UNIVERSITY OF CALIFORNIA, SAN DIEGO

Neural Correlates of Conspecific Vocal Recognition in the Caudomedial Nidopallium

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by

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ABSTRACT OF THE DISSERTATION

Neural Correlates of Conspecific Vocal Recognition in the Caudomedial Nidopallium

by

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Vocal recognition is important for communication in several species, including humans. Songbirds learn to recognize the vocalizations of conspecifics for social behaviors such as mate attraction and territorial defense. We examined the role of the caudomedial nidopallium (NCM), a forebrain region analogous to secondary auditory cortices, in song recognition. We trained European starlings to recognize conspecific songs and recorded the activity of single neurons in NCM. In ventral NCM, neurons responded stronger to unfamiliar songs than to songs that starlings had learned to recognize. While in dorsal NCM, neurons responded

similarly to learned and unfamiliar songs. In a second experiment we trained starlings to recognize songs and exposed them to an equal number of songs passively. Recognition learning weakened the firing rates to the learned songs, while passively hearing songs had no effect, indicating that the response suppression in NCM requires associative learning. These results show that song recognition learning weakens the responses to learned songs in ventral NCM.

Local inhibition is involved in the plasticity of sensory systems that results from altered sensory input such as deprivation. To investigate the mechanisms underlying the plasticity in NCM, we manipulated local inhibition in NCM of starlings that were trained to recognize conspecific songs. Blocking inhibition enhanced the preference for unfamiliar songs in ventral NCM and uncovered a preference for learned songs in dorsal NCM. Disrupting inhibition reduced the selectivity for songs and uncovered responses to specific regions of song that are normally masked. In dorsal NCM, a greater frequency of these unmasked responses occurred during learned songs. Blocking inhibition also increased the nonlinearities associated with spectro-temporal receptive fields. This demonstrates that local inhibition modulates learning-related plasticity by inactivating plasticity throughout NCM.

I. Introduction

Vocal Recognition

Vocal recognition is a fundamental behavior for communication in several animal species, including humans. Throughout life, humans learn to recognize the acoustic structure of specific sounds as they memorize words for language. By adulthood, many humans have learned to recognize over 10,000 words (Goulden et al., 1990). Humans also use vocal recognition to associate the sound of a speaker's voice with individual identity. While communicating with conspecifics, humans can recognize identity and other characteristics such as age, appearance and sex based on voice quality (Kreiman et al., 2005).

Vocal recognition is involved in different types of recognition behaviors related to animal communication. Vocal recognition is used by many species to detect conspecifics. For example, tungara frogs recognize the vocalizations of individuals to discriminate other tungara frogs from members of different frog species (Ryan and Rand, 1993). Vocal recognition is also important for recognizing members of groups within species. Barking foxes learn to recognize members of social groups based on long-distance barks (Frommolt et al., 2003), and African elephants recognize family members by infrasonic contact calls (McComb et al., 2000). Many species of animals use vocalizations to recognize specific members of their species. Kin recognition using vocalizations has been demonstrated in several species. Mother Vervet monkeys recognize their offspring based on vocalizations

(Cheney and Seyfarth, 1980). In northern fur seals, vocal recognition is mutual between mothers and offspring (Trillmich, 1981), and can last for several years (Insley, 2000). Kin recognition has been studied in detail in penguins, where adult penguins produce display calls that chicks must recognize in a noisy environment in order to feed (Aubin and Jouventin, 2002; Jouventin et al., 1999). Vocal recognition is also necessary for individual recognition outside of parent-offspring pairs. For example, several species of primates recognize individual conspecifics using vocalizations (Cheney and Seyfarth, 1980; Rendell et al., 1996; Snowdon and Cleveland, 1980). Vocal recognition of conspecifics has also been identified in cetaceans and birds. Wild battle nose dolphins recognize conspecifics by their signature whistles (Sayigh et al., 1990) and king penguins can recognize conspecifics from their calls (Lengagne et al., 2000).

