tsBOS and tutor song obtained here. If true, then neurons with equivalent responses to tsBOS and tutor song should have come from birds with tsBOS acoustically similar to tutor song. Furthermore, neurons with strong preferences for either tsBOS or tutor song ought to have come from birds with little similarity between their tsBOS and tutor song. This "similarity hypothesis" predicts a negative correlation between the absolute value of $d'_{\text{tsBOS-tutor}}$ and the acoustic similarity between tsBOS and tutor song (dotted line, Figure 4-6A). To test this hypothesis, we compared the mean $|d'_{\text{tsBOS-tutor}}|$ value of the X neurons recorded from each ts cut adult to the acoustic similarity measured between its song and its tutor song. When the percentage of correct matches between each ts cut adult song and tutor song was compared to the mean $|d'_{\text{tsBOS-tutor}}|$ value for each bird, no correlation was evident (Figure 4-6A) (r^2=0.02). The correlations were similarly low when other measures of similarity with tutor song were used (data not shown) (r^2=0.03 for spectral similarity scores, and r^2=0.01 for temporal similarity scores). Thus, the neural preference for tsBOS over tutor song was not accounted for by the acoustic similarity between these two song stimuli.

Neural preferences for tsBOS over tutor song recorded from X cells also did not depend on the song maturity of a bird, as estimated by age and song stereotypy. Although all birds were adults, their ages ranged from 92-288 d. Differences in age did not influence the neural preferences for tsBOS over tutor song, however, as the age of each ts cut adult did not correlate with the mean $d'_{\text{tsBOS-tutor}}$ value obtained from X cells in each bird (r^2=0.01; data not shown). Song stereotypy of these birds also could not account for the neural preferences for tsBOS over tutor song recorded from X. There was no strong correlation between mean $d'_{\text{tsBOS-tutor}}$ values obtained from each bird and its song stereotypy, as measured by human scoring (Figure 4-6B; r^2=0.02) or by the automated overlap measure (data not shown; $r^2=0.03$). Thus, the song maturity of a bird did not predict its neural preferences for tsBOS and tutor song in X.

Selectivity in ts cut adults was less than that for normal adults

The selectivity of X neurons in ts cut adults had not increased to normal adult levels. This was apparent when the average selectivity of all X neurons (n=54), regardless of their tsBOS versus tutor song preference, was compared to that obtained from 34 X neurons recorded from 13 normal adults (Figure 4-7A). The ts cut adult selectivity was significantly less than that from normal birds in all but the BOS-tutor song category of selectivity (unpaired t-tests; for $d'_{\text{BOS-tutor}}$ $p=0.456$, for $d'_{\text{BOS-reverse}}$ $p<0.0001$, for $d'_{\text{BOS-con}}$ $p<0.001$, for $d'_{\text{tutor-reverse}}$ $p<0.0001$, for $d'_{\text{tutor-con}}$ $p<0.0002$). For normal adults, the low level of selectivity in the BOS-tutor category relative to the other categories may reflect the acoustic similarities clearly evident between normal adult songs and their tutor songs. In addition, X neurons from ts cut adults had not increased their selectivity relative to that measured for 61 neurons from ts cut juveniles (open circles in Figure 4-7A; data from Solis and Doupe, 1999). None of the slight increases of average selectivity for ts cut adult neurons relative to ts cut juveniles seen for some selectivity categories were statistically signficant.

As was true for the subset of X neurons with similar responses to tsBOS and tutor song, the entire population of X neurons clearly exhibited selectivity. The average selectivity for all X neurons recorded from ts cut adults was significantly greater than that measured for 51 X neurons from 30 d birds (30 d data from Doupe, 1997; mean $d'_{\text{tutor-reverse}}=-0.17$ and mean $d'_{\text{tutor-con}}=-0.01$) (unpaired t-tests; $p<0.0004$ for $d'_{\text{tutor-reverse}}$ and $p<0.022$ for $d'_{\text{tutor-con}}$ comparisons). Furthermore, unlike 30 d neurons, paired comparisons found significantly greater responses to tutor song than to reverse tutor song or conspecific song (data not shown; paired t-tests, $p<0.004$ for tutor-reverse comparisons, n=33; and $p<0.01$ for tutor-conspecific comparions, n=47). Thus, the unusually low selectivity of X neurons from ts cut adults did not indicate that these neurons could not discriminate between different song stimuli. These neurons had an intermediate level of selectivity, similar to that observed for 60 d juveniles.

Given the small sample size of LMAN neurons from ts cut adults (n=3) it was difficult to make conclusive comparisons of their selectivity. The individual d′ values obtained for each neuron are shown for three of the selectivity categories in Figure 4-7B (gray triangles); no data were obtained for tutor song categories of selectivity. Their values spanned the range of selectivity between ts cut juveniles (52 LMAN neurons; from Solis and Doupe, 1999) and normal adults (15 LMAN neurons, recorded from 6 birds). On average, LMAN neurons from ts cut adults had significantly increased selectivity in the tsBOS-tutor and tsBOS-conspecific categories relative to LMAN neurons from ts cut juveniles (unpaired t-tests; $p<0.028$ for $d'_{\text{tsBOS-tutor}}$ and $p<0.036$ for $d'_{\text{tsBOS-con}}$). Furthermore, the average LMAN selectivity in ts cut adults was not significantly different from that in normal adults. Thus, the selectivity obtained from 3 LMAN neurons in ts cut adults approximated normal adult levels; however, the low sample size undermines the significance of this increase.

Because d′ measures of selectivity compare a cell's responses to pairs of stimuli, we examined the response strengths (RS) elicited by each stimulus alone in order to gain insight on potential mechanisms underlying the difference in selectivity between ts cut and normal adult X neurons. For example, an increase in selectivity for BOS over reverse could be mediated by an increased response to BOS, a decreased response to reverse BOS, or both. For ts cut adults, responses to the non-preferred stimuli (conspecific, reverse BOS and reverse tutor song) were significantly greater than those in normal adults (Figure 4-8) (unpaired t-tests; $p<0.010$ for conspecific, $p<0.0001$ for reverse BOS, and $p<0.0003$ for reverse tutor). For preferred stimuli (tsBOS and tutor song), the responses of X neurons from ts cut adults were on average less than that found in normal adults; however neither difference, reached statistical significance (unpaired t-tests; $p=0.063$ for BOS and $p=0.074$ for tutor). Thus, X neurons in ts cut adults have not lost their responses to non-preferred stimuli. Similarly, normal 60 d birds respond more to non-preferred stimuli than do normal adults (Solis and Doupe, 1997). In fact, the mean RS to non-preferred stimuli were not significantly different between ts cut adults and normal 60 d birds. Thus, the changes in RS that normally happen during song learning seem not to have occurred for neurons in ts cut adults.

Possible causes of intermediate selectivity in X of ts cut adults

Athough ts cut adults and normal birds were both used within a similar age range (ts cut adults: 92-288 d, normal adults: 105-336 d), on average ts cut adults were younger than normal adults (mean age±SD for ts cut adults=147±63 d; for normal adults=241±71 d) (unpaired t-test; $p<0.004$). If selectivity increases with age, even in adulthood, then this could contribute to the substantial difference in X selectivity found between ts cut adults and normal adults. No evidence for a positive correlation between age and selectivity was apparent for these adults, however. For normal adults, the strongest positive correlation between age and selectivity was quite weak ($r^2=0.01$); this was obtained when mean $d'_{\mathrm{tsBOS\text{-}con}}$ values for each bird were compared to age. Among ts cut adults, there were no positive correlations between any measure of selectivity and age. The strongest correlation was found between age and mean $d'_{\mathrm{tsBOS\text{-}reverse}}$ values ($r^2=0.15$), and its negative direction does not explain the low selectivity of ts cut adults. Overall, our data did not support an effect of age on selectivity in adulthood.

Alternatively, the abnormally low selectivity of X neurons from ts cut adults may have been due to their inability to match their vocalizations to the tutor song template . Results from the five ts cut adults which produced fairly accurate copies of tutor song were consistent with this idea: 14 X neurons recorded from these ts cut adults exhibited normal levels of adult selectivity for some selectivity categories (Figure 4-9A). For BOS comparisons of selectivity, average d′values from ts cut adults with high similarity to tutor song were not significantly different from those obtained from normal adults (unpaired t-tests; $p=0.425$ for $d'_{tsBOS\text{-}tutor}$, $p=0.658$ for $d'_{tsBOS\text{-}reverse}$, and $p=0.163$ for $d'_{tsBOS\text{-}con}$). Furthermore, these X neurons had increased selectivity relative to those from ts cut birds with low similarity to tutor song in two comparisons of BOS selectivity (unpaired t-tests; $p<0.0001$ for $d'_{tsBOS\text{-}reverse}$, and $p<0.001$ for $d'_{tsBOS\text{-}con}$). In contrast, selectivity for tutor song among neurons from ts cut birds with high similarity to tutor song remained significantly lower than that found in normal adults (unpaired t-tests, $p<0.007$ for $d'_{tutor\text{-}reverse}$ and $p<0.004$ for $d'_{tutor\text{-}con}$ comparisons), and not significantly different from the levels found in the ts cut birds with low similarity to the tutor song. Thus, only the high BOS selectivity apparent for X neurons from ts cut adults with similar songs to the tutor song was consistent with the idea that selectivity development depends on matching between BOS and the tutor song template.

Auditory responses of LMAN neurons were more readily recorded from ts cut birds with high song similarity to their tutor song. Three out of the 5 ts cut adults (all had passes through LMAN) yielded auditory data from LMAN; this percentage (60%) was similar to that in normal adults (67%), and greater than that obtained for ts cut birds with low similarity to tutor song (25%). Thus, the presence of auditory responses in these birds may have been related to their ability to match their vocalizations to the tutor song template. In all, auditory responses were obtained from five LMAN neurons in these ts cut birds, and their selectivity was clearly similar to the level found for normal adult birds in all selectivity categories (Figure 4-9B) (unpaired t-tests; $p=0.991$ for $d'_{tsBOS\text{-}tutor}$, $p=0.605$ for $d'_{tsBOS\text{-}reverse}$, $p=0.392$ for $d'_{tsBOS\text{-}con}$, $p=0.734$ for $d'_{tutor\text{-}reverse}$, and $p=0.699$ for $d'_{tutor\text{-}con}$). Although this selectivity was not significantly increased relative to ts cut adults with low similarity to tutor song, the small sample sizes in both groups limit this comparison.

If mismatching somehow compromises selectivity, it might be expected that neural selectivity would increase as song similarity with the tutor song increased. We examined this by comparing each ts cut adult's degree of similarity to tutor song to its mean d′ value obtained from X neurons for each selectivity category (ts cut adults with low and high similarity to tutor song both included). The strongest correlations occurred between $d'_{tsBOS\text{-}reverse}$ values and song similarity (Figure 4-10) ($p<0.047$ for percent correctly matched, $p<0.029$ for spectral similarity, $p<0.007$ for temporal similarity). Although this trend is consistent with the hypothesis that selectivity increases with matching, the correlations were considerably weaker for other measures of selectivity (Table 4-1).

Alternatively, the low selectivity in ts cut adults relative to normal adults could have reflected non-specific, trophic effects induced by ts cuts on upstream neurons. We estimated potential trophic effects with syrinx weight, which indicates the extent of reinnervation of the muscle after the ts cut. The syrinx weights of all ts cut adults were significantly less than those of normal adults, including those ts cut adults with high similarity to the tutor song (Figure 4-11A) (unpaired t-tests; $p<0.0001$ for ts cut adults with low similarity, and $p<0.019$ for ts cut adults with high similarity). Thus, the low syrinx weights of ts cut birds with normal adult selectivity were not consistent with idea that retrograde, trophic effects of the ts cut had limited selectivity. Furthermore, syrinx weight did not predict any measure of selectivity obtained for ts cut adults (data not shown). Another estimate of potential trophic effects of ts cuts was the spontaneous firing rate of AF cells. The spontaneous rates of X cells recorded in ts cut adults were highly similar to those from normal adults (Figure 4-11B). The distributions were highly similar, including both X cell types with low (<10 spikes/s) and high spontaneous firing rates, and the mean spontaneous rate were not significantly different between the two bird groups (Mann-Whitney U test, $p=0.670$). Moreover, the subset of X neurons with the lowest levels of selectivity (ts cut adults with low similarity to tutor song) had spontaneous rates similar to those from normal adults (Mann-Whitney U test, p=0.939). There was also no significant difference between the spontaneous rates of LMAN neurons recorded from the two ts cut groups (Mann-Whitney U test; p=0.478). Although we did not measure the spontaneous rates of non-auditory LMAN neurons, they did not seem different from spontaneous rates of auditory units. Because the extent of reinnervation did not vary with the selectivity obtained from a bird, and because the cells from ts cut birds had normal spontaneous rates, a non-specific, trophic effect as a result of ts transections did not obviously influence X selectivity.

Comparisons of LMAN and X selectivity in ts cut adults

The comparisons of selectivity from both kinds of ts cut adults suggested that LMAN was more selective than X. Among ts cut adults with low similarity to tutor song, there was a hint of increased selectivity for LMAN cells relative to ts cut juveniles that was not apparent for X (Figure 4-7). Also, among the ts cut adults with high similarity to tutor song, LMAN cells clearly exhibited adult levels of selectivity for all selectivity categories; in X this was true for only two categories. Thus, LMAN and X selectivity was compared in the five ts cut adult experiments (both low and high similarity) for which data from each nucleus was obtained. Paired comparisons of mean d′values for LMAN and X neurons did not reveal significant differences in selectivity for any category (data not shown). This agrees with the similar selectivity levels found between LMAN and X for both normal and ts cut 60 d birds (Solis and Doupe, 1997; Solis and Doupe, 1999).

DISCUSSION

This study addresses the separate contributions of BOS and tutor song experience to AF selectivity in adults. In ts cut adults with songs acoustically different from the tutor song, many X neurons continued to respond as well to the tutor song as to BOS. Furthermore, their X neurons were considerably less selective than those from normal adults. In LMAN, auditory properties were unusually rare. These results distinguish ts cut adults from normal adults, indicating that their AF neural properties have been altered as a result of ts transections.

Stereotypy and similarity to tutor song of ts cut adult songs

Adults receiving ts transections as juveniles attained adult levels of song stereotypy, despite the abnormality of their songs. Although stereotypy and song similarity to the tutor song covary during song development in normal birds (Scharff and Nottebohm, 1991; Solis and Doupe, 1997), this result indicates that stereotypy development does not depend on matching to the tutor song. This has also been shown for isolate birds, which develop stereotyped, though abnormal, song in the absence of a tutor song model (Price, 1979; Morrison and Nottebohm, 1993).

Despite ts transections as juveniles, some birds produced songs that clearly resembled the tutor song. Although these birds tended to have more reinnervation of the syrinx than the other ts cut birds, their overall syrinx weight was less than that of normal adults. Thus, the ability of some birds to mimic tutor song serves as a reminder that song acquisition does not rely exclusively on an intact syrinx. Temporal similarity can also be accomplished through patterned contractions of respiratory muscles, whose innervation is not altered in the ts cut manipulation (Hartley and Suthers, 1989; Goller and Suthers, 1996b; Vicario, 1991; Wild, 1997). Furthermore, it is remarkable that some birds were ultimately able to produce similar song to the tutor despite their loss of syrinx control early in sensorimotor learning. Although the development of ts cut song was not studied here, it would be interesting to know whether these birds experienced relatively quick reinnervation, such that they mimicked tutor song with a normal time course, or whether they only began to produce a copy of the tutor song later than normal sensorimotor learning. Detailed studies of ts cut song development could address the issue of critical periods for sensorimotor learning, which has been suggested in a previous study (Pytte and Suthers, 1996).

The song preferences of neurons recorded from ts cut adults

The ts cut birds with low similarity to the tutor song allowed us to test whether neurons in adult birds had developed a strong preference for tsBOS over tutor song. Unexpectedly, X neurons from these ts cut adult birds exhibited a range of tsBOS versus tutor song preferences that was similar to that found in 60 d birds: some neurons preferred tsBOS over tutor song, a few neurons preferred tutor song over BOS, and many neurons responded equally well to tsBOS and tutor song. In adult birds, such neurons could function as a reference that the song system uses to continuously evaluate BOS. Such a reference has been demonstrated behaviorally: when adult zebra finches receive altered auditory feedback of their song, they will change their songs, and when auditory feedback returns to normal, they will produce their original song (Leonardo and Konishi, 1998); this plasticity may be mediated by the AF (Brainard and Doupe, 1997).

Alternatively, the maintenance of neural selectivity for both tsBOS and tutor song may indicate that neurons in ts cut adults remained in a juvenile state, poised to participate in song learning. This is consistent with the juvenile state of X selectivity also found in ts cut adults. During song learning, neurons tuned to tsBOS could provide information about the current state of BOS, neurons tuned to tutor song could store information about the memorized tutor song, and neurons responding well to both could compare the two songs during sensorimotor learning. Nevertheless, due to the low selectivity in these neurons relative to adults, it is unclear whether the range of neural preferences for BOS and tutor song found in ts cut adults would also be true for normal adults.

Abnormally low selectivity in ts cut adults

Although songs of ts cut adults were substantially more stereotyped than songs from ts cut juveniles, there was no parallel increase in selectivity of X neurons from ts cut adults. These neurons, however, were not unselective: they had selectivity similar to that found in ts cut and normal 60 d birds, which reliably discriminated between different song stimuli, unlike X neurons from 30 d birds.

The ts cut manipulation could have resulted in lower neural selectivity in several ways. If the ts cut delayed song development, the ts cut birds may have produced crystallized song for a shorter time than normal adults, which might have slowed selectivity development. Among normal adults, however, a relation between selectivity and age has never been reported, nor did we find such a relation within our ts cut or normal adults. Alternatively, ts transections may have caused retrograde, trophic effects that eventually compromised the health and selectivity of AF neurons. We did not find evidence for such a trophic effect, however: the amount of reinnervation (and thus potential trophic effect), as assayed by syrinx weight, did not correlate with selectivity among ts cut birds. Furthermore, these neurons had spontaneous firing rates and response strengths to song stimuli similar to those found in normal birds. This suggests that the intermediate selectivity of the cells was not accompanied by a loss of cell health.

The chronic mismatch between what a ts cut bird sings and its tutor song template may have compromised AF selectivity. Some data supported this: unlike ts cut adults with low similarity to the tutor song, those ts cut adults with high tutor song similarity exhibited adult levels of BOS selectivity. Also, among all ts cut birds, there was a tendency for tsBOS-reverse selectivity to increase as the songs from ts cut adults increased their similarity to the tutor. Correlations between selectivity and similarity may not have been stronger because there was not a continuous gradient of similarity among ts cut birds (i.e., their songs were either clearly like the tutor song, or not at all like tutor song); alternatively, it is possible that selectivity itself does not increase gradually, but rather in steps. Another study has suggested a relation between song matching and selectivity: LMAN selectivity is not apparent in adult isolate birds that have stereotyped songs (Maekawa, 1998). Isolates are similar to ts cut birds in that they have not been able to match their songs to a tutor song template; however, unlike ts cut birds, this condition was brought about by raising these birds without tutor song experience.

Notably, tutor song selectivity was markedly lower than normal adult levels for both ts cut adults that achieved good tutor song matches and those that did not. One explanation is that the tutor song selectivity in ts cut adults reflects the upper limit of tutor song selectivity, and the much higher selectivity seen in normal adults is an artifact of the acoustic similarity between normal adult BOS and tutor song. For example, even if all neurons were tuned to BOS in adulthood, they could respond well and selectively to the tutor song if it were similar enough to BOS. X neurons from ts cut adults with high similarity to the tutor song, however, still had lower tutor song selectivity than normal adults. It could be that, although these songs were easily matched to the tutor song, they were still sufficiently different such that neurons could discriminate between tsBOS and tutor song (e.g., ts cut song in Figure 4-2E, which is missing the high tonal note). Studies in adult HVc, the input nucleus of the AF, show that neurons are extremely sensitive to the precise spectral and temporal features of song (Theunissen and Doupe, 1998). Lower tutor song selectivity relative to adult BOS selectivity may be more optimal for guiding plastic song development (Doupe, 1997). Alternatively, tutor song selectivity development may have been limited by any delay in song development among ts cut adults, including those with songs similar to the tutor song.

The simpler structures of ts cut song could theoretically contribute to the low neural selectivity measured in this study. Neurons from ts cut adults could actually be sharply tuned, but because of the simple structure of the tsBOS stimuli, they do not appear very selective. For example, a neuron that is sharply tuned to a simple harmonic stack would likely respond equally well to a reverse version of the stack, which is highly similar to the forward version. Some data in this study, however, suggest that low selectivity in ts cut birds was not an artifact of the simplicity of ts cut song. First, although ts cut songs were composed of simple syllables, they were sung in complex temporal patterns, which can provide more cues by which a neuron can distinguish tsBOS stimuli from reverse or conspecific stimuli. Neurons in the song system are senstive to the intervals between syllables and particular syllable combinations (Margoliash, 1983; Margoliash and Fortune, 1992; Lewicki and Arthur, 1996; Doupe, 1997). Second, the lower X selectivity of ts cut adults relative to normal adults was due in part to higher responses to non-preferred stimuli and lower responses to preferred stimuli. If lower selectivity reflected sharp tuning to simple stimuli, then one would expect higher responses to non-preferred stimuli only. Third, if ts cut songs were sufficiently simple, then neurons tuned to them might not be able to discriminate between tsBOS and the songs of other ts cut birds. Yet, X neurons were selective for tsBOS over other ts cut songs (data not shown). Furthermore, they discriminated their own songs from other ts cut song just as well as X neurons from normal adults did (no significant differences between $d'_{\text{BOS-ts cut con}}$ values, unpaired t-test, $p=0.204$). Thus, the abnormally low selectivity in ts cut adults could represent a real impairment in sharpness of tuning, rather than being an artifact of the tsBOS stimulus itself.

In other systems, reductions in selectivity are apparent after experience has been altered. In the barn owl, recalibration of auditory maps to altered visual input can cause a broadening of ITD tuning curve width for some sites (Brainard and Knudsen, 1995). In the visual system, changing the eye through which visual experience is received can reduce orientation tuning of cortical neurons (Mioche and Singer, 1989). Furthermore, upon receiving visual input through the formerly deprived eye, some neurons passed through a phase in which they were unresponsive to input from either eye. This result is reminiscent of the unusual lack of auditory responses in LMAN of ts cut adult birds. In general, continued exposure to experiences that are different from what the neuron expects may degrade neural responsiveness and/or selectivity, perhaps through morphological rearrangements of the its input axons.

Although LMAN neurons were readily recorded in ts cut adults, auditory responses were obtained less often than in normal adults. If this is also an effect of the ts cut manipulation, it is possible for a loss of auditory responses in LMAN alone to cause the unusually low selectivity apparent in X of these ts cut adults. Given its projection to X, LMAN could sharpen X selectivity; however, the role of this recurrent projection is unknown. Alternatively, ts transections could independently affect both auditory responses in LMAN and selectivity in X.

The properties of AF neurons in ts cut adults were highly unusual for adult birds, even though their ts cut songs were highly stereotyped. In X, neurons from ts cut adults resembled juvenile neurons: a population of neurons that responded equally well to tsBOS and tutor song was maintained, and X selectivity was much lower than expected from adult neurons. LMAN selectivity was difficult to characterize, given the rarity of auditory responses there. These results suggest that ts transections had somehow hindered normal selectivity development, perhaps by preventing ts cut adults from matching their songs to their tutor song model.

Figure 4-1 A) The two phases of song learning. For zebra finches, the sensory phase ends at ~60 d, and the sensorimotor phase begins at ~ 30 d, when juveniles begin to sing. Song learning is complete at 90+ days. Note the overlap between the two song phases. B) Anatomy of the song system: motor pathway nuclei are gray, and AF nuclei are black. C) At 60 d, AF neurons from ts cut juveniles at 60 d have a range of tsBOS versus tutor song preferences, as quantified with a $d'_{\text{tsBOS-tutor}}$ values for each neuron; LMAN cells are represented with white circles, and X cells with black circles. D) In normal adults, AF neurons display a range of preferences for the acoustically similar BOS and tutor song. Conventions as in C.

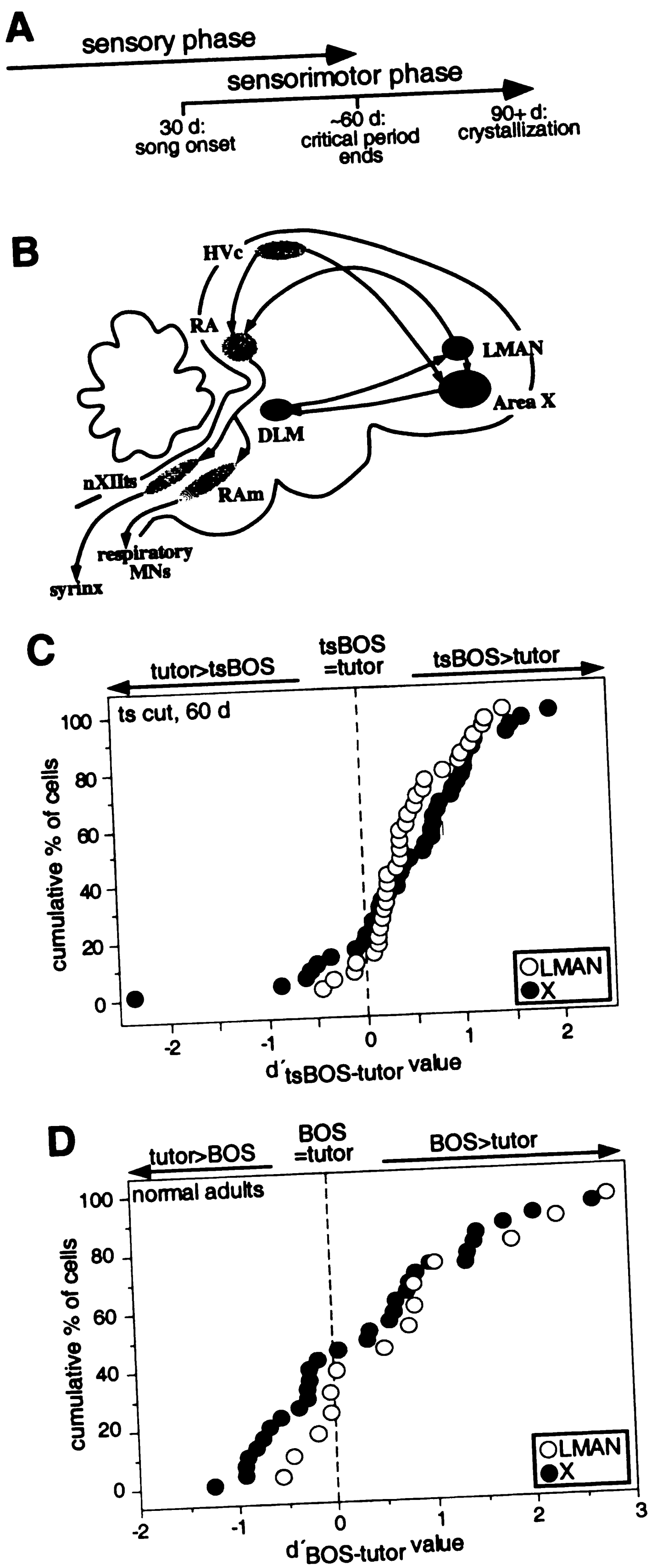

A
sensory phase
sensorimotor phase
30 d:
song onset
~60 d:
critical period
ends
90+ d:
crystallization
B
HVc
RA
LMAN
Area X
DLM
nXIIts
RAm
respiratory
MNs
syrinx
C
tutor>tsBOS
tsBOS
=tutor
tsBOS>tutor
ts cut, 60 d
cumulative % of cells
100
80
60
40
20
0
-2
-1
0
1
2
d′tsBOS-tutor value
○LMAN
●X
D
tutor>BOS
BOS
=tutor
BOS>tutor
normal adults
cumulative % of cells
100
80
60
40
20
0
-2
-1
0
1
2
3
d′BOS-tutor value
○LMAN
●X

Figure 4-1

Figure 4-2 Long term effects of juvenile NXIIts (ts) transections on adult song. A) The tutor song of two sibling ts cut adults is illustrated with a sonogram and oscillogram. Sonograms plot frequency versus time, and the intensity of each frequency band is indicated by its darkness; oscillograms show the sound waveform. Syllables are labelled with capital letters. This bird was the tutor for the birds whose songs are shown in B and C. B) Adult song of a bird that received ts transections at ~30 d; this bird was exposed to the tutor in A during song learning. The labelled syllable ("d") bears a slight resemblance to syllable D in the tutor song, but overall the song was not similar to the tutor song. C) Song of another ts cut adult that had little similarity to its tutor song, shown in A. One syllable ("c") was slightly similar to syllable C in the tutor song. D) The tutor song of the two birds whose songs are shown in E and F. Syllables are labelled with capital letters; note the high tonal note in syllable C (circled). E) The song of a ts cut adult that developed high similarity with its tutor song (in D). Syllables resembling those found in the tutor song are labelled with lower case letters; the high tonal note is missing (circle). Introductory notes are labelled with "i". F) The degree of similarity that normally develops between adults and tutor song is illustrated with the song of a normal adult bird that also had the tutor shown in D. Syllables resembling those found in the tutor song are labelled with lower case letters; the high tonal note is present (circle).

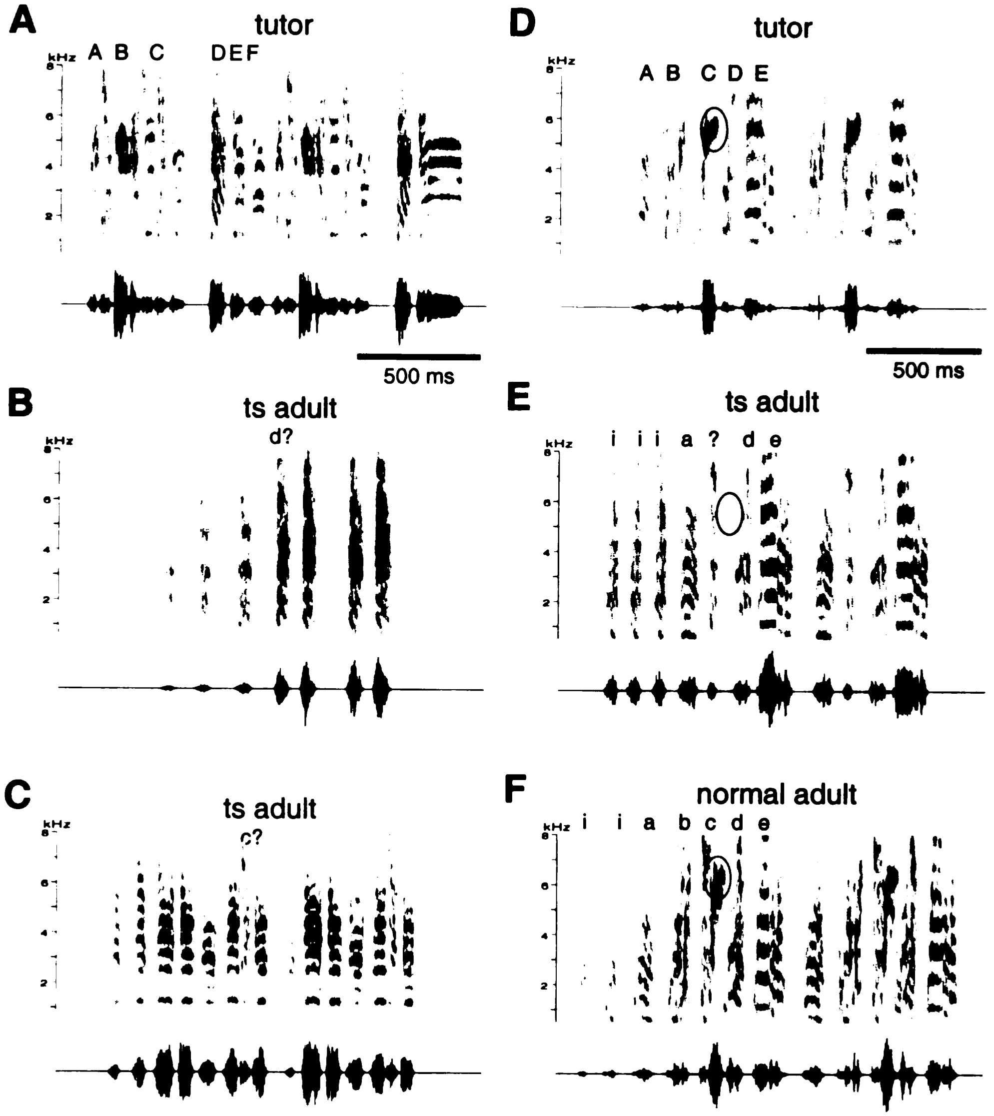

A
tutor
kHz
A B C DEF
8
6
4
2
500 ms
B
ts adult
d?
C
ts adult
c?
D
tutor
A B C D E
500 ms
E
ts adult
i i i a ? d e
F
normal adult
i i a b c d e

Figure 4-2

Figure 4-3 Effects of juvenile ts transections on adult song quality. A) Measures of similarity to tutor song are shown for different bird groups. Black triangles show the mean percentage of observers that matched a song to the correct tutor song (left-hand ordinate). This value averages the frequency of matching across all songs in each song type. There is no percentage for random matches because their correct tutor was never present among the tutor song choices. The mean spectral (open squares) and temporal (open circles) similarity scores are plotted along the right-hand ordinate. Error bars are SEM. B) Measures of song stereotypy are shown for different bird groups. Solid circles show the human scores of stereotypy; solid squares show the automated overlap values of stereotypy. The open symbols show the average stereotypy score and automated overlap value obtained from only those birds with low similarity to the tutor song; error bars are SEM.

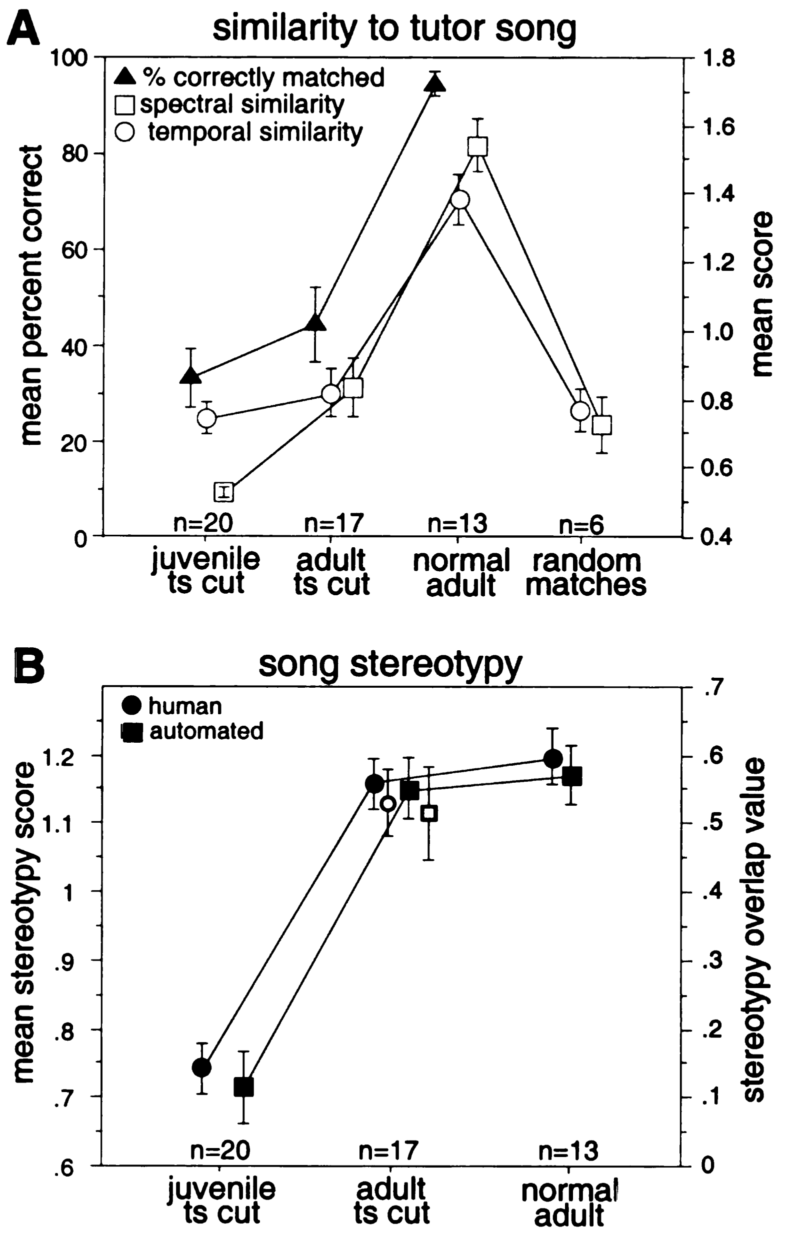

A
similarity to tutor song
▲ % correctly matched
□ spectral similarity
○ temporal similarity
mean percent correct
100
80
60
40
20
0
mean score
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
n=20
n=17
n=13
n=6
juvenile ts cut
adult ts cut
normal adult
random matches
B
song stereotypy
● human
■ automated
mean stereotypy score
1.2
1.1
1
.9
.8
.7
.6
stereotypy overlap value
.7
.6
.5
.4
.3
.2
.1
0
n=20
n=17
n=13
juvenile ts cut
adult ts cut
normal adult

Figure 4-3

Figure 4-4 Preferences for tsBOS versus tutor song recorded in X neurons from ts cut adults. A) Peristimulus time histograms (PSTHs) show the responses to tsBOS and tutor song of three different X neurons. The left-hand pair shows the cumulative responses of a neuron that preferred tutor song over tsBOS; 20 trials of each song were presented, and the ordinate of the PSTH begins at 35 spikes/s. The middle pair shows the responses of a neuron the responded equally well to tsBOS and tutor song; 15 trials of each song are shown. The right-hand pair shows the responses of a neuron that preferred tsBOS over tutor song; 15 trials of each stimulus are shown, and the ordinate of the PSTH begins at 25 spikes/s. B) The cumulative distribution of tsBOS versus tutor song preferences for all X neurons recorded, as quantified with $d'_{\text{tsBOS-tutor}}$ values, is shown with white circles. For comparison, the distribution of $d'_{\text{tsBOS-tutor}}$ values from ts cut juvenile birds (60 d) is shown with black circles. Gray shading highlights those cells considered to respond equally well to tsBOS and tutor song. C) The cumulative distribution of $d'_{\text{tsBOS-tutor}}$ values for the three LMAN neurons recorded in ts cut adults is shown with white circles. For comparison, the same distribution is shown for ts cut juveniles with black circles.