Songbirds

Songbirds have emerged as a useful model for the study of vocal recognition. Vocal recognition in songbirds is necessary for several behaviors, including female choice, kin recognition, territoriality, and individual recognition (Beecher et al., 1994; Godard, 1991; Lind et al., 1996; O'Loghlen and Beecher, 1997; Stoddard, 1996b; Stoddard et al., 1991). Most often, individual vocal recognition of songbirds occurs in the service of territoriality and mate attraction. In many species of songbirds, males sing songs to attract female conspecifics for mating. Females learn to recognize songs to evaluate the fitness of conspecifics males and choose mates.

For example, female zebrafinches are able to recognize their mate by song (Miller, 1979a). Song is also used to maintain territoriality. In many species, males learn the songs that other males sing to repel conspecifics males from their territory (Brenowitz et al., 1997; Kroodsma and Byers, 1991; Searcy, 1986). Songbirds also learn to recognize the songs of individuals to perform other behaviors. For example, female zebrafinches learn to recognize the songs of their father, which may be important for sexual imprinting or the development of mate preferences (Miller, 1979b). Individual vocal recognition is widespread amongst species of songbirds. Recognition based on song has been found in all species of songbirds that have been examined (Stoddard, 1996b). Several recognition strategies have been identified in songbirds. Some species learn to associate specific songs with singers for individual recognition, while others rely on more general acoustical structure such as frequency differences (Beecher et al., 1994; Brooks and Falls, 1975; Nelson, 1989).

Several parallels to human language make songbirds an interesting model for the study of communication (Doupe and Kuhl, 1999). Songbirds, like humans are vocal learners, meaning that their vocalizations must be acquired from experience with conspecifics (Doupe and Kuhl, 1999). This was first demonstrated when young chaffinches that were isolated from adult conspecifics developed abnormal songs (Thorpe, 1958). Song learning has been broken into several stages. First, during the memory acquisition or sensory stage, young birds listen to the songs of an adult model or tutor and form a memory of the song. Next, in the sensorimotor stage, the songbird sings and begins the process of matching the song to the memory of the

tutor song. The sensorimotor phase is broken into the subsong phase and the plastic song phase. During the subsong phase, which has been compared to human babbling, songbirds sing variable and quiet songs while calibrating their vocal production system. In the plastic song stage, songbirds sing louder songs with more structure and use auditory feedback to adjust their song. The final stage of song learning is crystallization, where the song becomes stereotyped (Marler, 1981). In several species of songbirds, called age-limited learners, this process occurs a single time during a critical period in development. While other songbird species, called open-ended learners, can repeat the song learning stages several times either seasonally or throughout the year to add new song material to their repertoires. Examples of age-limited learners include zebrafinches and white crown sparrows, while examples of open-ended learners include European starlings and canaries (Brenowitz et al., 1997; Marler and Peters, 1987; Williams, 2004).

Like human language, the songs of songbirds have complex acoustic structure and are organized hierarchically. Though there is great diversity in the complexity of songs among different species both in the acoustical structure of song units and the number and variability of song units sung by individuals (Brenowitz et al., 1997), a general hierarchical structure has been used to describe songs. The simplest individual sounds, such as a single frequency sweep are called notes. Several notes combined in a sequence are called a syllable and a sequence of syllables is called a motif or phrase. An entire song bout, an episode of singing from

start to finish is composed of sequences of phrases or motifs (Brenowitz et al., 1997).