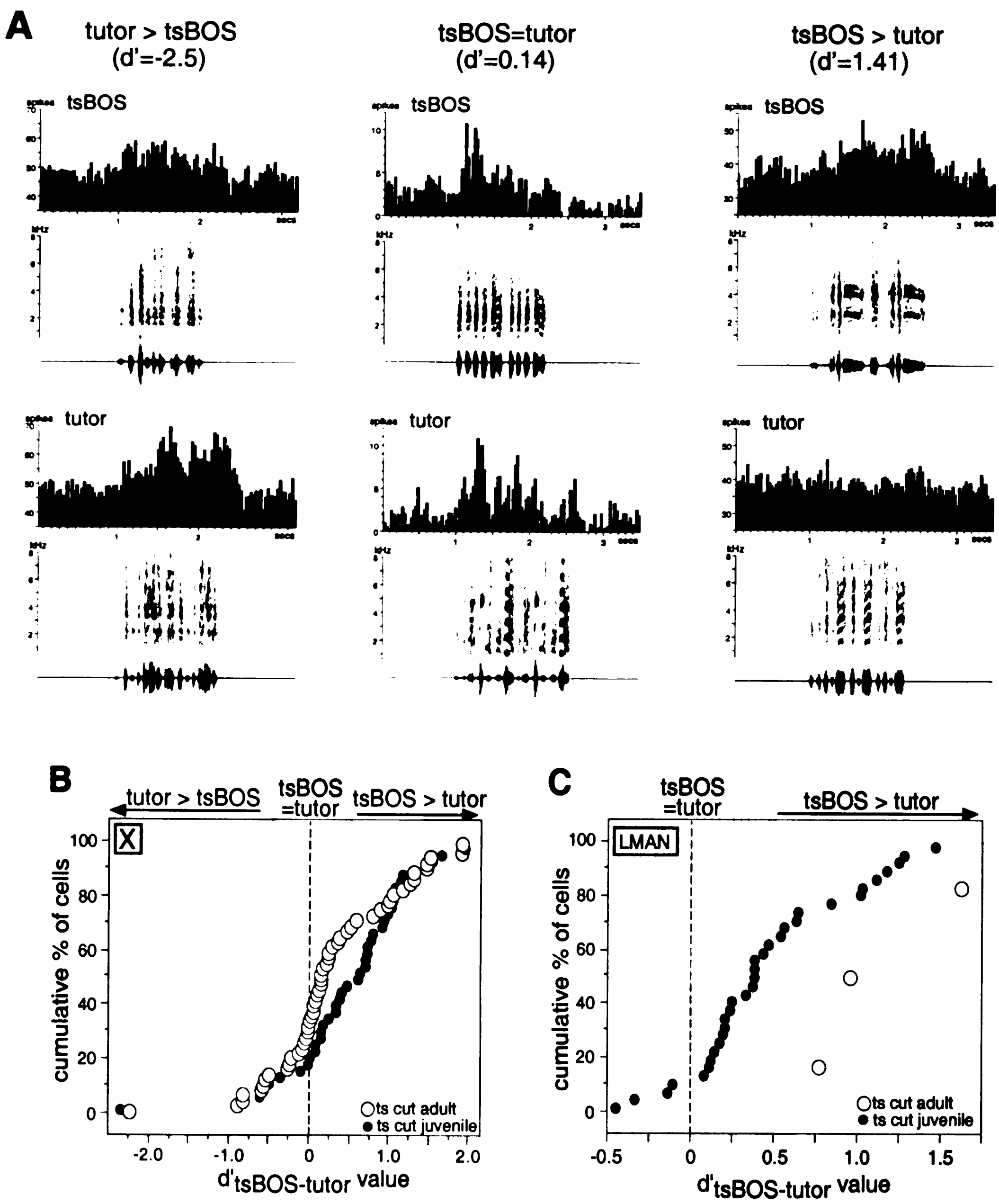

A
tutor > tsBOS
(d'=-2.5)
tsBOS=tutor
(d'=0.14)
tsBOS > tutor
(d'=1.41)
tsBOS
tutor
B
tutor > tsBOS
tsBOS =tutor
tsBOS > tutor
X
cumulative % of cells
100
80
60
40
20
0
-2.0
-1.0
0
1.0
2.0
d'tsBOS-tutor value
○ts cut adult
● ts cut juvenile
C
tsBOS =tutor
tsBOS > tutor
LMAN
cumulative % of cells
100
80
60
40
20
0
-0.5
0
0.5
1.0
1.5
d'tsBOS-tutor value
○ts cut adult
● ts cut juvenile

Figure 4-4

Figure 4-5 Equivalent responses to tsBOS and tutor song. A) PSTHs show the responses of a single X neuron to 20 presentations of each song. While this neuron responded equally well to tsBOS and tutor song ($d'_{\text{tsBOS-tutor}}$ =-0.05), it did not respond well to either reverse stimulus ($d'_{\text{tsBOS-reverse}}$=1.14 and $d'_{\text{tutor-reverse}}$=1.64), nor to two different conspecific songs ($d'_{\text{tsBOS-con1}}$=1.69, $d'_{\text{tsBOS-con2}}$=1.45, $d'_{\text{tutor-con1}}$=2.52, $d'_{\text{tutor-con2}}$=2.00). The white dashed line indicates the neuron's average spontaneous firing rate. Note that the ordinate of the PSTH begins at 40 spikes/s. This particular tsBOS was correctly matched to the tutor by only 2/9 observers. B) The $d'_{\text{tsBOS-tutor}}$ value of each X neuron is plotted against two measures of selectivity: $d'_{\text{tsBOS-reverse}}$ (black circles) and $d'_{\text{tutor-reverse}}$ (white circles). The gray region highlights those neurons considered to have responded equally well to tsBOS and tutor song. The dashed vertical line marks the criterion for selectivity (d'=0.5). C) For those neurons considered to respond equally well to tsBOS and tutor song ($-0.5<d'_{\text{tsBOS-tutor}}<0.5$), histograms show paired comparisons of the mean RS to tutor song and conspecific (black bars) or to tutor song and reverse tutor song (white bars). Error bars are SEM, and asterisks indicate significant differences.

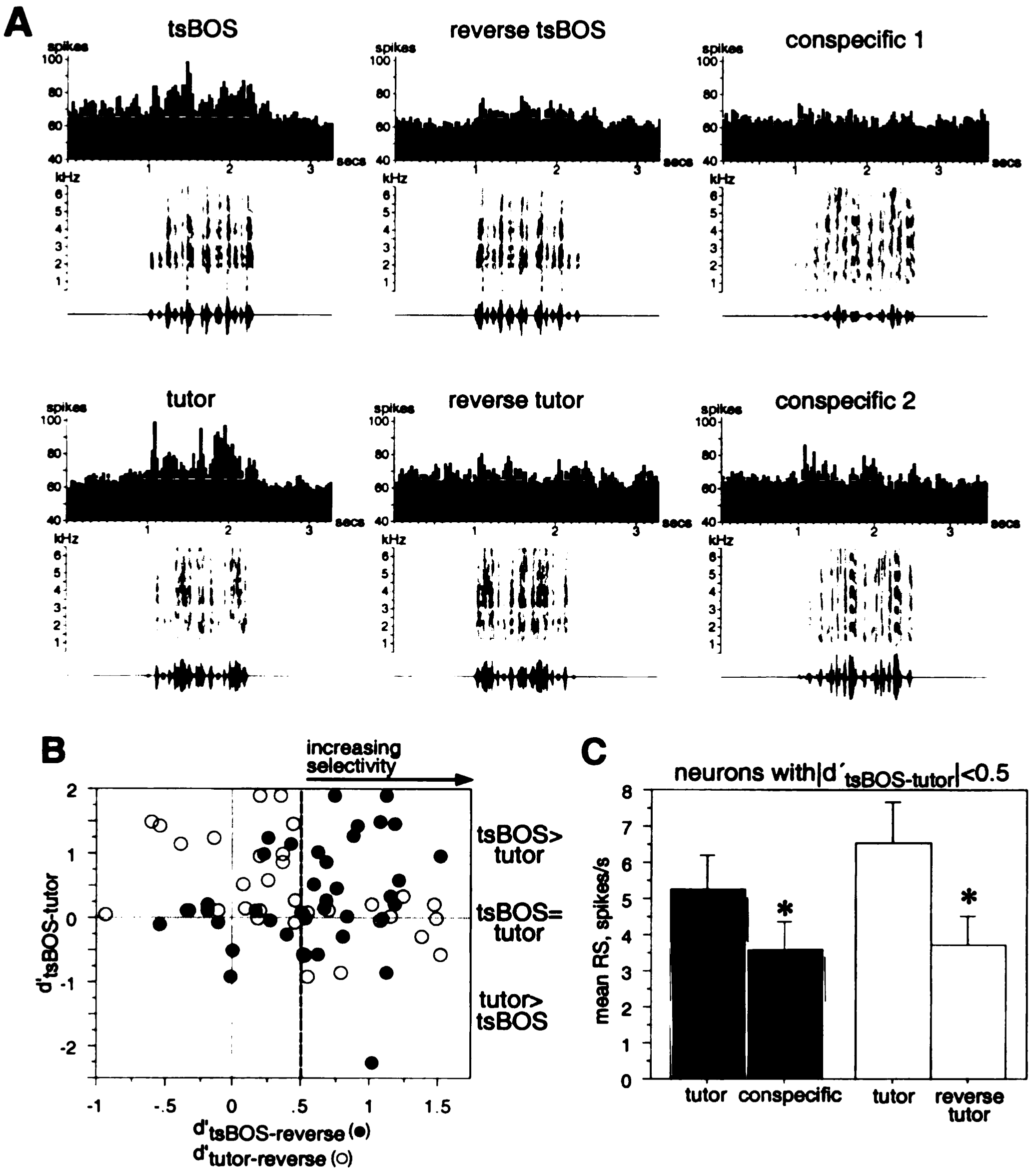

A
tsBOS
reverse tsBOS
conspecific 1
tutor
reverse tutor
conspecific 2
spikes
100
80
60
40
secs
1
2
3
kHz
6
5
4
3
2
1
B
increasing selectivity
2
1
0
-1
-2
-1
-.5
0
.5
1
1.5
d'tsBOS-tutor
d'tsBOS-reverse (●)
d'tutor-reverse (○)
tsBOS> tutor
tsBOS= tutor
tutor> tsBOS
C
neurons with |d'tsBOS-tutor|<0.5
mean RS, spikes/s
8
7
6
5
4
3
2
1
0
*
*
tutor
conspecific
tutor
reverse tutor

Figure 4-5

Figure 4-6 A) If residual acoustic similarity between tsBOS and tutor song accounts for the neural responses to tsBOS and tutor song, then the trend shown with the gray dashed line is expected ("similarity hypothesis"): equivalent responses to tsBOS and tutor song would occur when tsBOS and tutor song are similar, and strong preferences for one song or the other (quantified by the absolute value of $d'_{\text{tsBOS-tutor}}$) would result when tsBOS and tutor song are dissimilar. When the percent correctly matched is plotted against the mean $|d'_{\text{tsBOS-tutor}}|$ values obtained for each bird, the resulting linear least squares fit of the data (black line) does not approximate the trend predicted by the similarity hypothesis. B) The human score of song stereotypy for each ts cut adult bird is compared to its mean $d'_{\text{tsBOS-tutor}}$ value obtained from the responses of X neurons in each bird. The black line is the linear least squares fit of the data.

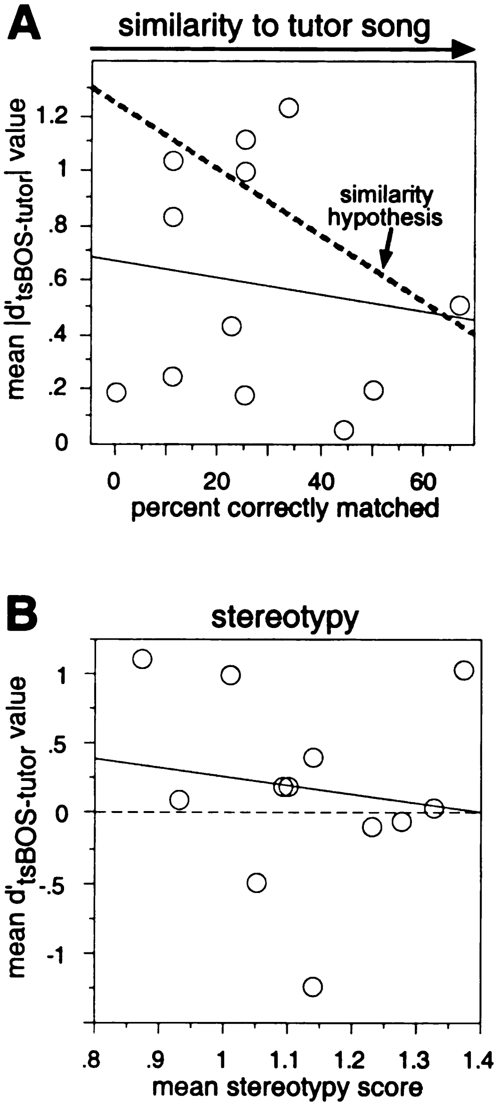

A
similarity to tutor song
mean |d'tsBOS-tutor| value
1.2
1
.8
.6
.4
.2
0
similarity hypothesis
0
20
40
60
percent correctly matched
B
stereotypy
mean d'tsBOS-tutor value
1
.5
0
-.5
-1
.8
.9
1
1.1
1.2
1.3
1.4
mean stereotypy score

Figure 4-6

Figure 4-7 Comparisons of selectivity. A) The degree of selectivity of X neurons from ts cut adults (black triangles) is compared to that from ts cut juveniles (white circles), and normal adults (black circles). The mean d′ values for each selectivity category (listed on the abscissa) are plotted along the ordinate; error bars are SEM. Lines connect the mean d′ values obtained for each bird group. Asterisks indicate significant differences in selectivity between ts cut adult and normal adult X neurons. B) The selectivity of each of the three LMAN neurons obtained from ts cut adults (gray triangles) is compared to average selectivity measured for ts cut juveniles (white circles) and normal adults (black circles); lines connect the d′ values obtained from each neuron in ts cut adults. Error bars are SEM, and asterisks denote the selectivity categories for which there were significant differences between the mean d′ values (not shown) of ts cut adult and ts cut juveniles.

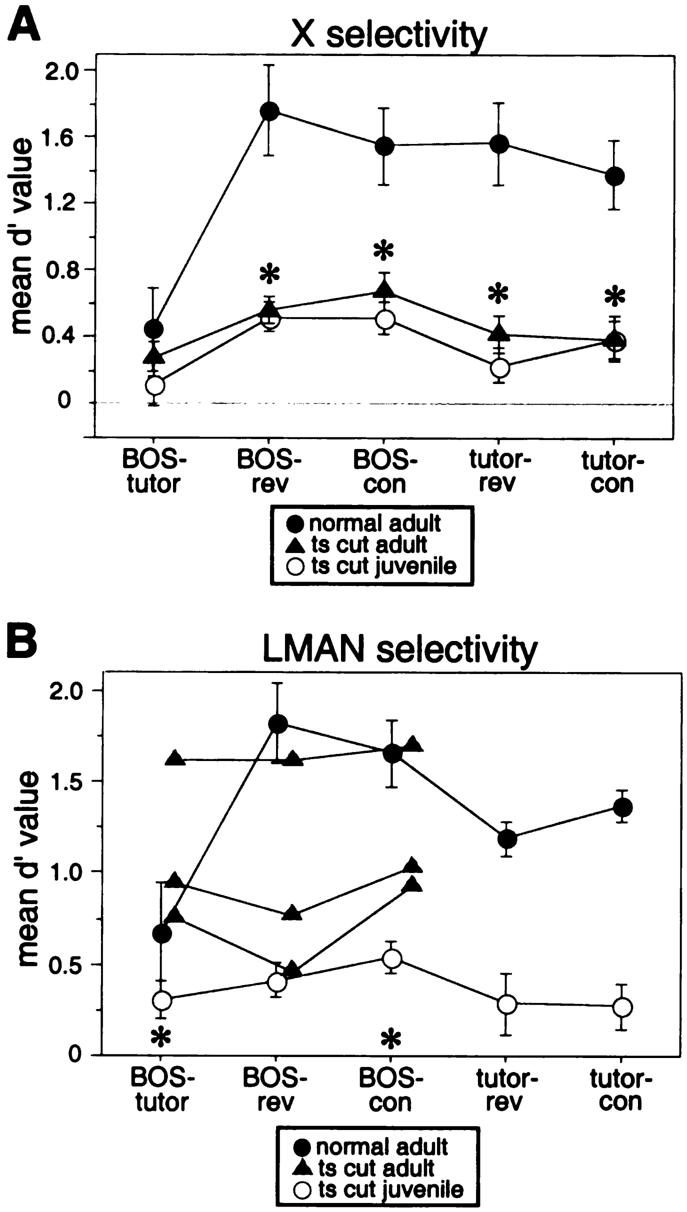

A
X selectivity
mean d' value
2.0
1.6
1.2
0.8
0.4
0
*
*
*
*
BOS-tutor
BOS-rev
BOS-con
tutor-rev
tutor-con
● normal adult
▲ ts cut adult
○ ts cut juvenile
B
LMAN selectivity
mean d' value
2.0
1.5
1.0
0.5
0
*
*
BOS-tutor
BOS-rev
BOS-con
tutor-rev
tutor-con
● normal adult
▲ ts cut adult
○ ts cut juvenile

Figure 4-7

Figure 4-8 A histogram compares the mean RS to different stimuli measured from X neurons in ts cut adults (white bars) and normal adults (black bars); error bars are SEM. Asterisks indicate significant differences between ts cut and normal responses.

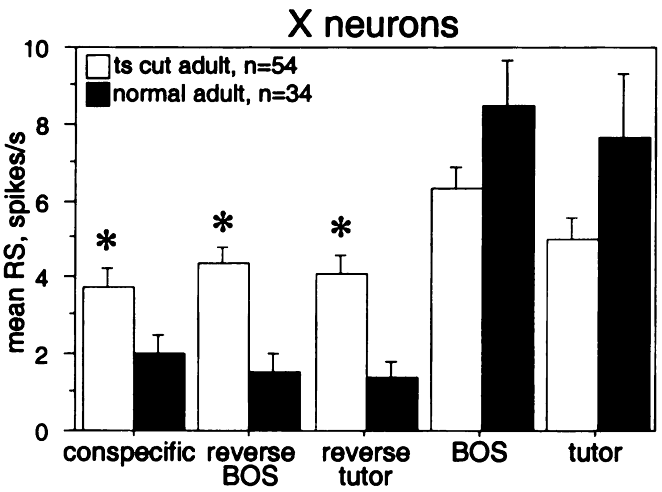

X neurons
☐ ts cut adult, n=54
■ normal adult, n=34
mean RS, spikes/s
0
2
4
6
8
10
*
*
*
conspecific
reverse BOS
reverse tutor
BOS
tutor

Figure 4-8

Figure 4-9 The selectivity of ts cut adults with high similarity to the tutor song. A) The degree of selectivity of X neurons from ts cut adults with high similarity to the tutor song (white triangles) is compared to that measured for normal adults (black circles). The mean d´ values for each selectivity category (listed on the abscissa) are plotted along the ordinate; lines connect mean d´ values obtained for each bird group. Error bars are SEM, and asterisks denote significant differences between these ts cut adults and normal adults. B) The degree of selectivity of LMAN neurons from ts cut adults with high similarity to the tutor song (white triangles) is compared to that measured for LMAN neurons from normal adults (black circles). Conventions are as in A.

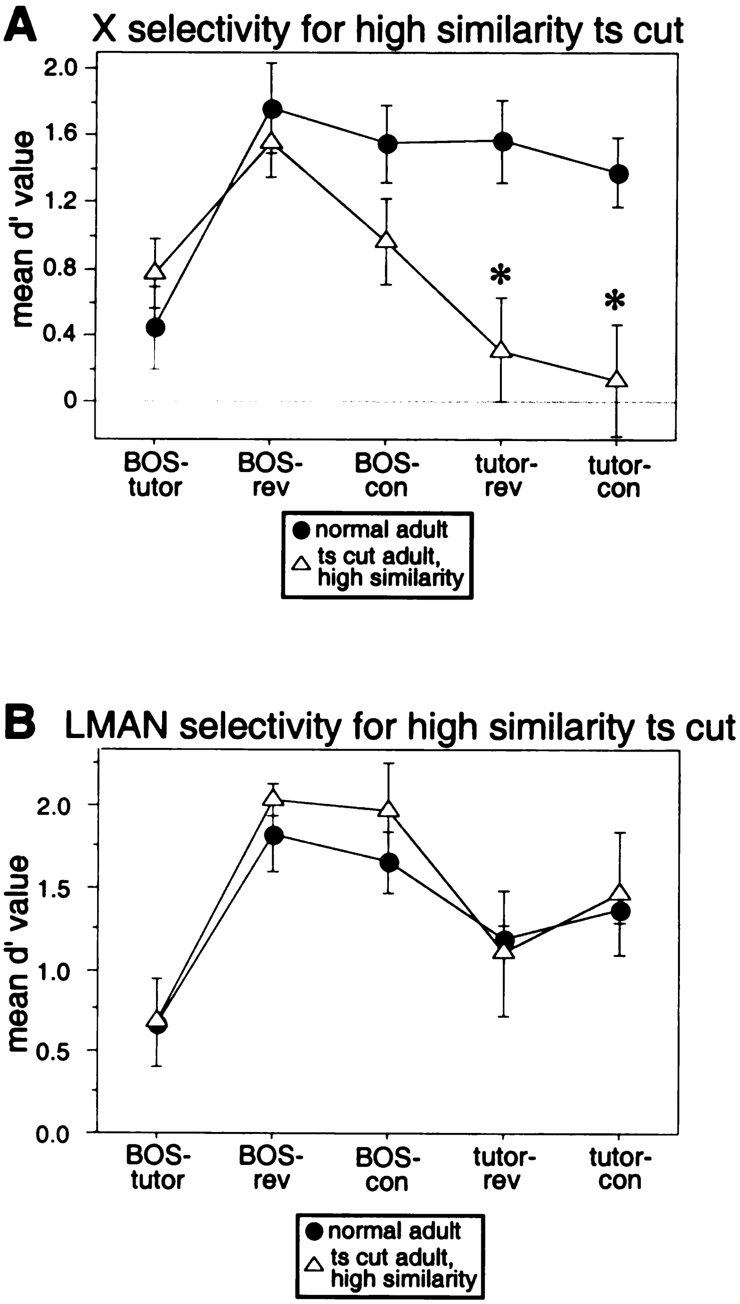

A
X selectivity for high similarity ts cut
mean d' value
2.0
1.6
1.2
0.8
0.4
0
*
*
BOS-
tutor
BOS-
rev
BOS-
con
tutor-
rev
tutor-
con
● normal adult
△ ts cut adult,
high similarity
B
LMAN selectivity for high similarity ts cut
mean d' value
2.0
1.5
1.0
0.5
0.0
BOS-
tutor
BOS-
rev
BOS-
con
tutor-
rev
tutor-
con
● normal adult
△ ts cut adult,
high similarity

Figure 4-9

Figure 4-10 Comparisons of X selectivity to tsBOS similarity to the tutor song. Scatterplots compare the mean $d'_{\text{tsBOS-reverse}}$ values for each ts cut bird to the acoustic similarity measured between its tsBOS and tutor song, using percent correctly matched (A), mean spectral similarity scores (B), and mean temporal similarity scores (C). Open and black circles mark ts cut adults with low and high similarity to tutor song, respectively. Lines are the linear least squares fit through the data.