European Starling Song Behavior

Vocal recognition has been studied extensively in European starlings (*Sturnus vulgaris*). Starlings have become a popular subject for the study of song behavior for several reasons. Starlings are a convenient choice because they are available throughout the world and adapt well to captivity (Eens, 1997). Most importantly, starlings are an interesting model for vocal recognition due to their complex song behavior. Starlings sing long songs composed of acoustically complex elements that often include mimicry of other species (Eens, 1997). A useful depiction of starling song from the first half of the twentieth century described starling song as, "a lively rambling melody of throaty warbling, chirruping, clicking and gurgling notes interspersed with musical whistles and pervaded by a peculiar creaking quality" (Witherby et al., 1943). Despite the complexity of starling song, the general organization of song, described above, mostly applies. Starlings sing long song bouts that can last around one minute in length. The song bout is composed of motifs, which last between half a second and one second long and may be repeated several times. Each motif is a fixed combination of notes and notes are the simplest sound in starling songs (Eens, 1997). In addition, a large-scale temporal structure has been identified in starling songs. The first section of starling song bouts contains pure-toned whistles, which may be separated by brief periods of silence. Next, in the

most variable section, are complex warble motifs that may include heterospecific sounds. The third section contains click trains or rattles and the fourth and final section contains high frequency and high amplitude motifs and often includes many repetitions. As the song bout progresses, both the tempo and the frequency of the song increase (Adrethausberger and Jenkins, 1988; Chaiken et al., 1993; Eens et al., 1989).

Communication with conspecifics is essential in starlings because they are one of the more social species of songbirds. Starlings breed in colonies, live in large flocks and sing all year long (Feare, 1984). Starling songs serve a similar function as the songs of most other songbird species. Most evidence points towards female attraction as the major function of male starling song. Males often sing to females prior to copulation and then reduce their singing immediately following (Eens et al., 1990, 1994). Male starlings that are trying to court females will also increase singing if the female is removed from their view (Cuthill and Hindmarsh, 1985). Males also sing more to females than males when presented with conspecifics from both sexes (Eens et al., 1993). The behavior of female starlings also points to song as a factor in mate selection. Females will go to nest boxes with male song over boxes with silence (Mountjoy and Lemon, 1991). Females choose their mates based on characteristics of song and males with larger repertoires, meaning more motifs, are more likely to mate and mate faster (Eens et al., 1991; Mountjoy and Lemon, 1996). Similarly, females prefer to spend time hearing longer songs with more motifs than hearing shorter songs (Gentner and Hulse, 2000a). The role of starling song in territoriality

is less clear. While one study describes male starlings singing to keep other males away, there is minimal evidence overall (Eens et al., 1993). Further studies are required to understand the role of starling song for male-to-male territorial interactions, however, the role of song in attracting female mates is well established (Eens, 1997).

Starlings sit at one extreme of the continuum of the different types of song learners. Starlings are open-ended learners that learn new song motifs throughout life (Chaiken et al., 1994). As starlings age, they learn to sing new motifs and the size of their repertoires increases (Eens et al., 1992). While new motifs are added, others are dropped or modified (Mountjoy and Lemon, 1995). Remarkably, there can be a long delay, up to eighteen months, between exposure to new song material and incorporation into the starling's repertoire (Chaiken et al., 1994). Open-ended song learning contributes to the variety of motifs sung by each individual starling. In addition, starlings learn motifs from numerous conspecific males throughout their life (Eens et al., 1992). In the wild, the song (or motif repertoire) of each individual starling is unique and motif sharing is rare (Chaiken et al., 1993; Eens et al., 1989). Despite the large variability in song motifs both between individuals and within and individual starling's repertoire, the song bouts for starlings are fairly repeatable (Eens, 1997). The unique, but stable nature of starling songs make them a useful signal for individual recognition and suggest that memorizing the motifs of an individual is a useful recognition strategy (Ball et al., 2006). This is supported by vocal recognition experiments in the laboratory. When starlings are trained with

operant conditioning to recognize the motifs of conspecific individuals, they generalize to novel songs with overlapping motifs, but not other songs from the same singer (Gentner and Hulse, 1998). If the starlings are asked to recognize songs with varying numbers of familiar motifs, song recognition ability varies directly with the proportion of familiar motifs (Gentner and Hulse, 2000b). In addition, disrupting the order of notes that comprise familiar motifs reduces recognition performance, indicating that starlings learn the acoustic structure of individual motifs (Gentner, 2008). While the studies on the mechanism of song recognition learning have taken place in the laboratory, there is evidence that starlings use individual vocal recognition in the wild. For example, after mating, female starlings can recognize the songs of their mate and interrupt attempts of the male to attract new mates with song (Eens and Pinxten, 1995).

Songbird Auditory System

Understanding the neural mechanisms of vocal recognition in songbirds requires understanding how the auditory system processes songs. The auditory system of songbirds is similar across other avian groups and vertebrates including mammals (Hodos and Butler, 1997; Theunissen and Shaevitz, 2006). The same general organization from the ear to the auditory cortex in mammals can be identified in the auditory regions of birds. As in mammals, auditory information arises in the ear and travels to the cochlear nucleus, then both directly and indirectly to the midbrain, then to the auditory portion of the thalamus and on to

further processing regions. In birds, auditory information travels from the ear to the cochlear nuclei in the medulla and then travels to the olivary nuclei and lemniscal nuclei and then contralaterally to the nucleus mesencepahlicus lateralis (MLd), the analogue to the inferior colliculus in the midbrain. From the midbrain, information travels to the medial portion of nucleus ovoidalis in the thalamus and on to the field L complex, which is the analogue to primary auditory cortex. The field L complex is subdivided in to five cytoarchitectonic subregions that are densely interconnected: L1, L2a, L2b, L3 and L. Connections from Ovoidalis first arrive in L2a and L2b, which project to L1 and L3. The field L subregions project to the secondary auditory forebrain regions. L2a and L3 project to the caudomedial nidopallium (NCM), while L1, L2a, L2b and L3 project to the lateral caudal mesopallium (CLM). Both NCM and CLM have reciprocal projections to the other secondary auditory forebrain region, the medial caudal mesopallium (CMM) (Fortune and Margoliash, 1992; Vates et al., 1996). Our understanding of the similarities between the auditory systems of mammals and birds has been advanced by recent work that identified columnar structure in the auditory system of chicks that is similar to the layered structure of the auditory cortex (Wang, Brzozowska-Prechtl & Karten, in press).

Electrophysiological experiments have characterized the functional properties of neurons in several regions of the songbird auditory system. There is a functional hierarchy in the songbird auditory system, where neurons become increasingly selective in successive processing stages. In the midbrain region MLd, neurons are broadly tuned. MLD neurons respond strongly to synthetic sounds such

as tones and natural sounds including conspecifics song stimuli (Woolley and Casseday, 2004). Despite this broad responsiveness, the spike patterns in responses to natural sounds are more discriminable and have higher information rates (Hsu et al., 2004). Recently it was shown that as an ensemble, MLd neurons accurately encode the temporal changes in the envelope of songs suggesting that MLd neurons are tuned to low-level characteristics of song (Woolley et al., 2006). Like MLd, neurons in the thalamic nucleus Ov respond to a wide range of auditory stimuli including synthetic sounds (Biederman-Thorson, 1970; Bigalke-Kunz et al., 1987). Neurons in Ov have spectro-temporal receptive fields that are intermediate in complexity to MLd and field L (Amin et al., 2010). Several sources have demonstrated that neurons in the field L complex have a higher degree of selectivity than MLd or Ov. Early studies reported that neurons in field L responded rarely to tones, while responding stronger to more complex sounds such as hisses, clicks or conspecifics songs (Biederman-Thorson, 1970; Bonke et al., 1979; Leppelsack and Vogt, 1976). These results have been confirmed by recent studies that found selectivity for conspecifics songs over synthetic sounds that were designed to match the overall power spectra and amplitude modulation spectra of song (Grace et al., 2003). Selectivity also increases throughout the nuclei of the field L complex. Neurons in L2a and L2b display some selectivity for conspecifics songs, but still respond to synthetic sounds, while neurons in L1 and L3 respond even less to synthetic sounds (Bonke et al., 1979; Müller and Leppelsack, 1985). These differences can also be seen in the receptive fields of field L neurons. Neurons in the

input and output layers of field L have different types of spectro-temporal receptive fields (Nagel and Doupe, 2008). The selectivity for conspecifics songs in field L breaks down for synthetic sounds that preserve the joint spectro-temporal statistics of song, as these sounds drive field L neurons as well as song (Hsu et al., 2004). In addition, responses of neurons in field L are informative for song discriminations, in that the identity of songs can be read-out from the spike trains of single neurons (Narayan et al., 2006; Wang et al., 2007). Overall, neurons in the field L complex are selective for conspecifics songs and represent information about song identity, suggesting that they are important for processing vocalizations in conspecifics recognition behaviors (Theunissen and Shaevitz, 2006).