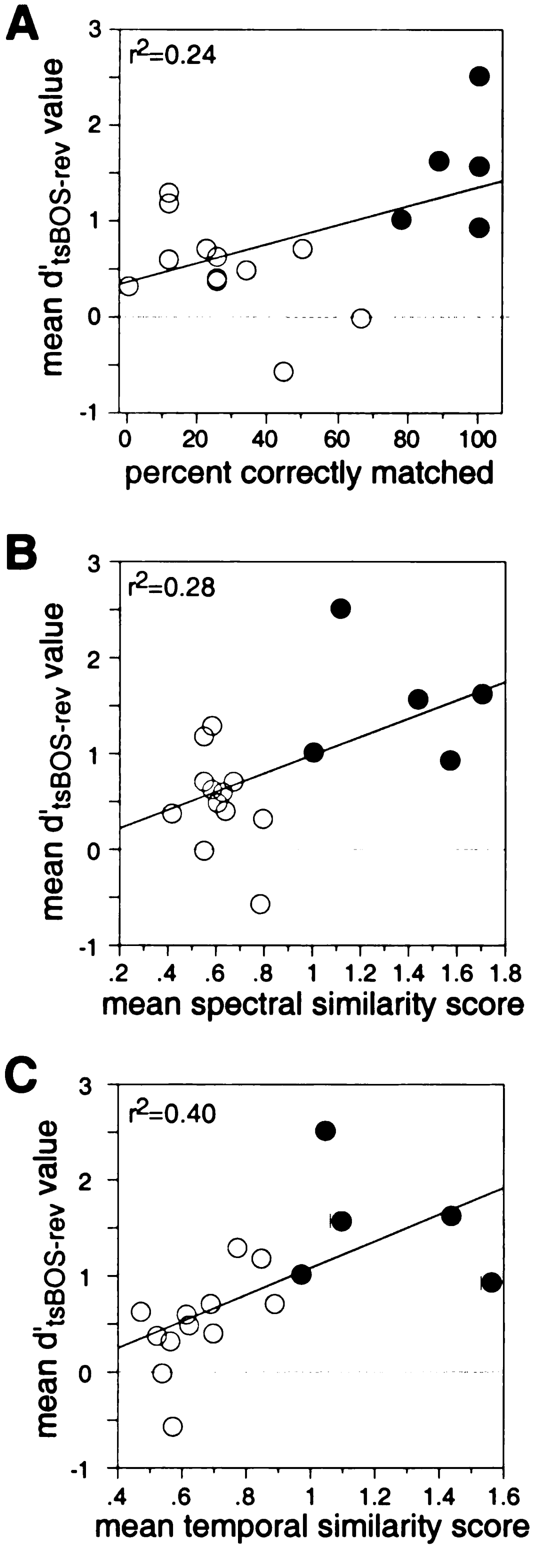

A
r²=0.24
mean d'tsBOS-rev value
3
2
1
0
-1
0
20
40
60
80
100
percent correctly matched
B
r²=0.28
mean d'tsBOS-rev value
3
2
1
0
-1
.2
.4
.6
.8
1
1.2
1.4
1.6
1.8
mean spectral similarity score
C
r²=0.40
mean d'tsBOS-rev value
3
2
1
0
-1
.4
.6
.8
1
1.2
1.4
1.6
mean temporal similarity score

Figure 4-10

Figure 4-11 Assessing potential trophic effects of ts transections on selectivity. A) The histogram compares the mean syrinx weight of normal adults (black), ts cut adults with low similarity to the tutor song (white), and ts cut adults with high similarity to the tutor song (gray). Error bars are SEM, and asterisks mark significant differences from normal adult weights. B) The cumulative distributions of spontaneous firing rates are shown for X cells recorded from all ts cut adults (white triangles) and normal adults (black circles).

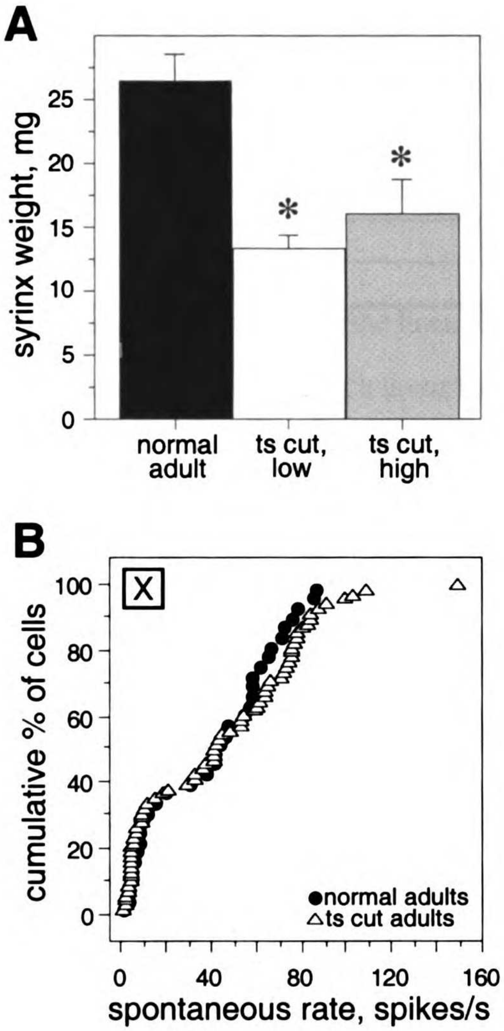

A
syrinx weight, mg
0
5
10
15
20
25
*
*
normal adult
ts cut, low
ts cut, high
B
X
cumulative % of cells
0
20
40
60
80
100
●normal adults
△ts cut adults
0
40
80
120
160
spontaneous rate, spikes/s

Figure 4-11

Table 4-1: Correlations between selectivity and song similarity

	ts cut adult song similarity to tutor song		
selectivity category	percent correct	spectral similarity	temporal similarity
$d'_{tsBOS\text{-}reverse}$	0.24	0.28	0.40
$d'_{tsBOS\text{-}con}$	0.02	0.07	0.10
$d'_{tsBOS\text{-}tutor}$	0.03	0.10	0.05
$d'_{tutor\text{-}reverse}$	0.01	0	0.01
$d'_{tutor\text{-}con}$	0	0	0.01

The coefficient of determination (r^2) is shown for the linear least squares fit of each comparison between the mean selectivity from each ts cut bird (dependent variable) and its measured similarity to tutor song (independent variable). Entries of 0 refer to r^2 values less than 0.004.

Chapter 5:

Issues in Measuring Selectivity in the Anterior Forebrain

ABSTRACT

The songbird anterior forebrain (AF) may process the auditory experiences necessary for song learning. At an intermediate stage of song learning, AF neurons are selective for the bird's own song (BOS) and tutor song, preferring these songs to reverse versions of song. The ability of a neuron to discriminate between two stimuli depends on the strength and variability of its response, which are both incorporated in a d′ measure of selectivity. This measurement of selectivity, however, also depends on what is considered a neural response. Although neural responses are normally measured over the entire song duration, responses measured over smaller durations may reveal increased selectivity. In order to find the time scale yielding maximum selectivity, responses of AF neurons were measured over a series of time windows, ranging from 10 to 2000 ms. In general, long windows (≥500 ms) gave the highest discrimination between forward and reverse song. This reflects the complex nature of these cells, whose responses probably encode entire syllable combinations.

To understand the reliability with which auditory responses in the AF could signal behaviorally relevant stimuli during learning, response variability was measured. For responses measured over entire song stimuli, high variability was apparent: the variance of the spike count exceeded the mean count by a factor of three. There was also no strong difference in variability between two different nuclei in the AF. During development, however, response variability decreased for both nuclei: AF neurons from adult birds were less variable in their responses to song than those from juvenile birds. This increase in response reliability could contribute to the increase in selectivity apparent for AF neurons during song learning.

INTRODUCTION

Neurons in the zebra finch anterior forebrain (AF) are highly selective for extremely complex song stimuli. In adults, these neurons respond with higher firing rates to presentation of the bird's own song (BOS) stimuli than to other zebra finch songs, or to the reversed versions of these songs (Doupe and Konishi, 1991; Doupe, 1997). This sensitivity to the spectral and temporal features of song makes these selective AF neurons well suited to process the essential auditory experiences of BOS and tutor song during song learning (Konishi, 1965; Price, 1979). Consistent with this, LMAN and X, two nuclei within the AF, must be intact for normal song learning to occur (Bottjer et al, 1984; Sohrabji et al, 1990; Scharff and Nottebohm, 1991). Furthermore, these neurons are auditory throughout development, and acquire selectivity for both BOS and tutor song by an intermediate stage of song learning (Solis and Doupe, 1997). Thus, AF neurons could mediate the effects of these essential auditory experiences on the nervous system during song learning.

The degree of selectivity could also depend on the time interval over which a neural response is measured. Song selectivity is apparent when neural responses are measured over the entire stimulus duration, as is commonly done in studies of song system neurons. The firing rate of an AF neuron, however, can fluctuate dramatically during its response to a relatively long stimulus (2sec is a typical duration for zebra finch song). This raises the possibility that neural responses measured over time intervals shorter than the song itself could yield higher selectivity. To address this, we have analyzed how selectivity varies as a function of the time interval used to measure neural responses. This analysis estimates the time intervals over which maximum discrimination occurs between two song stimuli, which not only guides our decisions about how best to measure a neural response, but also informs our ideas about the decoding capabilities of neurons downstream from LMAN and X.

Whether AF neurons could encode BOS or tutor song stimuli depends on the strength and variability of their responses. For example, although a neuron may on average respond more to BOS than to BOS played in reverse, it would not reliably signal the occurrence of BOS if its response is highly variable from trial to trial. Thus, a good description of a neural response would consider both response strength and variability. A measure of selectivity that incorporates these two factors is d´, which compares the responses to two different stimuli. The metric d´ expresses the relative difference in response strength to two stimuli, normalized by the variability of their responses; this results in a measure of the discriminability between two different stimuli, like BOS and reverse BOS.

AF selectivity increases between 60 d and adulthood, and changes in response strength, response variability, or both, could mediate this increase. Understanding how these factors contribute to increases in selectivity may yield insight onto the mechanisms underlying selectivity development. For example, it has been shown that the increase in selectivity for BOS over reverse song between 60 d and adulthood is accompanied by an increase in response strength to BOS, and by a decrease in response strength to the reverse BOS (Solis and Doupe, 1997); this suggests that changes in synaptic strength could underlie selectivity development. In addition, this increase in selectivity could be mediated by decreases in response variability; such a change could reflect increased synaptic reliability, perhaps due to a change in the probability of release for a presynaptic neuron. Thus, to address the contributions of response variability to AF selectivity, response variability was characterized in LMAN and X neurons to behaviorally relevant song stimuli from 60 d birds. As a beginning, the response variability over the entire song stimulus was measured. The response variability for 60 d neurons was then compared to that measured for AF neurons recorded in adult birds.

MATERIALS AND METHODS

We analyzed data collected for previous studies of selectivity in the zebra finch anterior forebrain (Solis and Doupe, 1997). In brief, responses of single neurons to different song stimuli were recorded extracellularly in anesthetized juvenile (60 d of age) or adult zebra finches. In this study, only the responses to BOS, reverse BOS, tutor song and reverse tutor song were used.

Neural responses to a stimulus were quantified for the period during which the stimulus was presented, offset by an estimate of the latency of the response (for details, see Solis and Doupe, 1997). The number of spikes obtained during this period was used as the "spike count" response of the neuron. Spike counts were obtained for each presentation of a stimulus, and the mean count and variance were calculated. In order to compare spike counts elicited by different stimuli that had different durations, the firing rate was calculated: each count was normalized by duration (in ms), then multiplied by 2000 ms (typical length of a zebra finch song). This estimates the spike count for a 2 sec stimulus for every cell. Count variance was also estimated in this way. Mean spike count and variance were obtained for a file of song, containing typically 15-20 trials of bird in same state, usually equivalent to 15-20 minutes of recording time. The mean counts and variances of each file were averaged across the same stimulus type. These were not significantly less from mean spike counts calculated over longer times (~1 hour), however.

The firing rate for each cell was to measure selectivity. The difference between the firing rate during a stimulus and the spontaneous firing rate (details on measurement in Solis and Doupe, 1997) was called the response strength (RS). The selectivity of a cell for stimulus A over stimulus B was quantified with a d′ value, a measure adapted from psychophysics. Adapted from psychophysics (Green and Swets, 1966), the d′ measure quantifies the preference of a neuron for stimulus A over stimulus B such that $d'_{A-B} = \frac{2\left(\overline{RS_A} - \overline{RS_B}\right)}{\sqrt{\sigma_A^2 + \sigma_B^2}}$. In this equation, $\overline{RS_A}$ and $\overline{RS_B}$ are the mean RS to stimulus A and B, respectively, and σ^2 is the variance of each mean RS. If d'_{A-B} is positive, then A elicited a stronger response, if d'_{A-B} is negative, then B elicited a stronger response, and d'_{A-B} values of ~0 indicated no difference in the responses evoked by stimulus A and stimulus B. The d′ value can distinguish between two means that come from largely overlapping distributions and those that do not. Thus, d′ is a measure of discriminability between two stimuli given the two neural responses.

For each cell, the cumulative responses to a stimulus were displayed as a peristimulus time histogram (PSTH). To measure the maximum response of a neuron to a particular stimulus, a Gaussian curve of a specified width was slid across the cumulative responses PSTH, so that the rate was calculated at every 1 ms increment. The place in the stimulus eliciting the maximum rate was noted, and then the peak rate was calculated at this place for each trial; in addition, the same procedure was done during background. The difference between the peak response and peak background gave a maximum response strength (RS) for each trial; this was then averaged over trials to obtain a mean maximum RS. This was done for each stimulus, and resulted in maximum responses for the same time windows; however, the maximum RS of different song types reflected different parts of the songs relative to song onset. The maximum RS and variance for different stimuli were then compared using the d′ measure in order to quantify a neuron's ability to discriminate between two stimuli. This procedure for maximum RS was repeated for windows ranging from 10 ms to 2000 ms in width.

RESULTS

The data analyzed here came from 60 d old birds, and included 56 LMAN cells and 49 X cells. Neural responses to BOS, tutor song, and reverse versions of these songs (both the notes within the syllables and the sequence of syllables within the song are reversed) were analyzed.

Time scale for maximum selectivity

When quantifying neural responses to song, it is common to normalize the number of spikes fired during the entire song by its duration. Although AF neurons are selective for forward song over reverse song when measured this way, the time-varying response profiles of neurons during the relatively long song stimulus (2 sec) raised the possibility that responses measured over shorter durations might yield higher selectivity. To examine this, we calculated selectivity over a series of time windows, ranging from 10 to 2000 ms. Each time window was moved across the cumulative responses of a cell to a stimulus until the maximum firing rate was obtained. At that point in the stimulus, the response strength and variance was measured across trials, and used in the d′ measure of selectivity. Two measures of selectivity were analyzed: $d'_{\text{BOS-rev}}$ and $d'_{\text{tutor-rev}}$ (see Methods).

Selectivity varied as a function of the time interval over which neural responses were measured. The flucuations of $d'_{\text{BOS-rev}}$ values over different time windows is shown for 2 different LMAN cells in Figure 5-1A. The upper panel shows the most typical curve, where d′ values rise as window length increases, and plateau at 1000 ms. The lower panel shows a rare example of a cell for which a short window (100 ms) yielded highest selectivity; similar peak-type curves were apparent for only 5/56 LMAN neurons. Although the exact shapes of the curves varied for each cell, in general long windows gave higher d′values than short time windows. This is shown in Figure 5-1B, which compares the maximum $|d'_{\text{BOS-rev}}|$ and $|d'_{\text{tutor-rev}}|$ values among the group of long windows (500-2000 ms) to that from the short windows (10-250 ms) for each cell; the absolute value of the d′ values indicates the cell's ability to discriminate between the two stimuli, regardless of which stimulus evoked the larger response. The majority of points lie above the dashed line representing unity, indicating that most cells had higher discrimination between forward and reverse song when their responses were measured over long windows rather than short windows. The average of maximum d′ values obtained from long windows were significantly greater than that obtained for short windows (Wilcoxon test, $p<0.0001$ for $|d'_{\mathrm{BOS\text{-}rev}}|$ and $p<0.009$ for $|d'_{\mathrm{tutor\text{-}rev}}|$). In X, another nucleus in the AF, both plateau- and peak-type curves were also obtained from individual cells (Figure 5-1C); however, only 6/49 X cells had peak-type curves. Typically, long windows gave the maximum discrimination between forward and reverse song (Figure 5-1D): the majority of the points lie above the dashed line. The average of the maximum $|d'|$ values from long windows was significantly greater than that obtained for short windows (Wilcoxon tests, $p<0.0001$ for $|d'_{\mathrm{BOS\text{-}rev}}|$ and $p<0.04$ for $|d'_{\mathrm{tutor\text{-}rev}}|$).

To compare the relation between selectivity and time window across nuclei, the d′ values for each window were averaged across all LMAN and all X cells. The resulting average curves were quite similar between nuclei. In Figure 5-2A, the average discriminability between BOS forward and reverse stimuli increased for time windows between 10 ms and 750 ms, upon which it reached and maintained a plateau out to 2000 ms. The average discriminability between tutor song stimuli also increased as time windows lengthened for both LMAN and X, but a plateau was less evident (Figure 5-2B); this may suggest that the two stimuli are encoded differently within the AF. Although the shapes of the curves for tutor song selectivity were similar between the nuclei, on average X neurons consistently exhibited higher $|d'_{\mathrm{tutor\text{-}rev}}|$ than LMAN neurons for each time interval tested. The higher selectivity for long time windows obtained here indicates that normalizing spike count by the duration of the entire stimulus does not grossly underestimate the neural response to the stimulus.

Response variability of AF neurons

Response variability limits the reliability with which a neuron can encode a stimulus. Although this is incorporated in d′ measures of selectivity, we examined response variability alone to investigate the extent to which it characterized AF responses in birds at an intermediate stage of learning. As a beginning, we have measured the variability of spike counts obtained over an an entire song stimulus. Figure 5-3A shows an example of this response variability obtained from the responses of a single LMAN neuron from a 60 d bird; the rasters show the spike events for 15 presentations of BOS. To quantify the variability of this response, the mean spike count during the stimulus and the variance across trials were compared in a variance/mean spike count ratio; for the response in Figure 5-3A, this was 2.3. Because slow changes in responsiveness during recording could lead to an overestimation of response variability, the mean count and variance calculations in this study were based on 15-20 trials collected over a time during which the neuron appeared to be in the same state of responsivity. This was subjectively judged during the experiment, based on the spontaneous rate of the cell. In all, this represented 15-20 minutes of recording. The mean counts and variance over this collection time were then averaged with those values obtained for the same stimulus from other collection times.

To characterize the response variability of the population of LMAN neurons, the mean spike count and associated variance was plotted for each LMAN neuron's response to BOS, tutor song, and the reverse versions of these songs (Figure 5-3B); thus, data from a single neuron is represented by four points, each corresponding to a response to each stimulus type. These data were best fit with the power law function: $\text{variance}=2.0\text{count}^{1.3}$ ($r^2=0.86$). The relation between mean spike count and variance was summarized for each data point with the variance/mean spike count ratio; the frequency distribution of the resulting ratios are shown in Figure 5-3C. On average, the variance exceeded the mean count by a factor of three.

To examine whether the degree of response variability was conserved within the AF pathway, the spike count and variance of 48 single X cells was analyzed. An example of a single neuron's variable response to repeated BOS presentations is shown in Figure 5-4A; the variance/mean spike count for this response was 3.1. For the population of X neurons, however, the relation between mean spike count and variance measured for the four stimuli was not as well described by a power law function (solid diagonal line, Figure 5-4B): variance=4.9count$^{.79}$ (r^2=0.49). The poor quality of this fit was in part due to the inclusion of two types of X neurons, based on their spontaneous rates. In this data set, 13 neurons had spontaneous rates that were less than 10 spikes/sec and 35 neurons had spontaneous rates 10 spikes/sec or greater. A power law reasonably described for X cells with low spontaneous rates the relation between mean count and variance (upper dashed line, Figure 5-4B): variance=2.1count$^{1.2}$ (r^2=0.71). In contrast, data from X cells were still not fit well by a power law (lower dashed line, Figure 5-4B): variance=0.6count$^{1.2}$ (r^2=0.35). Instead, these data were best fit by the linear equation: variance=2.4count (r^2=0.61). The relation between mean spike count and variance for each datum was summarized with variance/mean count ratios; the distribution of these ratios is shown in Figure 5-4C. As in LMAN, there was a large range of ratios, and on average the variance exceeded the mean count by a factor of 3. Furthermore, responses from low spontaneous X cells (black bars, Figure 5-4C) were more variable than those recorded from high spontaneous cells (gray bars) ($p<0.0001$, Mann-Whitney test).

The relation between mean spike count and variance was compared between responses to different stimulus types. Figure 5-5A shows the distributions of variance/mean count ratios for each stimulus type obtained from LMAN neurons. These ratios were similar across stimulus type (Friedman test, $p=0.241$), indicating that response variability was the same, whether the cell was responding to a behaviorally relevant stimulus (BOS or tutor) or not (reverse songs). In contrast, for X cells, small differences in response variability was apparent between different stimuli (Friedman test, $p<0.001$). The distributions of the variance/mean ratios for different stimuli obtained from X neurons are shown in Figure 5-5B. Specifically, the ratios obtained from BOS responses were significantly more greater than the ratios obtained from either reverse BOS or reverse tutor song (Wilcoxon rank, $p<0.006$ and $p<0.005$, respectively). Thus, the higher variability for BOS suggests that song stimuli are not all processed equivalently at this age.

Response variability within the AF

To examine whether the degree of response variability was conserved within the AF, a mean count and variance was obtained for each LMAN and X cell by taking the average of spike counts and variances originally calculated for each stimulus types. Figure 5-6A shows the relation between mean spike count and variance for each LMAN and X cell. Although X cells had much higher spike counts, for several X cells the variance was similar to that expected from the relation between mean count and variance for LMAN cells. These cells tended to be low spontaneous X cells, and the power law function describing the relation between mean count and variance was overlapping with that for X data. Many X cells, however, fell below the line describing the power law fit for the LMAN data, indicating that they were less variable than LMAN cells; these tended to have high spontaneous rates. Similarly, the power law fit of data from high spontaneous X (dashed line) was shifted downward relative to the LMAN fit, indicating lower variance. A comparison of the variance/mean count ratios obtained for each cell also shows that although the distributions were overlapping, data for X cells were slightly shifted toward smaller ratios, or less variability. There was no significant difference between the mean ratio between LMAN and X, however ($p=0.146$, Mann-Whitney test). When X cells were divided by cell type, the variance/mean count ratios from X cells with high spontaneous rates were significantly less than those from LMAN cells ($p<0.005$, Mann-Whitney test).

The mean variance/ count ratio ±SD for X cells with high spontaneous rates was 2.4±1.6 and for X cells with low spontaneous rates was 4.5±2.5. These means were signifcantly different ($p<0.001$, Mann-Whitney U test), as was the case when ratios from responses to all stimulus types were compared. Thus, although on average there was not a significant difference in variability when compared across all cells in LMAN and X, X cells with high spontaneous rates showed the least variability in their responses.

Changes in response variability during development

During song development, selectivity increases in both LMAN and X: adult AF neurons exhibit a higher degree selectivity than neurons from 60 d juveniles. To examine whether a change in response variability could contribute to this increase in selectivity, the mean count and variance of each LMAN cell recorded from 60 d birds was compared to those obtained from 14 neurons recorded in adult birds (Figure 5-7A). In general, LMAN neurons from adults had lower response variability than those from 60 d juveniles: most adult cells had lower variance than many 60 d neurons, and the power law fit of the adult data was shifted downward compared to the fit obtained for 60 d cells. This trend for lower response variability in adult neurons is also evident by comparing the distributions of variance/mean count ratios (Figure 5-7B): ratios from adult neurons lacked the higher ratios prevalent in the juvenile neural responses. The average variance/mean count ratio from adults, although from a limited sample, was significantly less than that from 60 d birds ($p<0.001$, Mann-Whitney U test). Further comparisons of variance/mean count ratios for different stimuli revealed that the decrement in variability for adults was equivalent across stimulus type (Figure 5-7C). Thus, a decrease in response variability seemed to accompany the increase in selectivity for LMAN neurons between 60 d and adulthood.

A similar decrease in variability between 60 d and adulthood was apparent for X cells. Response variance from 29 X neurons from adults tended to be lower than that measured for 60 d neurons (Figure 5-7D). Furthermore, the distribution of variance/mean count ratios obtained from each adult neuron was shifted toward lower values relative to the distribution from 60 d neurons (Figure 5-7E); the average ratio from adults was significantly less than that from juveniles ($p<0.0001$, Mann-Whitney U test). This difference in response variability between adults and juveniles was also true when each type of X neuron was considered separately ($p<0.002$ for high spontaneous and $p<0.003$ for low spontaneous; Mann-Whitney U test). X cells with both high and low spontaneous rates were found in adults in proportions similar to that found in 60 d birds: of all X neurons, 28% had low spontaneous rates in adults, compared to 27% in 60 d birds. Comparisons of variance/mean count ratios for different stimuli revealed equivalent decreases in variability across stimulus type (Figure 5-7F). Thus, the decrease in response variability for adults was prevalent in the AF, occurring in both LMAN and X, for both X cell types, and for responses to all stimuli.

DISCUSSION

In this chapter, we have extended our analysis of AF neural responses in birds at an intermediate stage of song learning. We found that the time interval over which neural responses to song stimuli were measured could greatly influence the measured selectivity. In general, long windows (≥500 ms) optimized selectivity for forward song over reverse song in both LMAN and X. One component of a neuron's selectivity, its response variability, was also measured separately over the entire stimulus duration. In juveniles, the variance exceeded the mean count by a factor of three in both LMAN and X. Within the AF, this degree of response variability was conserved between all LMAN and X cells; however, a subpopulation of X cells exhibited significantly lower levels of variability. Finally, response variability decreased between 60 d and adulthood, and thus could mediate the increase in selectivity that occurs during song learning.

Relevant time intervals for measuring AF neural responses

The time varying responses of AF neurons raised the possibility that these neurons were most sensitive to particular components of song, like syllables. While these features would allow the neuron to identify and thus encode the song, measuring the neural response over the entire song would then underestimate the neuron's selectivity for the song. We found that measurements of selectivity were sensitive to the time interval over which the original neural responses were calculated. Longer duration intervals resulted in optimal selectivity, however: maximum discrimination between forward and reverse stimuli was obtained over intervals of 750 ms and maintained up to 2sec for both LMAN and X neurons. These longer time intervals may reflect the ability of AF neurons to respond to combinations of syllables within a song (Doupe, 1997); the average duration of a song syllable is ~120 ms. Similarly, the weak selectivity obtained for windows shorter than 100 ms could be due to splitting a neuron's response to an entire syllable or syllable combinations. Furthermore, the consistently high selectivity obtained for neural responses measured over long time intervals indicate that normalizing a response by the duration of the stimulus, as has been done in most studies of song system neurons, does not seriously underestimate the selectivity of a neuron.

The appropriate time interval over which a neural response should be measured depends on the integration times of downstream neurons. Our results suggest that the downstream neurons could have relatively long integration times, given that discrimination between stimuli is increased for longer time intervals. The actual integration time of a neuron, however, is difficult to determine. It is the product of complex interactions between cellular properties such as membrane time constants, PSP durations, and polysynaptic connectivity and the particular inputs to that neuron. Although experiments in slice preparations of downstream nuclei (Mooney, 1992) measure these cellular properties, understanding how they interact with song input will require intracellular recordings *in vivo*. Integration times of a neuron can also be approximated by measuring the neural response to systematically decomposed songs. Such experiments would find the combinations of syllables required to reconstitute the response to the entire song. In this way, HVc neurons have been found to have integration times from between 80 ms to 350 ms (Margoliash and Fortune, 1992). Similar syllable decomposition experiments in downstream targets of LMAN and X would complement the information gained from slice experiments in these areas, and guide our thinking about how song stimuli are encoded by these neurons.

Possible sources of response variability

In 60 d birds in the process of song learning, response variance was high, approximately three times the mean spike count. In adult birds response variance was reduced to about twice the mean count. Although this variability constrains the capability of AF neurons to reliably encode stimuli, it did not abolish selectivity. Several factors may have caused an overestimation of response variability, however. First, these recordings were made in anesthetized animals, which may have experienced changes in arousal leading to fluctuating states of cellular excitability. Several studies have found that presumably anesthesia-related changes in cellular responsiveness while collecting responses from a cell increases measurements of response variability (Tolhurst et al, 1983; Bradley et al, 1987; Arieli et al, 1996). These fluctuations in cellular excitability may also occur in awake animals, however: in the visual cortex, differences in response variability were not observed between awake and anesthetized preparations (Vogels et al, 1989). Although we tried to minimize these effects on our measurement of response variability, it will be important to measure the variability of auditory responses of AF neurons in awake birds in order to clarify its impact on neural selectivity during learning. Furthermore, because AF neurons exhibit both auditory and premotor activity in awake birds, another possible source of variability in the anesthetized bird could be due to the decoupling between premotor activity and auditory feedback of BOS. Normally, birds would not experience auditory feedback of their own song without singing themselves. This would not explain the variability of tutor song responses, however.

Our calculation of mean spike counts and variance over the entire stimulus duration is likely to have inflated our measurement of response variability. For example, if 10 spikes normally occur during the entire stimulus presentation, trials in which no spikes occur, perhaps due to a state change, will greatly increase the variance of spike count over trials; however, when spike counts are measured over smaller time intervals, then trials with no spikes will have less impact on the variance. This effect of time interval on measured response variability has been demonstrated for the auditory nerve and brainstem (Teich et al, 1990), and for the H1 neuron in the fly visual system (de Ruyter van Steveninck et al, 1997). Thus, measuring response variability over smaller time intervals could find a lower bound of response variability; in addition, it can approximate the degree of response variability that is more likely to confront downstream neurons, which are unlikely to have integration times of 2000 ms. Moreover, given the time-varying properties of song stimuli, it is possible that response variability would fluctuate consistently during stimulus presentation, perhaps reaching a minimum for those places in the song evoking the maximum number of spikes.

The response variability of AF neurons measured here is difficult to compare to that found in higher order neurons because of differences in how variability is measured. In the visual cortex, the response variance is approximately 1-1.5 times the mean spike count (Shadlen and Newsome, 1998; Dean, 1981; Bradley et al, 1987; Tolhurst et al, 1983; Vogels et al, 1989; Snowden et al, 1992). Two of these studies measured variability of spike counts occuring during a 2 sec interval (Shadlen and Newsome, 1998; Bradley et al, 1987), which most resembles our estimation of response variability over a 2 sec song stimulus. Thus, AF neurons from adults had somewhat similar response variability (~1.6 for X and ~2 for LMAN in adult birds) to that measured in the visual cortex. That AF neurons share similar variability probably reflects their high order place in sensory processing of song stimuli, and may reflect the extent of their intrinsic circuitry (Stratford et al, 1996). It has been suggested that input variability could actually improve the ability of neural circuits to extract information about a stimulus (Douglas et al, 1995).

Inclusion of response variability in a selectivity measure

Whether to include response variability in a measure of selectivity depends on the extent to which response variability compromises the decoding capabilities of downstream neurons. The impact of response variability on downstream neurons is influencedby the connectivity with their inputs. For example, a downstream neuron could increase the signal coming from noisy inputs, by averaging over several, independent inputs. In this case, it would be more appropriate to ignore response variability in a measurements of selectivity for one stimulus over another. A "selectivity index" does this by comparing the average response of a neuron to two different stimuli, in ratio form (Volman, 1996; Doupe, 1997; Solis and Doupe, 1997). In contrast, if downstream neurons only receive input from one or a few neurons, then they would be more susceptible to each neuron's response variability. In this case, it would be more appropriate for the song selectivity measure to consider response variability, such as the metric d′ (Solis and Doupe, 1997; Theunissen and Doupe, 1998). Thus, the connectivity between AF neurons and their downstream targets has important implications for the signal extraction capabilities of downstream neurons.

Unfortunately, little is known about the precise connectivity between LMAN and X cells and their downstream targets. Recent data from the DLM neurons downstream of X, however, suggests that a DLM neuron receives input from only one X projection neuron (David Perkel, personal communication); this would make them extremely sensitive to the response variability of the X projection neuron. Another factor affecting a downstream neuron's ability to average out noise is the correlation between inputs. If their variability is correlated, then the neuron would not be able to average out the noise quite as well (Zohary et al, 1994; Shadlen and Newsome, 1998). Although the extent of correlation between LMAN neurons or between X neurons is unknown, this could be a factor in the AF. Recordings from pairs of neurons in HVc, the input nucleus to the AF, show higher correlation between pairs of neurons within a trial than across trials (Janata and Margoliash, 1998).

Response variability decreases during song learning

The increase in song selectivity for AF neurons between 60 d and adulthood was also accompanied by a decrease in response variability. A difference in the effects of anesthesia between these ages probably does not account for the decreased variability, since a difference in reaction to anesthesia has not been observed between 60 d and adult birds. The interpretation of this decrease is limited, however, by the fact that 60 d and adult data were collected for different studies and thus in potentially varied conditions. Ideally, one would record from the same neuron throughout development, obtaining measures of RS and variance at each stage (Mioche and Singer, 1989). With this caveat in mind, however, comparisons across ages suggest that selective circuits can be constructed by changing response strength, like synaptic strength (Solis and Doupe, 1997), as well as by decreasing response variability. An increase in synaptic reliability occurs for thalamic inputs onto LMAN neurons between 20 and 60 d of age, as measured by a decrease in probability of release (Boettiger and Doupe, 1998b). The increased response variability for 60 d AF neurons relative to adults may serve a special function during song learning. Response variability could limit the amount of correlated activity reaching a cell, perhaps preserving plasticity. This plasticity may be helpful during song learning, allowing premotor circuitry to explore motor space during plastic song. Alternatively, even if the decrease in variability is simply an outcome of development, response variability itself could indicate the maturity of these neural circuits.

Figure 5-1 Selectivity varied over the time interval used to measure a response. A) The selectivity for BOS over reverse BOS, as quantified with a d′ value, is plotted for each time interval used to measure the neural response and variance. Two examples of curves are shown for two different LMAN cells: the top panel shows a neuron that had best discrimination for longer windows, whereas the bottom panel shows a neuron with best discrimination with a short window. B) For each LMAN cell, the maximum |d′| obtained among those calculated for short windows (10-250 ms) is compared to the maximum |d′| from long windows (500-2000 ms). The dotted line marks where points would lie if the maximum |d′| values were the same. The d′ values measure discrimination between BOS and reverse BOS (solid circles), and between tutor song and reverse tutor (open circles). C) The relation between selectivity for BOS over reverse stimuli and the time interval used to measure the neural response is shown for two X neurons. The top panel shows a neuron that had best discrimination for longer windows, whereas the bottom panel shows a neuron with best discrimination with a short window. D) For each X cell, the maximum |d′| from short windows is compared to that obtained from long windows. Conventions are as in B.

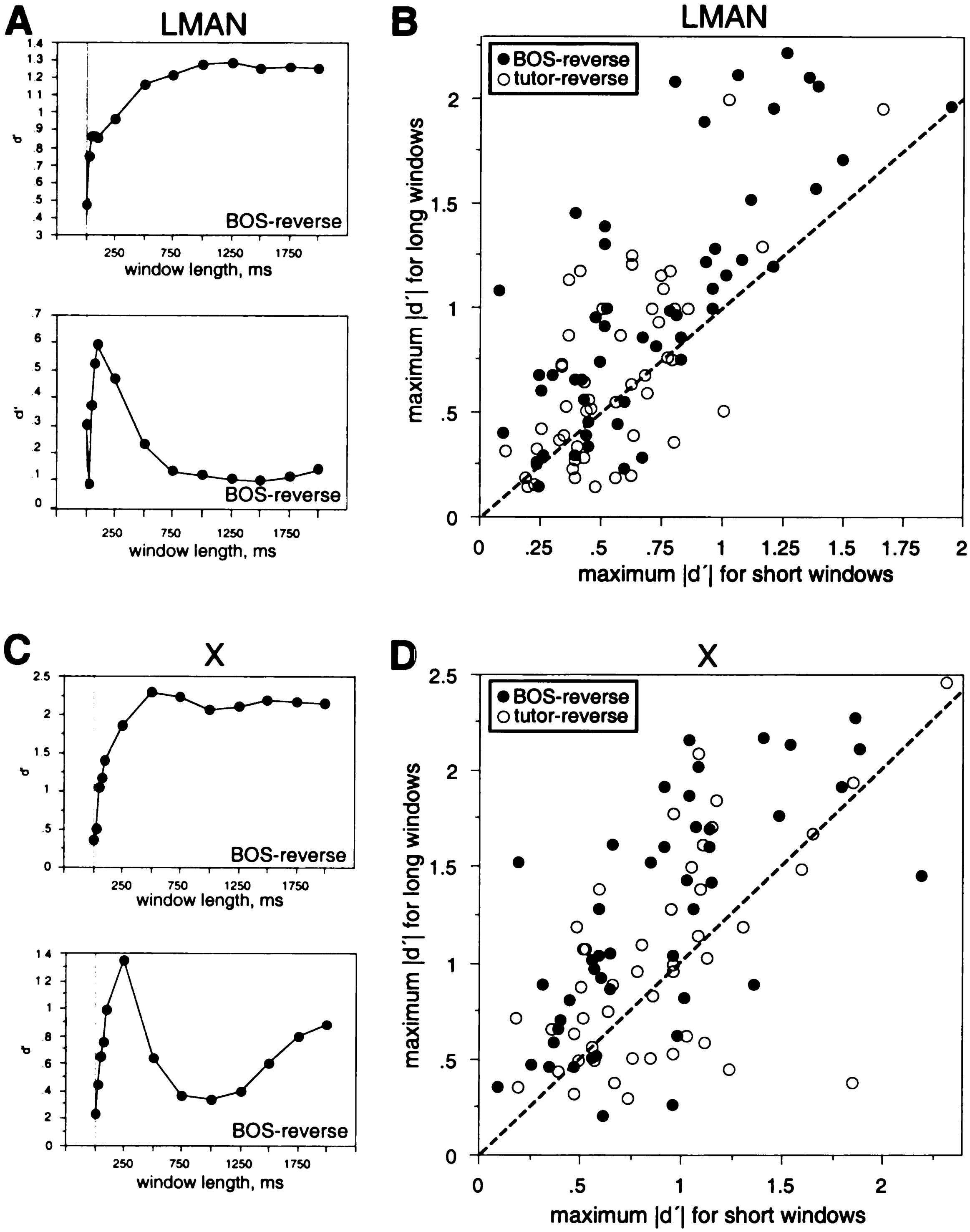

A
LMAN
d'
window length, ms
BOS-reverse
250
750
1250
1750
B
LMAN
● BOS-reverse
○ tutor-reverse
maximum |d′| for long windows
maximum |d′| for short windows
0
.25
.5
.75
1
1.25
1.5
1.75
2
C
X
D
X
2.5

Figure 5-1

Figure 5-2 Discrimination increased between forward and reverse song as the response window duration increased A) The mean |d′| values for discrimination between BOS and reverse BOS are compared for LMAN (solid circles) and X (open circles) cells for a series of time intervals over which the neural response to each stimulus was measured; error bars are SEM. B) The mean |d′| values for discrimination between tutor song and reverse tutor song are compared for LMAN and X neurons for a series of time intervals; conventions are as in A.