The selectivity of neurons continues to increase in the secondary auditory regions CLM, CMM and NCM. Neurons in CLM are more selective for conspecific songs and other complex stimuli than neurons in field L (Grace et al., 2003; Müller and Leppelsack, 1985). Further analysis revealed that neurons in CLM were more selective for the natural order in time and frequency in song (Hsu et al., 2004). Receptive field modeling also points to an increase in selectivity in CLM, because the ability of linear spectro-temporal receptive fields to model the responses of neurons is worse for CLM than field L (Gill et al., 2006; Sen et al., 2001). Recent work has advanced our understanding of the function of CLM neurons. A study that used different types of receptive field models demonstrated that CLM neurons are sensitive to the recent history of auditory stimulation and the expectations based on the properties of conspecific songs (Gill et al., 2008). The authors demonstrated that

this sensitivity is not present in neurons in MLd and field L, suggesting that it arises in CLM (Gill et al., 2008). In another recent study, the authors recorded from CLM and field L neurons in singing songbirds, while they manipulated auditory feedback. They found that some of the neurons in field L and CLM were sensitive to vocal perturbations of the bird's own song indicating that these neurons were important for auditory feedback (Keller and Hahnloser, 2009). Neurons in CMM are also highly selective for conspecific songs. CMM neurons respond more strongly to conspecific song than other stimuli, but most individual neurons are also highly selective for particular conspecific songs (Gentner and Margoliash, 2003; Müller and Leppelsack, 1985). When song motifs are broken up into notes, CMM neurons respond to specific notes and the responses to song can be explained by the linear combination of excitation and suppressed to individual notes. Individual CMM neurons were tuned to respond to specific, but distinct notes, suggesting a convergence of inputs that respond to discrete sets of notes (Meliza et al., 2010). There is also evidence for selectivity for conspecific song in NCM neurons. NCM neurons respond more strongly to conspecific song than to synthetic sounds or heterospecific songs (Chew et al., 1996; Müller and Leppelsack, 1985; Stripling et al., 1997). This result has also been found in studies that measured neural activity in less direct ways. In NCM, there is increased expression of the immediate early gene (IEG) ZENK in response to conspecific songs than to either heterospecific songs or synthetic stimuli (Mello et al., 1992). In addition, ZENK expression in NCM suggested that NCM neurons are tuned to specific syllables of songs (Ribeiro et al., 1998). FMRI of songbirds revealed

stronger BOLD activity in NCM during the presentation of conspecific songs than other auditory stimuli (Van Meir et al. 05).

Learning and the songbird auditory system

The regions CMM and NCM have been implicated in various types of song learning including vocal recognition (Bolhuis and Gahr, 2006). The responses of neurons in CMM change with song recognition learning. Training starlings to recognize certain conspecific songs using both go no-go and two alternative choice procedures, changed the representation of songs in CMM. Following training, CMM neurons responded more strongly to the songs the starlings had learned to recognize than other unfamiliar conspecific songs. CMM responses were influenced not only by song exposure but also differences in reinforcement. CMM neurons responded stronger to the go songs that were paired with reinforcement, than to the no-go songs (Gentner and Margoliash, 2003). This study indicates that song recognition learning changes the representation of songs in CMM neurons in accordance with behavioral relevance. Similar results were found using the analysis of IEG expression in CMM. IEG expression is elevated in CMM following the recognition of learned songs and the acquisition stage of learning to recognize new songs (Gentner et al., 2004). These results suggest that CMM neurons are involved in learning to recognize songs. A potential role for NCM in song learning was first demonstrated in studies of IEG expression following song exposure. Exposure to song for short periods of time causes increased expression of the IEG ZENK. As