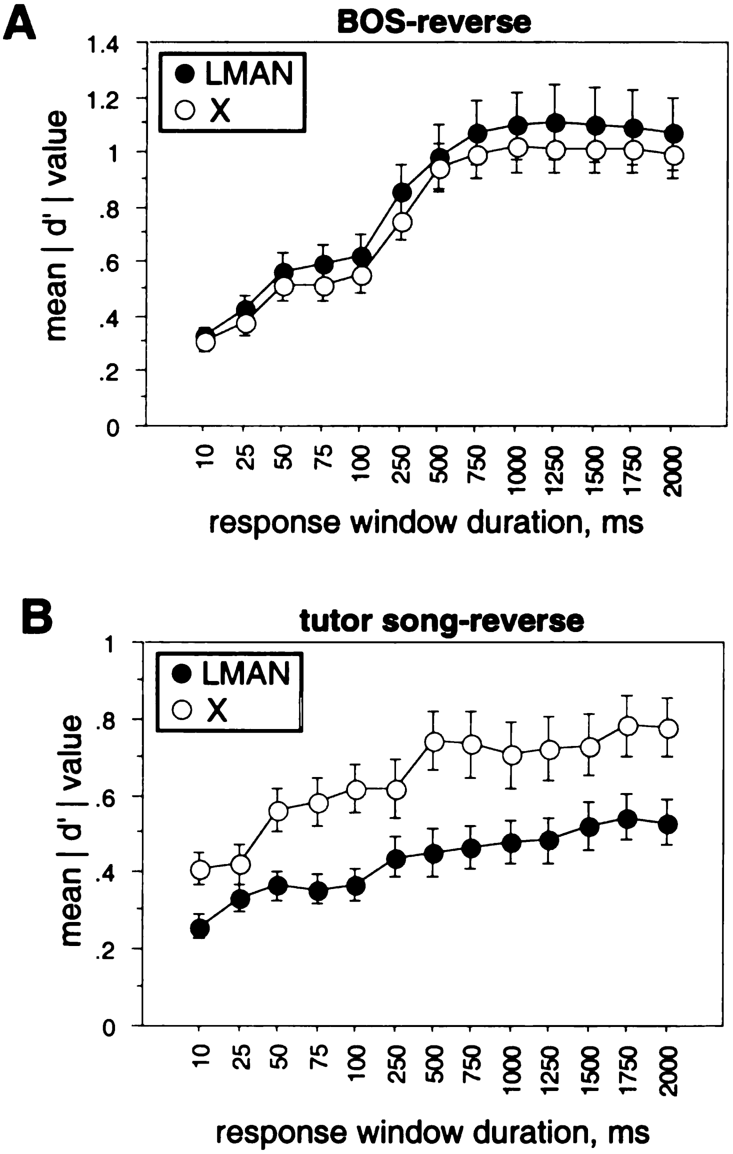

A
BOS-reverse
● LMAN
○ X
mean | d' | value
1.4
1.2
1
.8
.6
.4
.2
0
10
25
50
75
100
250
500
750
1000
1250
1500
1750
2000
response window duration, ms
B
tutor song-reverse
● LMAN
○ X
mean | d' | value
1
.8
.6
.4
.2
0
10
25
50
75
100
250
500
750
1000
1250
1500
1750
2000
response window duration, ms

Figure 5-2

Figure 5-3 Response variability of LMAN neurons recorded from 60 d old juvenile birds. A) Spike rasters show the spike events of a single LMAN cell for each trial of BOS. A peristimulus time histogram (PSTH) displays the cumulative response to BOS over 15 trials. The lower panel shows the BOS stimulus in sonogram (frequency vs time plot, with the energy in each frequency band indicated by the degree of darkness) and oscillogram (amplitude waveform vs time plot) form. B) The mean spike count is plotted against the variance of the responses from 56 LMAN neurons. Each neuron's response to four different stimuli is shown. The best fitting power law for all data points is shown by the diagonal line. C) A histogram shows the distribution of variance/mean spike count ratios obtained for each datum in B.

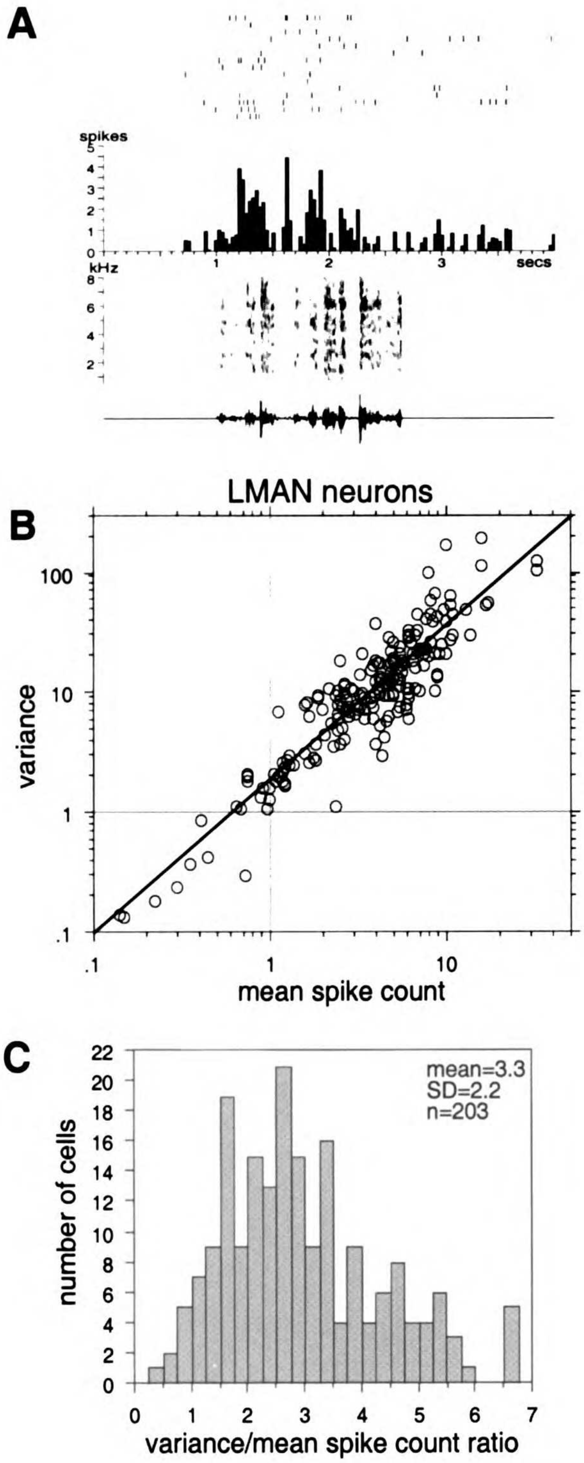

A
spikes
5
4
3
2
1
0
1
2
3
secs
kHz
8
6
4
2
B
LMAN neurons
variance
100
10
1
.1
.1
1
10
mean spike count
C
number of cells
22
20
18
16
14
12
10
8
6
4
2
0
mean=3.3
SD=2.2
n=203
0
1
2
3
4
5
6
7
variance/mean spike count ratio

Figure 5-3

Figure 5-4 Response variability of X neurons from 60 d birds. A) Rasters show the spike events of a single X cell for each presentation of BOS. A PSTH shows the cumulative spike counts to BOS over 15 trials. The variance/mean count ratio of the responses during BOS in this collection block of trials was 3.1. B) The mean spike count is plotted against the variance of the response obtained from 48 X neurons. Each neuron's response to four different stimuli is shown. The best fitting power law for all data points is shown by the solid diagonal line. The dashed gray lines indicate the fits for X neurons when subdivided by their spontaneous rate. Neurons with high rates are indicated with open circles; neurons with low rates are shown with black circles. C) A histogram shows the distribution of the variance/mean spike count ratios obtained for each X cell's responses to each stimulus. Gray and black bars indicate ratios from high and low spontaneous rate cells, respectively.

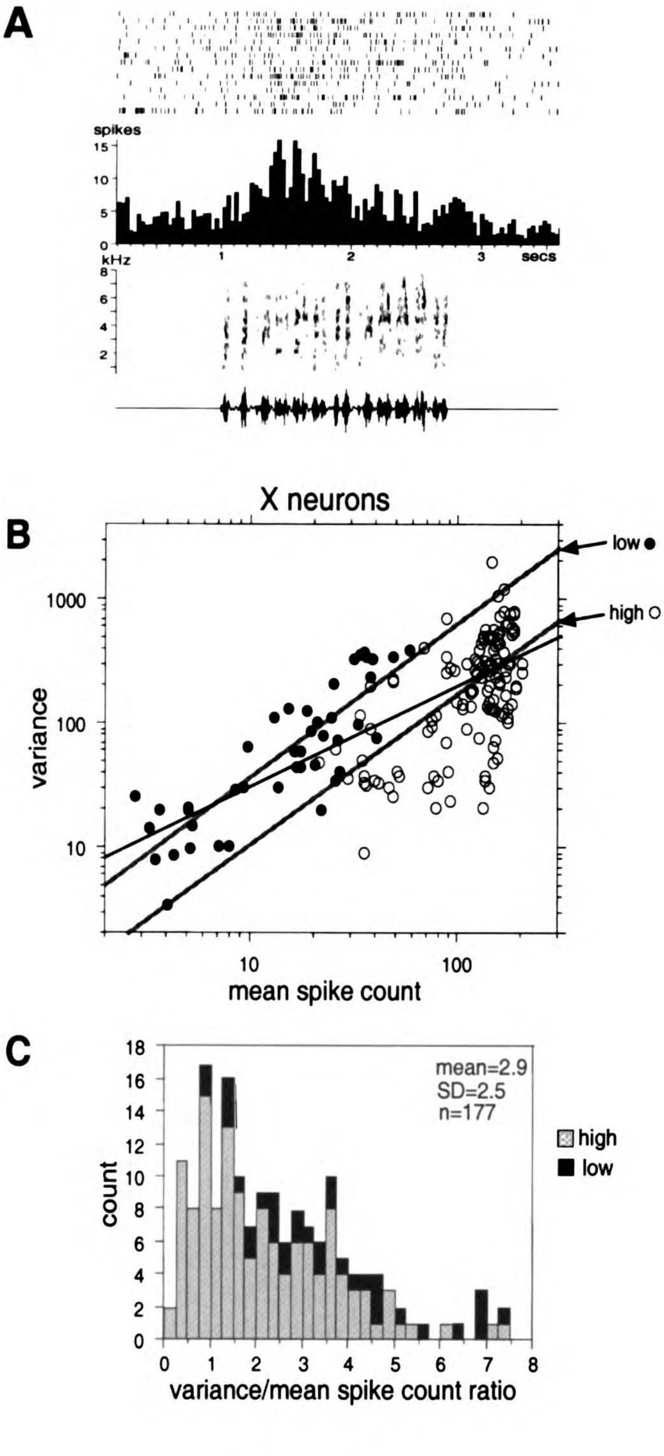

A
spikes
15
10
5
0
1
2
3
secs
kHz
8
6
4
2
B
X neurons
1000
100
10
variance
10
100
mean spike count
low ●
high ○
C
18
16
14
12
10
8
6
4
2
0
count
mean=2.9
SD=2.5
n=177
high
low
0
1
2
3
4
5
6
7
8
variance/mean spike count ratio

Figure 5-4

Figure 5-5 Response variability between stimulus types A) The distributions of variance/mean count ratios are shown for BOS, reverse BOS, tutor song, and reverse tutor song stimuli. In each box, the inner line indicates the median, the height of the box marks where 95% of the data lie, and the error bars embrace 99% of the data. The dotted line indicates the mean ratio across all stimulus types. B) The distributions of variance/mean ratios obtained for each stimulus type are shown for X neurons. Conventions are as in A. Asterisks denote those stimuli which had significantly lower ratios than BOS.

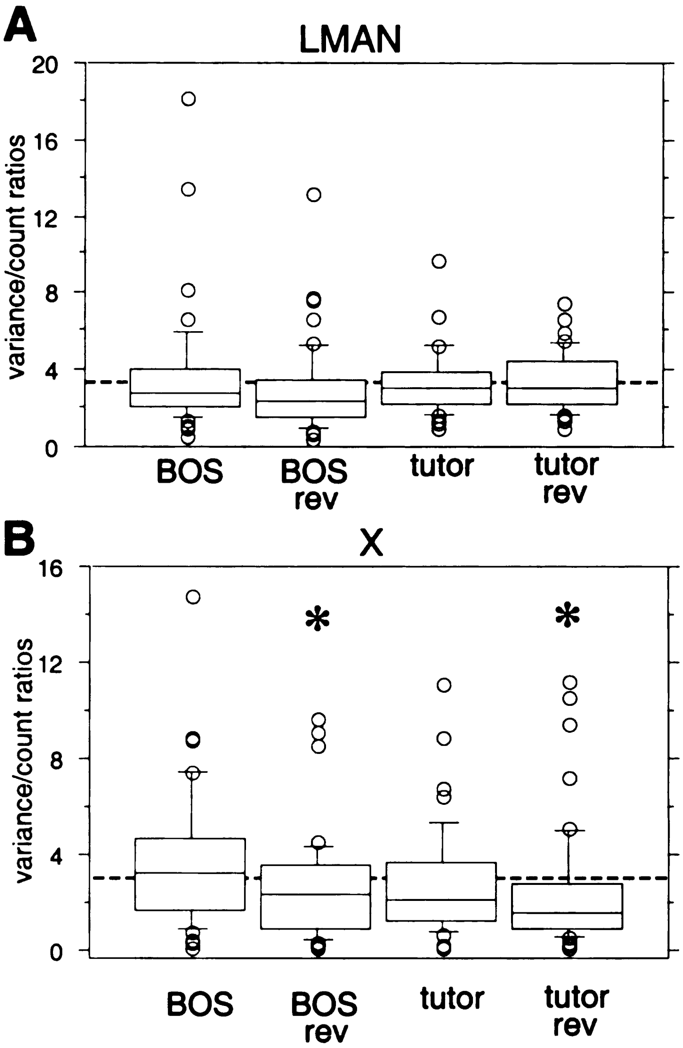

A
LMAN
variance/count ratios
20
16
12
8
4
0
BOS
BOS rev
tutor
tutor rev
B
X
variance/count ratios
16
12
8
4
0
*
*
BOS
BOS rev
tutor
tutor rev

Figure 5-5

Figure 5-6 Comparisons of response variability between LMAN and X neurons in 60 d birds. A) The mean spike count is compared to the variance obtained from each LMAN (open circles) and X cell (black circles for low spontaneous X and open triangles for high spontaneous X). Lines indicate the best power law fits of LMAN (solid), low spontaneous X (solid) and high spontaneous X (dashed) data. B) Histograms compare the variance/mean count ratios obtained from each LMAN cell (upper panel, white bars) and each X cell (lower panel, gray bars). Gray and black bars indicate ratios from high and low spontaneous rate cells, respectively.

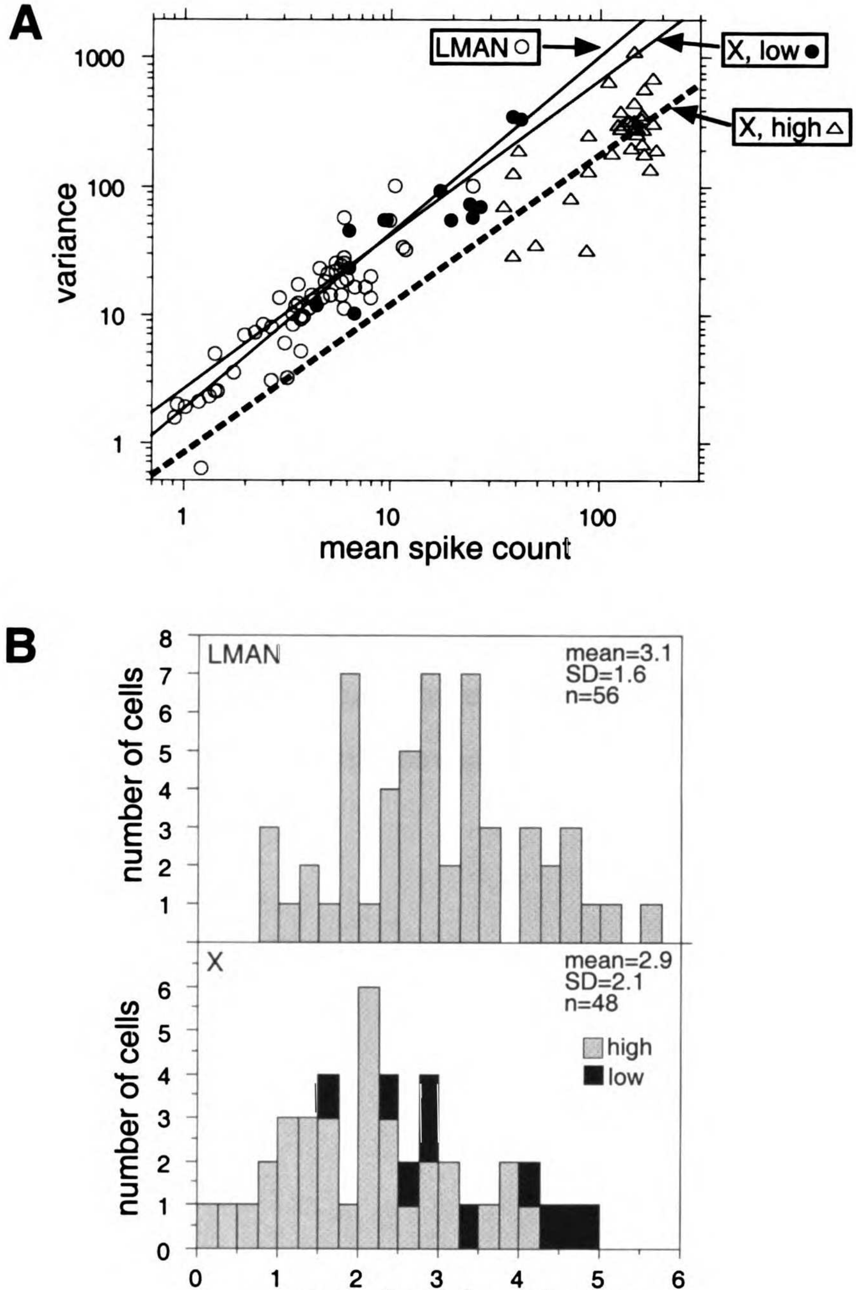

A
1000
100
10
1
variance
1
10
100
mean spike count
LMAN ○
X, low ●
X, high △
B
LMAN
mean=3.1
SD=1.6
n=56
8
7
6
5
4
3
2
1
number of cells
X
mean=2.9
SD=2.1
n=48
high
low
6
5
4
3
2
1
0
number of cells
0
1
2
3
4
5
6
mean variance/count ratio

Figure 5-6

Figure 5-7 Response variability decreases during song learning. A) The variance is plotted against the mean spike count obtained for each LMAN cell recorded from 60 d (open circles) and adult (black circles) birds. Lines indicate the best power law fits of the data. B) Histograms compare the distributions of variance/mean count ratios obtained for individual LMAN cells recorded from 60 d (white bars) and adults (gray bars). C) The average variance/mean count ratio for each stimulus type is compared for 60 d (open circles) and adult (black circles) LMAN neurons; error bars are SEM. D) The variance is plotted against the mean spike count obtained for each X cell recorded from 60 d and adult birds. E) Histograms compare the distributions of variance/mean count ratios obtained for individual X cells recorded from 60 d and adults. For adult neurons, high spontaneous X cells are denoted with white bars, and low spontaneous X cells are denoted with black bars. For 60 d neurons, high and low spontaneous X cells are marked by gray and black bars, respectively. F) The average variance/mean count ratio for each stimulus type is compared for 60 d and adult X neurons; conventions are as in C.

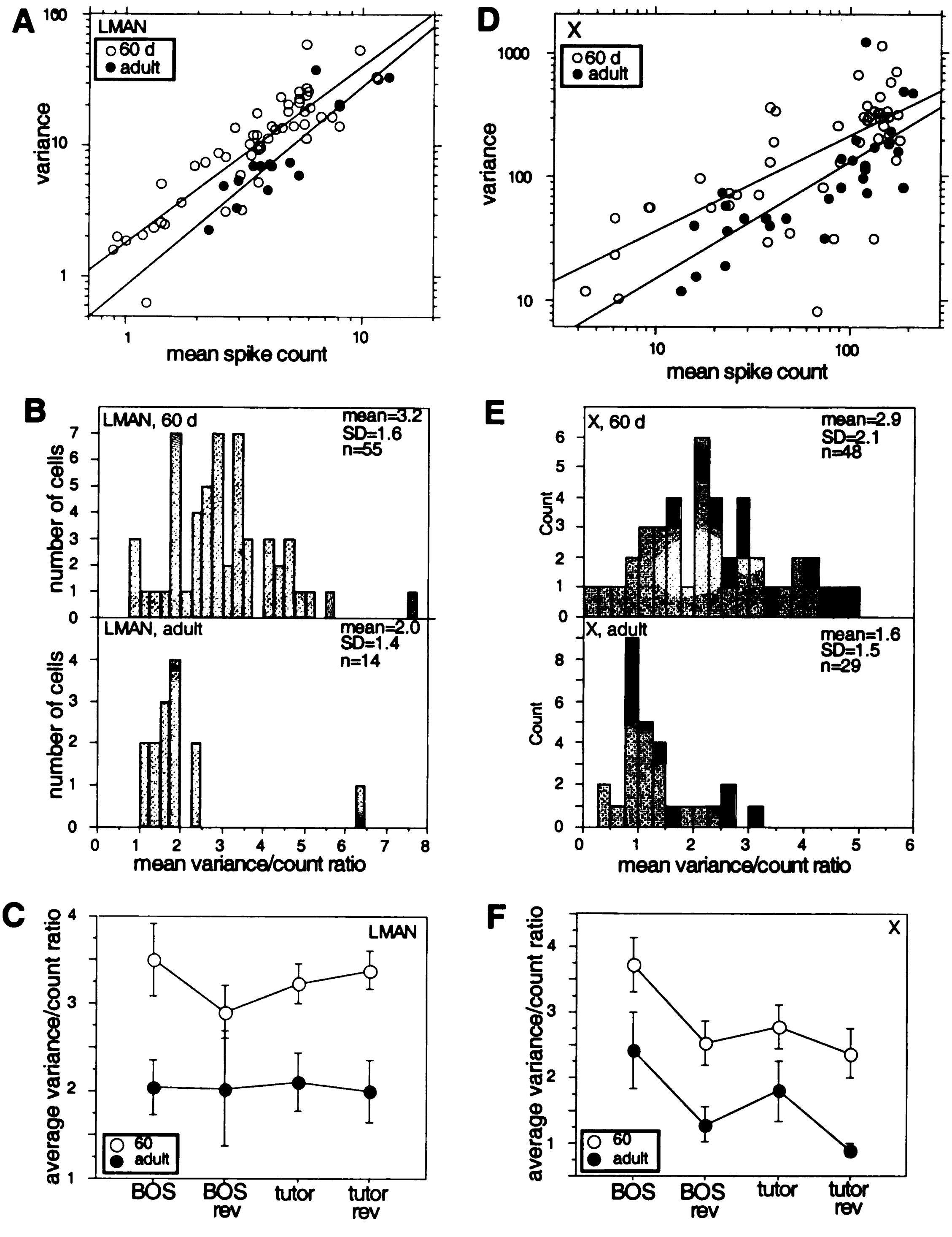

A
LMAN
○ 60 d
● adult
variance
mean spike count
100
10
1
1
10
D
X
○ 60 d
● adult
variance
mean spike count
1000
100
10
10
100
B
LMAN, 60 d
mean=3.2
SD=1.6
n=55
number of cells
LMAN, adult
mean=2.0
SD=1.4
n=14
number of cells
mean variance/count ratio
0
1
2
3
4
5
6
7
8
E
X, 60 d
mean=2.9
SD=2.1
n=48
Count
X, adult
mean=1.6
SD=1.5
n=29
Count
mean variance/count ratio
0
1
2
3
4
5
6
C
LMAN
average variance/count ratio
○ 60
● adult
BOS
BOS rev
tutor
tutor rev
F
X
average variance/count ratio
○ 60
● adult
BOS
BOS rev
tutor
tutor rev

Figure 5-7

Chapter 6:

Future Directions

The range of neural preferences for BOS versus tutor song

Testing the relative contributions of BOS and tutor song experiences on selectivity during learning, revealed that AF neurons in 60 d birds had selectivity for both stimuli. In both normal and ts cut juveniles, a range of preferences was found: neurons that responded equally well to BOS and tutor song were commonly recorded, as were neurons which preferred BOS over tutor song; neurons preferring tutor song did occur, but were rare. An explanation for this range of preferences has remained elusive! One possibility is that all neurons actually respond equally well to BOS and tutor song, but because some birds did not accurately copy the tutor song, the tutor song presented did not drive the neurons well. This would lead to neurons with a preference for BOS over tutor song. To test this possibility, it is necessary to determine what a bird knew about the tutor song at the time of the recording session. To do this requires the development of an anesthesia that will yield auditory responses in the AF and from which birds will recover. After characterizing AF selectivity at 60 d, birds would recover and continue song development in isolation. In this way, the bird's adult song will reflect what it knew about the tutor song at the time of the recording session. If neural preferences for BOS and tutor song recorded at 60 d reflect the accuracy of the tutor song template, then those birds yielding neurons with similar responses to BOS and tutor song are predicted to develop good copies of the tutor song, and birds with neurons that strongly preferred BOS over tutor song are expected to develop songs different from their tutor song.

Alternatively, this range of neural preferences for BOS versus tutor song could actually exist within each bird. Although $d'_{\text{BOS-tutor}}$ values of neurons clustered within a bird, it is possible that each nucleus was not sampled well enough to capture the entire range of responses. Comparing each neuron's $d'_{\text{BOS-tutor}}$ value to its location within LMAN or X did not reveal a topography shared by all birds; however, it is possible for individual birds to have unique maps of BOS versus tutor song preferences. An orderly transition in d' values was not found along the dorsal-ventral axis of individual birds, however; this was the most carefully sampled dimension. To investigate whether BOS versus tutor song preferences are organized in the medial-lateral or anterior-posterior dimensions will require more extensive sampling along these axes. Small electrode arrays could simultaneously record neurons over large portions of LMAN and X.

Alternatively, the range of neural preferences for BOS over tutor song may have been the outcome of neural state changes during recording sessions. In the visual system, neural state changes, as measured with EEG, correlate with the recorded receptive field properties (Worgotter et al, 1998). AF neurons, particularly in LMAN, are susceptible to state changes during recording. Although we try to record from neurons in a stable state, this is difficult to control. To begin to monitor neural state, the bird's EEG could be monitored during the recording session. EEG activity might covary with neural preference for BOS over tutor song, as well as the existence of auditory responses in LMAN. Eventually, it would be optimal to gain more control of the state of anesthesia; this is not possible with the current urethane protocol.

Finally, it is possible that the range of neural preferences for BOS over tutor song indicate different stages of song development. For example, neurons that respond equally well to BOS and tutor song could be in a transition state from exclusive tutor song tuning to BOS tuning. A transition in selectivity occurs in the barn owl optic tectum (Brainard and Knudsen, 1995). In 60 d birds, however, d′ values did not increase with age or song maturity. It is possible that this trend exists over a larger range of ages, however. If so, it is expected that birds between 30 and 60 d would have greater tuning to tutor song, and that birds older than 60 d would have more neurons tuned to BOS. Although many BOS-preferring neurons were found in adult birds, the mean d′ values were not significantly different from 60 d neurons.

Stages in selectivity development

At 60 d, neurons are clearly selective for their plastic BOS, in both ts cut and normal birds. Since plastic song itself is changing during the sensorimotor phase, the mechanisms underlying selectivity must be very flexible and fast in order to mold themselves to features of plastic song. In order to test how quickly selectivity for an earlier plastic BOS is overwritten by the current plastic BOS, playback studies could test neural responses to a series of plastic songs from different ages. For example, in a 70 d bird, neural responses could be recorded and compared between the current BOS and those plastic songs produced at 30 d, 40 d, 50 d, and 60 d. Furthermore, the ability to obtain chronic recordings in the AF make it possible to monitor selectivity changes throughout development for past and current plastic BOS in a single bird.

At 60 d, neurons reliably discriminated between forward and reverse song stimuli. In contrast, they discriminated less well between forward and reverse order song, which reverses syllable sequence only. This result suggests that neurons develop selectivity for individual syllables first, and syllable sequence later. Similar progressions of selectivity development might occur for conspecific and heterospecific song measures of selectivity. For example, heterospecific song is acoustically less like BOS than conspecific song, making it easier to discriminate. Thus, juvenile neurons might develop selectivity for BOS relative to heterospecific song prior to attaining selectivity for BOS relative to songs that resemble it, such as conspecific song. This kind of progression of selectivity development was not apparent at 60 d, however. This could reflect the intermediate stage of song learning and selectivity in 60 d birds. Selectivity studies in younger birds, such as 45 d olds, may capture neurons in a state of selectivity for one category, but not for another, more difficult category.

Auditory responses in LMAN of ts cut adult birds were extremely rare, suggesting that the inability of ts cut adults to match their tutor song model somehow limited auditory responses. This could be investigated with chronic recordings in LMAN, which would allow the auditory responses of a particular site to be monitored from juvenile stages to adulthood. Such experiments could determine whether previously auditory sites lost their auditory responses as ts cut birds matured. Because singing-related activity is also prevalent in chronic recordings of LMAN in awake birds, it would be interesting to see if this activity remains in ts cut adults. This could offer insight into the interdependence between auditory and premotor activity in LMAN.

The function of AF selectivity in juveniles

Neurons tuned to BOS and tutor song during learning could be extremely useful for mediating song learning. Neurons tuned to BOS could provide information about the current state of BOS, and neurons tuned to tutor song could store information about the tutor song model. Neurons tuned to both could be involved in comparing BOS to tutor song, which occurs behaviorally. One idea about how these neurons could compare BOS to the tutor song model is that they would register the amount of coincident activity between inputs tuned to BOS and inputs tuned to tutor song (Figure 6-1). For example, neurons tuned exclusively to BOS would be activated every time the bird sang, and would serve to filter out sounds that the bird itself did not make. Furthermore, this activity alone would be capable of driving the neurons responsive to both BOS and tutor song. The second input would arise from neurons tuned exclusively by tutor song experience. The degree to which these neurons respond would depend on the similarity that BOS shared with the tutor song: auditory feedback of BOS that was highly similar to tutor song would strongly activate tutor-tuned neurons, and would also drive the downstream neurons responsive to both BOS and tutor song. Thus, neurons that respond well to both tutor song or BOS presented alone in electrophysiology experiments would be activated differently during song behavior, depending on the degree of matching. Poor song copies would activate BOS inputs, but not the tutor-tuned inputs. This would result in a small degree of activity, or low coincident activity in neurons responsive to both songs. In contrast, when birds produce good copies of the tutor song, this would activate both BOS- and tutor-tuned inputs, and result in higher activity, or more coincident activity in the downstream neurons responsive to both songs. The overall amount of activity in this latter type of neuron would then indicate the degree of match, and perhaps provide an error signal to neurons downstream. This model posits that all types of neurons would be found in a bird, which may or may not be the case.

The role that selectivity plays during song learning, if any, is unclear. Consistent with a function during song learning, selectivity increases during song development. If selectivity is really related to song learning, then it should be affected by manipulations of song learning. wo findings consistent with this come from isolate and ts cut birds. Isolate birds, which develop an abnormal song in the absence of a tutor song model, have LMAN neurons that seem to completely lack selectivity, even that for BOS (Maekawa, 1998). In ts cut adults, which are prevented from producing a copy of their tutor song, X neurons exhibit abnormally low selectivity. These results suggest that the ability to copy a tutor song model influences the degree of selectivity in the AF, consistent with a relation between song learning and selectivity.

Other impairments in song learning may reveal a connection between learning and selectivity. Tutor song copying has been impaired by blocking excitatory transmission in LMAN during tutor song exposure (Basham et al, 1996); it would be interesting to know whether the selectivity of these cells was affected by this learning impairment. Song learning can also be interfered with by early testosterone injections, or perhaps by altering a bird's auditory feedback of its own vocalizations (Leonardo and Konishi, 1998). Characterizing the selectivity in such birds could strengthen the correlation between learning and selectivity. Like selectivity, synaptic properties in LMAN follow a progression of changes during song development (Boettiger and Doupe, 1998b). It is of interest to determine how these manipulations of learning manifest themselves at the synaptic level. One exciting possibility is to prepare slices from birds deafened during song development. A description of the synaptic properties of LMAN cells in deaf birds will address the basic effects of auditory experience on AF neural properties; the selectivity of AF neurons in deaf birds cannot be tested *in vivo* until a reversible method of deafening is developed.

A more conclusive role for selectivity in song learning might be established by inactivating selective neurons, and examining the effects on song development. It is possible that some pharmacological agents could specifically inactivate or decrease the contributions of selective neurons, but these have not yet been described. One study has shown that blocking inhibition within LMAN with bicuculline not only disrupts selectivity, but also eliminates auditory responses (Rosen and Mooney, 1998). Ideally, a pharmacological agent is wanted that decreases selectivity without abolishing auditory responses; the effects of potential agents on selectivity could be tested in acute experiments using iontophoresis techniques. Drugs that block glutamatergic transmission may decrease selectivity in LMAN, since LMAN neurons receive input through glutamatergic synapses (Boettiger and Doupe, 1998a; Livingston and Mooney, 1998).

The function of AF selectivity in adults

In adult birds, selectivity for BOS is prominent in both normal and ts cut birds. This BOS selectivity could reflect the crystallized song, and could function as a reference to maintain song stability in adulthood. Alternatively, BOS selectivity might reflect the current state of BOS, as suggested by the selectivity for plastic song in juvenile birds. These two possibilities could be distinguished in experiments using birds that received ts transections *as adults*. This manipulation severely alters BOS, disrupting syllable morphology. Prior to the ts cut, adults will have developed AF neurons highly tuned to their normal BOS. After prolonged experience of ts cut song, neural recordings would reveal whether the previous selectivity was altered. If neurons are tuned to the original adult BOS, then BOS selectivity might be a reference. If, instead, neurons are tuned to the new, ts cut song, then BOS selectivity would reflect the current state of BOS.

Selectivity for tutor song is also found in adult birds, although to a lesser degree in ts cut birds. This tutor song selectivity may reflect acoustic similarity to BOS, or the maintenance of tutor song information into adulthood. The existence of tutor song selectivity in ts cut adults supports the latter possibility; however, neurons in these birds may maintain juvenile properties. Behavioral experiments could explore the possibility that tutor song information is maintained and serves as a reference for song in adults. To do this, birds must be prevented from successfully mimicking the tutor song during learning; unlike the ts cut, however, this condition must be reversible. One potential manipulation is altered auditory feedback (Leonardo and Konishi, 1998). When artificial auditory feedback is superimposed onto the singing of an adult bird, song is dramatically altered; when the altered feedback is removed, song returns to normal. If altered auditory feedback is applied to juvenile birds throughout song development, it is likely to interfere with their ability to mimick the tutor song. Once they are adults, altered auditory feedback would be removed, and the adult song studied for changes. If the bird develops a song resembling the tutor, then this would suggest that tutor song information is maintained into adulthood, consistent with tutor song selectivity found in adults. If the song does not change, then it suggests that prior BOS information guides song in adulthood.

The circuitry underlying selectivity

As discussed above, iontophoresis studies could begin to describe the circuitry that underlies AF selectivity by interfering with specific types of neurotransmission. Candidate neurotransmitters are suggested from experiments done in slice preparations, such as glutamtate in LMAN (Boettiger and Doupe, 1998a). Neuromodulators might also influence selectivity in the AF: for example, both LMAN and X receive a catecholaminergic inputs (Lewis et al, 1981; Soha et al, 1996). Alternatively, different receptor types in AF nuclei may be important for gating auditory responses rather than constructing selective neurons, as suggested by experiments that blocked inhibition in LMAN (Rosen and Mooney, 1998).

The AF is composed of three nuclei which have highly song selective neurons (Doupe and Konishi, 1991). The purpose of a string of song selective nuclei is unclear: it is possible that selectivity is progressively sharpened as auditory responses move through the nuclei. Although no difference in selectivity has been found between LMAN and X in adults or juveniles, this may reflect the interconnectedness of AF nuclei. A feedback projection exists within the AF, linking LMAN to X. Thus, X responses reflect input both from HVc and from LMAN. Whether this feedback projection alters selectivity in X is unknown, and could be tested by reversibly inactivating LMAN while recording auditory responses in X. A similar experiment has tested the influence of HVc and LMAN inputs on RA responses (Doupe and Konishi, 1991; Vicario and Yohay, 1993).

Similarly, it is unclear whether AF nuclei are more selective than their HVc inputs. Although HVc neurons differ in their response properties, some neurons exhibit a degree of selectivity similar to that found in the AF (Doupe, 1997). Whether these selective neurons actually project to the AF, however, is unknown. Thus, to isolate the selective properties of HVc inputs to the AF, one could try to record from HVc afferents in X. Afferents could be isolated from X neurons by injecting X with muscimol, which could reversibly silence neural activity in X. This strategy has revealed the thalamic contributions to orientation selectivity in the visual cortex (Chapman et al, 1991).

Although the degree of selectivity is not largely different between X and LMAN, X neurons respond to a broader range of stimuli than LMAN neurons do. For example, X neurons readily respond with an increase in firing rate to conspecific or reverse song, whereas LMAN neurons either do not respond to these stimuli or are inhibited by them. To understand how these responses are transformed by AF processing will require extensive characterization of responses to different stimuli for the different cell types within each nucleus, including DLM.

Encoding of song stimuli by AF neurons

Information about how stimuli are encoded in the AF can be gained from detailed analyses of its neural responses. While AF responses have been commonly measured over the entire song duration, one could also examine the portion of each song eliciting the maximum response, such as been done for HVc neural responses (Sutter and Margoliash, 1994). LMAN neurons from the same bird have extremely similar time-varying responses during BOS presentation (Doupe, 1997); this has not yet been examined in X, however. Beyond describing the time-varying response characteristics of AF neurons to song, one could rigorously determine the syllable combinations that are essential for song responses in 60 d birds. In these experiments, the syllable components of song would be presented in isolation and in combination, in order to find the auditory features driving the neuron's response for song. This kind of experiment not only addresses the mechanisms underlying neural responses to song, but could also estimate the time scale over which neurons integrate information from their inputs. These syllable combination experiments have been done in LMAN of adult birds, and it would be interesting to see whether juvenile neurons have similar integration capabilities.

Similar syllable decompostion experiments in RA would be extremely informative for estimating how LMAN auditory responses are read out by downstream neurons. In addition to informing our ideas about the encoding of song stimuli, the results from this experiment could clarify the appropriate time window over which LMAN responses should be measured. Afterall, when measuring a neural response, we are trying to approximate the information it provides about a particular stimulus to a downstream neuron.

Figure 6-1 Model of neural comparison between BOS and tutor song. Each circle represents a different cell type: neurons preferring tutor song over BOS (T>B), neurons preferring BOS over tutor song (B>T), and neurons responding equally well to both songs (B=T). Auditory feedback of BOS production will always drive B>T neurons, and this activity will then drive B=T neurons downstream (solid arrow). In contrast, auditory feedback of BOS production will activate T>B neurons according to the similarity between BOS and tutor song. BOS with high similarity will be able to drive downstream neurons (B=T), whereas BOS with low similarity will not (dotted arrow indicates dependence of input on similarity to tutor song). Thus, BOS feedback that is similar to the tutor song will result in high or coincident activation of BOS and tutor song inputs in B=T neurons; BOS feedback that is a poor copy of the tutor song will result in a much lower degree of activation in B=T neurons.