songbirds were exposed to song for longer periods of time, ZENK expression decreases back to baseline levels. This adaptation was specific for the song stimulus used during exposure and could last for several days (Mello et al., 1995). Song exposure has similar effects on electrophysiological responses in NCM. When a single song stimulus is played repeatedly, the spiking response gradually decreases back towards the spontaneous firing rate. Like the adaptation of IEG responses, the adaptation of spiking is also long lasting and stimulus-specific (Chew et al., 1995; Stripling et al., 1997). These studies demonstrate that NCM responses to song are plastic and suggest that changes in NCM may be involved in behavioral habituation to song. However, because studies that have induced neuronal adaptation in NCM have never also measured behavior, it is unclear what if anything songbirds are learning through these manipulations. NCM has also been implicated in the memory of a songbird's tutor song. After song learning is complete, the amount of IEG expression in NCM is correlated with how well a songbird's song matches their tutor's song (Bolhuis et al., 2000; Terpstra et al., 2004). Relatedly, how well songbird's song matches their tutor song is also correlated with the rate of firing rate adaptation in NCM (Phan et al., 2006). Together these results suggest that NCM activity might underlie the memory of the tutor song. The evidence for the involvement of NCM in tutor song memory was increased by two recent studies that manipulated NCM activity. Lesioning NCM bilaterally reduced the preference of songbirds for their tutor song and spared singing ability and simple auditory discriminations, suggesting that the memory for tutor song was disrupted (Gobes

and Bolhuis, 2007). Similarly, disrupting a kinase that regulates ZENK induction in NCM of young songbirds caused them to sing songs that were poor copies of their tutor song as adults (London and Clayton, 2008). The above results show that both CMM and NCM are involved in different types of song learning. However, it is unclear how CMM is involved in tutor song learning and how NCM is involved in learning to recognize conspecifics songs.

Mammalian auditory system

Studies of auditory processing and plasticity in mammals have also informed the study of vocal recognition learning. While differences exist in the organization of the auditory system of different species of mammals, a general organization has emerged (Kaas et al., 1999; Semple and Scott, 2003). Auditory information is processed in brainstem nuclei such as the cochlear nuclei and transmitted to the inferior colliculus (IC) of the midbrain. From the IC, information travels to the auditory region of the thalamus, the medial geniculate nucleus (MG). From the MG, auditory information travels to the auditory cortex which is composed of several distinct regions (Semple and Scott, 2003). The exact number of cortical regions in the auditory cortex differs among species (Scheich, 1991). In the primate, the auditory cortex can be divided into a central core of 2-3 regions including the primary auditory cortex (AI), a surrounding belt of 7-8 regions and a lateral parabelt of 2 regions (Semple and Scott, 2003). In some species such as cats and mice, a secondary auditory cortex (AII) has also been identified in the core. AI and the

regions in the core receive the strongest input from the ventral MG. The belt regions receive less inputs from ventral MG and strong inputs from the dorsal and medial MG and the core. The parabelt regions receive strong inputs from the belt, and weaker inputs from the core and the dorsal and medial MG (Kaas et al., 1999).