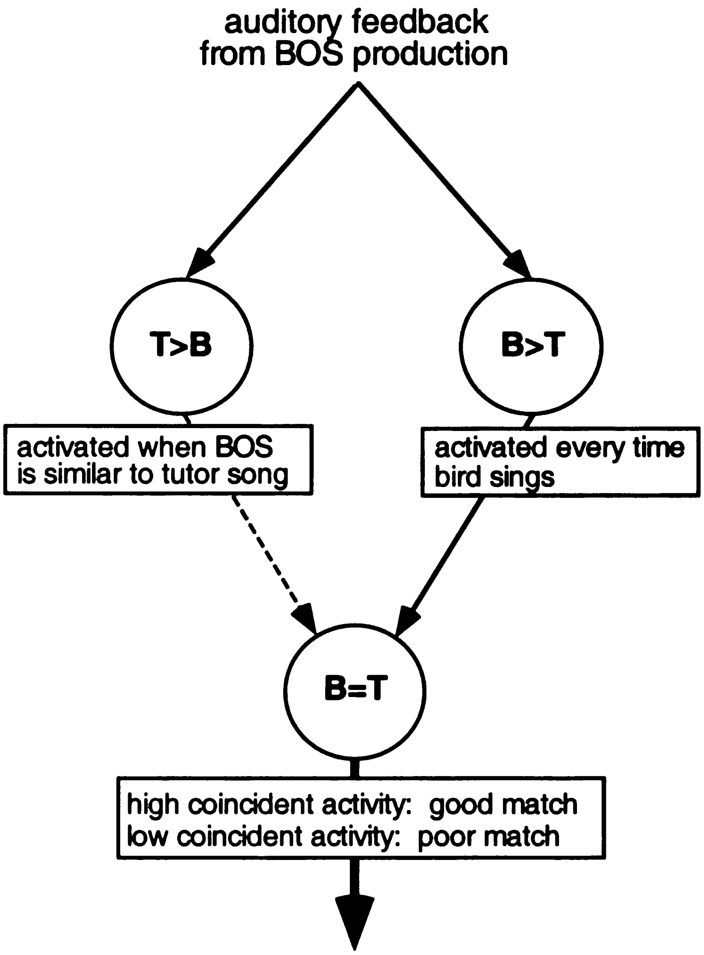

auditory feedback
from BOS production
T>B
B>T
activated when BOS
is similar to tutor song
activated every time
bird sings
B=T
high coincident activity: good match
low coincident activity: poor match

Figure 6-1

REFERENCES

Aamodt, S, Kozlowski, M, Nordeen, E, Nordeen, K (1992) Distribution and developmental change in [3-H]MK-801 binding within zebra finch song nuclei. J Neurobiol 23:997-1005.

Aamodt, S, Nordeen, E, Nordeen, K (1995) Early isolation from conspecific song does not affect the normal developmental decline of N-methyl-D-aspartate receptor binding in an avian song nucleus. J Neurobiol 27:76-84.

Antonini, A, Stryker, MP (1993) Rapid remodeling of axonal arbors in the visual cortex. Sci 260(5115):1819-21.

Arieli, A, Sterkin, A, Grinvald, A, Aertsen, A (1996) Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. Science 273:1868-1871.

Basham, M, Nordeen, EJ, Nordeen, KW (1996) Blockade of NMDA receptors in the anterior forebrain impairs memory acquisition in the zebra finch (Peophila guttata). Neurobiol Learn Mem 66:295-304.

Becker, P (1982). The coding of species-specific characteristics in bird sounds. In: Acoustic communication in birds (D. Kroodsma and E. Miller), 213-252. New York: Academic Press.

Boehner, J (1990) Early acquisition of song in the zebra finch, Taeniopygia guttata. Anim Behav 39:369-374.

Boettiger, C, Doupe, A (1998a) Intrinsic and thalamic excitatory inputs onto songbird LMAN neurons differ in their pharmacological and temporal properties. J Neurophysiol 79:2615-2628.

Boettiger, C, Doupe, A (1998b) Developmental changes in temporal dynamics of synaptic responses in the songbird nucleus LMAN. Soc Neurosci Abstr 24(Pt 1):191.

Bottjer, SW, Miesner, EA, Arnold, AP (1984) Forebrain lesions disrupt development but not maintenance of song in passerine birds. Science 224:901-903.

Bradley, A, Skottun, B, Ohzawa, I, Sclar, G, Freeman, R (1987) Visual orientation and spatial frequency disrcimination: a comparison of single neurons and behavior. J Neurophysiol 57(3):755-772.

Brainard, M, Doupe, A (1997) Anterior forebrain lesions eliminate deafening-induced song plasticity in adult finches. Soc Neurosci Abstr 23:796.

Brainard, MS, Knudsen, EI (1993) Experience-dependent plasticity in the inferior colliculous: a site for visual calibration of the neural representation of auditory space in the barn owl. J Neurosci 13(11):4589-4608.

Brainard, MS, Knudsen, EI (1995) Dynamics of visually guided auditory plasticity in the optic tectum of the barn owl. J Neurophysiol 73(2):595-614.

Carrillo, G, Doupe, AJ (1995) Developmental studies of glutamate receptor and peptide immunoreactivity in the zebra finch song system. Soc Neurosci Abstr 21(Pt 2):960.

Chapman, B, Zahs, K, Stryker, M (1991) Relation of cortical cell orientation selectivity to alignment of receptive fields of the geniculocortical afferents that arborize within a single orientation column in ferret visual cortex. J Neurosci 11(5):1347-1358.

Dave, A, Yu, A, Margoliash, D (1997) Reassessing auditory responses in the bird song system. Soc Neurosci Abstr 23:245.

Dave, AS, Yu, AC, Margoliash, D (1998) Behavioral state modulation of auditory activity in a vocal motor system. Science 282:2250-2254.

Dean, A (1981) The variability of discharge of simple cells in the cat striate cortex. Exp Brain Res 44:437-440.

de Ruyter van Steveninck, R, Lewen, G, Strong, S, Koberle, R, Bialek, W (1997) Reproducibility and variability in neural spike trains. Science 275:1805-1808.

Douglas, R, Koch, C, Mahowald, M, Martin, K, Suarez, H (1995) Recurrent excitation in neocortical circuits. Science 269:981-985.

Doupe, AJ (1997) Song- and order-selective neurons in the songbird anterior forebrain and their emergence during vocal development. J Neurosci 17(3):1147-1167.

Doupe, AJ, Konishi, M (1991) Song-selective auditory circuits in the vocal control system of the zebra finch. Proc Natl Acad Sci USA 88:11339-11343.

Doupe, A, Solis, M (1997) Song- and order-selective neurons develop in the songbird anterior forebrain. J Neurobiol 33(5):694-709.

Eales, LA (1985) Song learning in zebra finches; some effects of song model availability on what is learnt and when. Anim Behav 33:1293-1300.

Eales, LA (1987) Song learning in female-raised zebra finches: another look at the sensitive phase. Anim Behav 35:1356-1365.

Eales, LA (1989) The influences of visual and vocal interaction on song learning in zebra finches. Anim Behav 37:507-520.

Esser, K, Condon, C, Suga, N, Kanwal, J (1997) Syntax processing by auditory cortical neurons in the FM-FM area of the mustached bat Pteronotus parnellii. Proc Natl Acad Sci USA 88:11339-11343.

Farries, M, Perkel, D (1998) Electrophysiological similarities between Area X neurons in the zebra finch and neurons of the mammalian striatum. Soc Neurosci Abstr 24:191.

Fortune, ES, Margolish, D (1992) Neurons in the field L complex of the male zebra finch project directly into HVc. Third International Congress of Neuroethology Aug 9-14:345.

Fortune, ES, Margoliash, D (1995) Parallel pathways and convergence onto HVc and adjacent neostriatum of adult zebra finches (Taeniopygia guttata). J Comp Neurol 360:413-441.

Goller, F, Suthers, R (1996a) Role of syringeal muscles in controlling the phonology of bird song. J Neurophysiol 76:287-300.

Goller, F, Suthers, R (1996b) Role of syringeal muscles in gating airflow and sound production in singing brown thrashers. J Neurophysiol 75:867-876.

Green, DM, Swets, JA (1966). Signal Detection Theory and Psychophysics. New York: Wiley.

Gurney, ME (1981) Hormonal control of cell form and number in the zebra finch song system. J Neurosci 1:658-673.

Hartley, R, Suthers, R (1989) Airflow and pressure during canary song: direct evidence for minibreaths. J Comp Physiol [A] 165:15-26.

Hessler, N, Doupe, A (1997) Singing-related neural activity in anterior forebrain nuclei of adult zebra finch. Soc Neurosci Abstr 23:245.

Hessler, N, Doupe, A (1999) Social context modulates singing-related neural activity in the songbird forebrain. Nat Neurosci 2:209-211.

Immelmann, K (1969). Song development in the zebra finch and other estrildid finches.In: Bird Vocalizations (R. A. Hinde), 61-74. London: Cambridge University Press.

Janata, P, Margoliash, D (1998) Song-selective auditory responses in NIF: extending the sensorimotor integration hierarchy. Soc Neurosci Abstr 24:190.

Jarvis, E, Nottebohm, F (1997) Motor-driven gene expression. Proc Natl Acad Sci USA 94:4097-4102.

Jin, H, Clayton, D (1997) Localized changes in immediate early gene regulation during sensory and motor learning in zebra finches. Neuron 19:1049-1059.

Johnson, F, Bottjer, SW (1992) Growth and regression of thalamic efferents in the song-control system of male zebra finches. J Comp Neurol 326:442-450.

Jones, A, Ten Cate, C, Slater, P (1996) Early experience and plasticity of song in adult male zebra finches (Taeniopygia guttata). J Comp Psychol 110:354-369.

Knudsen, EI, Brainard, MS (1991) Visual instruction of the neural map of auditory space in the developing optic tectum. Sci 253:85-87.

Konishi, M (1965) The role of auditory feedback in the control of vocalization in the white-crowned sparrow. Z Tierpsychol. 22:770-783.

Kroodsma, D (1976) Reproductive development in a female songbird: differential stimulation by quality of male song. Science 192:574-575.

Kroodsma, DE, Konishi, M (1991) A suboscine bird (eastern phoebe, Sayornis phoebe) develops normal song without auditory feedback. Anim Behav 42:477 -487.

Leonardo, A, Konishi, M (1998) Decrystallization of adult birdsong by perturbation of auditory feedback. The Fifth International Congress of Neuroethology Abstracts.

Lewicki, MS (1994) Bayesian modelling and classification of neural signals. Neural Comput 6:1005-1030.

Lewicki, MS, Arthur, BJ (1996) Hierarchical organization of auditory temporal context sensitivity. J Neurosci 16(21):6987-98.

Lewis, JW, Ryan, SM, Arnold, AP, Butcher, LL (1981) Evidence for a catecholaminergic projection to area X in the zebra finch. J Comp Neurol 196:347-354.

Livingston, F, Mooney, R (1997) Development of intrinsic and synaptic properties in a forebrain nucleus essential to avian song learning. J Neurosci 17(23):8997-9009.

Luo, M, Perkel, DJ (1999) A long-range GABAergic projection in a circuit essential for vocal learning. J Comp Neurol 403:68-84.

Maekawa, M (1998) Response selectivity of neurons in the forebrain song control nucleus of the zebra finch reared in abnormal acoustic environment. Soc Neurosci Abstr 24 Pt1:190.

Margoliash, D (1983) Acoustic parameters underlying the responses of song-specific neurons in the white-crowned sparrow. J Neurosci 3:1039-1057.

Margoliash, D (1986) Preference for autogenous song by auditory neurons in a song system nucleus of the white-crowned sparrow. J Neurosci. 6:1643-1661.

Margoliash, D, Fortune, ES (1992) Temporal and harmonic combination-sensitive neurons in the zebra finch's HVc. J Neurosci 12(11):4309-4326.

Margoliash, D, Konishi, M (1985) Auditory representation of autogenous song in the song system of white-crowned sparrows. Proc Natl Acad Sci USA 82:5997-6000.

Mioche, L, Singer, W (1989) Chronic recordings from single sites of kitten striate cortex during experience-dependent modifications of receptive-field properties. J Neurophys 62:185-197.

Miyashita, Y (1988) Neuronal correlate of visual associative long-term memory in the primate temporal cortex. Nature 335:817-820.

Mooney, R (1992) Synaptic basis for developmental plasticity in a birdsong nucleus. J Neursci 12:2464-2477.

Mooney, R, Rao, M (1990) Waiting periods versus early innervation: the development of axonal connections in the zebra finch song system. J Neurosci 14(11):6532-6543.

Morrison, R, Nottebohm, F (1993) Role of a telencephalic nucleus in the delayed song learning of socially isolated zebra finches. J Neurobiol 24:1045-1064.

Newsome, WT, Britten, KH, Salzman, CD, Movshon, JA (1990) Neuronal mechanisms of motion perception. Cold Spring Harb Symp Quant Biol 55:697-705.

Nixdorf-Bergweiler, BE (1995). The establishment of sex differences in the lateral magnocellular nucleus of the anterior neostriatum (LMAN) of the zebra finch is multifaceted. Nervous Systems and Behavior: Proceedings of the 4th International Congress of Neuroethology, Cambridge, England, George Thieme Verlag.

Nixdorf-Bergweiler, BE, Wallhausser-Franke, E, DeVoogd, TJ (1995) Regressive development in neuronal structure during song learning in birds. J Neurobiol 27(2):204-215.

Nordeen, EJ, Nordeen, KW (1988) Sex and regional differences in the incorporation of neurons born during song learning in zebra finches. J Neurosci 8:2869-2874.

Nordeen, KW, Nordeen, EJ (1992) Auditory feedback is necessary for the maintenance of stereotyped song in adult zebra finches. Behav Neural Biol 57:58-66.

Nottebohm, F, Stokes, TM, Leonard, CM (1976) Central control of song in the canary, Serinus canarius. J Comp Neurol 165:457-486.

Ohlemiller, K, Kanwal, J, Suga, N (1996) Facilitative responses to species-specific calls in cortical FM-FM neurons of the mustached bat. Neuroreport 7:1749-1755.

Price, PH (1979) Developmental determinants of structure in zebra finch song. J Comp Physiol Psychol 93(2):260-277.

Pytte, C, Suthers, R (1996) Evidence for a sensitive period for sensorimotor integration during song development in the zebra finch. Soc Neurosci Abstr 22:693.

Rauschecker, J, Tian, B, Hauser, M (1995) Processing of complex sounds in the macaque nonprimary auditory cortex. Science 268:111-114.

Reinke, H, Wild, J (1998) Identification and connections of inspiratory premotor neurons in songbirds and budgerigar. J Comp Neurol 391:147-163.

Riquimaroux, H, Gaioni, S, Suga, N (1991) Cortical computational maps control auditory perception. Science 251:565-568.

Rosen, M, Mooney, R (1998) Local inhibition modulates song-selective auditory responses within the song nucleus LMAN. Soc Neurosci Abstr 24(Pt1):189.

Scharff, C, Nottebohm, F (1991) A comparative study of the behavioral deficits following lesions of the various parts of the zebra finch song system: Implications for vocal learning. J Neurosci 11:2896-2913.

Scharff, C, Nottebohm, F, Cynx, J (1998) Conspecific and heterospecific song discrimination in male zebra finches with lesions in the anterior forebrain pathway. J Neurobiol 36:81-90.

Schmidt, MF, Konishi, M (1998) Gating of auditory responses in the vocal control system of awake songbirds. Nature Neuroscience 1:513-18.

Seidemann, E, Zohary, E, Newsome, W (1998) Temporal gating of neural signals during performance of a visual discrimination task. Nature 394:72-5.

Shadlen, M, Newsome, W (1998) The variable discharge of cortical neurons: implications for connectivity, computation, and information coding. J Neurosci 18(10):3870-3896.

Simpson, HB, Vicario, DS (1990) Brain pathways for learned and unlearned vocalizations differ in zebra finches. J Neurosci 10:1541-56.

Snowden, R, Treue, S, Andersen, R (1992) The response of neurons in areas V1 and MT of the alert rhesus monkey to moving random dot patterns. Exp Brain Res 88:389-400.

Soha, JA, Shimizu, T, Doupe, AJ (1996) Development of the catecholaminergic innervation of the song system of the male zebra finch. J Neurobiol 29(4):473-489.

Sohrabji, F, Nordeen, KW, Nordeen, EJ (1989) Projections of androgen-accumulating neurons in a nucleus controlling avian song. Brain Res 488:253-259.

Sohrabji, F, Nordeen, EJ, Nordeen, KW (1990) Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch. Behav Neur Biol 53:51-63.

Sohrabji, F, Nordeen, EJ, Nordeen, KW (1993) Characterization of neurons born and incorporated into a vocal control nucleus during avian song learning. Brain Res 620:335-338.

Solis, M, Doupe, A (1997) Anterior forebrain neurons develop selectivity by an intermediate stage of birdsong learning. J Neurosci 17(16):6447-6462.

Solis, M, Doupe, A (1999) Contributions of tutor and bird's own song experience to neural selectivity in the songbird anterior forebrain. J Neurosci (in press).

Sossinka, R, Bohner, J (1980) Song types in the zebra finch poephila guttata castanotis. Z Tierpsychol 53:123-132.

Stratford, K, Tarczy-Hornoch, K, Martin, K, Bannister, N, Jack, J (1996) Excitatory synaptic inputs to spiny stellate cells in cat visual cortex. Nature 382:258-261.

Suga, N, O'Neill, W, Manabe, T (1978) Cortical neurons sensitive to combinations of information-bearing elements of biosonar signals in the mustache bat. Science 200:778-781.

Suthers, R (1997) Peripheral control and lateralization of birdsong. J Neurobiol 33:632-652.

Sutter, M, Margoliash, D (1994) Global synchronous response to autogenous song in zebra finch HVc. J Neurophysiol 72(5):2105-2123.

Teich, M, Johnson, D, Kumar, A, Turcott, R (1990) Rate fluctuations and fractional power-law noise recorded from cells in the lower auditory pathway of the cat. Hearing Res 41:41-52.

Theunissen, F, Doupe, A (1998) Temporal and spectral sensitivity of complex auditory neurons in the nucleus HVc of male zebra finches. J Neurosci 18(10):3786-3802.

Tolhurst, DJ, Movshon, JA, Dean, AF (1983) The statistical reliability of signals in single neurons in cat and monkey visual cortex. Vision Res 23(8):775-785.

Troyer, TW, Doupe, AJ, Miller, KD (1996) An associational hypothesis for sensorimotor learning of birdsong. In: Computational Neuroscience: Trends in Research 1995 (JM Bower), 409-414. New York: Academic Press.

Vates, G, Nottebohm, F (1995) Feedback circuitry within a song-learning pathway. Proc Natl Acad Sci USA 92:5139-5143.

Vicario, DS (1991) Contributions of syringeal muscles to respiration and vocalization in the zebra finch. J Neurobiol 22:63-73.

Vicario, DS, Yohay, KH (1993) Song-selective auditory input to a forebrain vocal control nucleus in the zebra finch. J Neurobiol 24: 488-505.

Vogels, R, Spileers, W, Orban, G (1989) The response variability of striate cortical neurons in the behaving monkey. Exp Brain Res 77:432-436.

Volman, SF (1993) Development of neural selectivity for birdsong during vocal learning. J Neurosci 13:4737-47.

Volman, SF (1996) Quantitative assessment of song-selectivity in the zebra finch "high vocal center". J Comp Physiol (A) 178:849-862.

Wallhausser-Franke, E, Nixdorf-Bergweiler, B, DeVoogd, T (1995) Song isolation is associated with maintaining high spine frequencies on zebra finch LMAN neurons. Neurobiol Learn Mem 64:25-35.

Wang, X, Merzenich, M, Beitel, R, Schreiner, C (1995) Representation of a species-specific vocalization in the primary auditory cortex of the common marmoset: temporal and spectral characteristics. J Neurophysiol 74:2685-2706.

Wild, JM (1993) Descending projections of the songbird nucleus robustus archistriatalis. J Comp Neurol 338:225-41.

Wild, J (1997) Neural pathways for the control of birdsong production. J Neurobiol 33(5):653-670.

Williams, HW (1990) Models for song learning in the zebra finch: fathers or others? Anim Behav 39:745-757.

Williams, H, McKibben, J (1992) Changes in stereotyped central motor patterns controlling vocalization are induced by peripheral nerve injury. Behav Neural Biol 57:67-78.

Worgotter, F, Suder, K, Zhao, Y, Kerscher, N, Eysel, U, Funke, K (1998) State-dependent receptive-field restructuring in the visual cortex. Nature 396:165.

Zohary, E, Shadlen, M, Newsome, W (1994) Correlated neuronal discharge rate and its implications for psychophysical performance. Nature 370:140-143.