Studies of the auditory system in mammals have revealed a hierarchical organization in the representation of increasingly complex sounds. In the IC, sharply tuned neurons are arranged tonotopically and form a map of best frequency (Clopton and Winfield, 1973; Hind et al., 1963; Merzenich and Reid, 1974). Though, IC neurons in some species display selectivity for more complex sounds. For example, IC neurons in the bat are selective for specific frequency modulation sweeps (Fuzessery, 1994; Fuzessery and Hall, 1996). The MG also has a tonotopic organization and neurons are frequency selective (Aitkin and Webster, 1971). Spectro-temporal receptive fields have shown that MG neurons are highly selective temporally and spectrally (Winer et al., 2005). Another property of MG neurons, which is not found in AI, is the ability to phase-lock to stimuli even at high frequencies (Winer et al., 2005). While MG neurons respond robustly to simple tonal stimuli, there is no selectivity for more complex sounds (Symmes et al., 1980). Despite earlier ideas, several lines of evidence indicate that the MG is not a simple relay stage to the cortex as was once thought (Winer et al., 2005). Simultaneous recordings of functionally connected neurons in MG and AI demonstrated how spectro-temporal receptive fields changed between the two regions. Some AI neurons had receptive fields similar to those in MG, implying a simple inheritance

mechanism. However, a large portion of neurons in AI had receptive fields that could be explained by combining several small receptive fields of MG neurons into a larger receptive field, demonstrating a computation performed in the MG (Miller et al., 2001). One functional role of the MG may be increasing the efficiency of the representation of sounds. A study that compared recordings made in the IC, MG and AI found that neurons in the MG and AI represented information with less redundancy than neurons in the IC (Chechik et al., 2006). Several regions of the auditory cortex have a tonotopic organization, though the number of tonotopic regions in the cortex varies among different species (Hackett et al., 1998; Merzenich and Brugge, 1973; Scheich, 1991). Tonotopy in the core regions including AI is a general finding among many species (Bonke et al., 1979; Merzenich et al., 1976; Scheich, 1991). The organization of the tonotopic maps is different in each region of the core, suggesting different types of processing (Kaas et al., 1999; Polley et al., 2007). Besides the general tonotopy, single neurons in the core regions are characterized by sharp frequency tuning, though not as sharp as neurons in IC or MG (Semple and Scott, 2003). While AI neurons are tuned to frequency, a standard simple stimulus that drives AI neurons strongly, such as oriented bars in the primary visual cortex, has not been identified, indicating that the understanding of AI neurons remains incomplete (King and Nelken, 2009). Neurons in AI are not able to phase-lock to repetitive sounds at high frequencies like neuron in the MG and IC. Instead, some neurons in AI represent repetitive sounds with a rate code instead of a timing code, indicating a transformation in the type of representation between MG

and AI (Lu et al., 2001). In addition, there is evidence for hierarchical processing within AI. The STRFs of AI neurons show increasing complexity across the layers of the cortex (Atencio et al., 2009). Overall there is less tonotopic organization in the belt and parabelt regions of the cortex (Semple and Scott, 2003). Neurons in the lateral belt regions are more broadly tuned for frequencies and have more complex response properties. Belt neurons are selective for frequency bandwidth and the direction and rate of frequency modulations (Rauschecker et al., 1995; Tian and Rauschecker, 1998). A framework has been described for the auditory cortex that divides the processing of "what" and "where" information into hierarchical streams (Rauschecker and Tian, 2000). However, several studies have shown that these divisions are not supported by the data and that the processing of spatial and non-spatial information is interspersed (Recanzone and Cohen, 2010; Semple and Scott, 2003).

Similar to the songbird system, several studies have examined the responses to conspecific vocalizations in the mammalian auditory system. In the MG, neurons respond similarly to simple synthetic sounds and conspecific vocalizations (Symmes et al., 1980). Early reports found very small numbers of neurons in the primate auditory cortex that responded selectively to vocalizations over simple tones (Winter and Funkenstein, 1973; Wollberg and Newman, 1972). More recently, these results were confirmed by a study that compared the responses of AI neurons to conspecifics vocalizations and synthetic sounds with the same spectral but different temporal characteristics (Wang et al., 1995). Interestingly, when the same

experiment was performed in cats, selectivity was absent and AI neurons responded similarly to primate vocalizations and synthetic sounds (Wang and Kadia, 2001). In the lateral belt, selectivity for conspecific vocalizations is more prominent. Recordings in the belt revealed selectivity for complex sounds and vocalizations over tonal stimuli in most neurons (Rauschecker et al., 1995). Together, these results provide evidence for a hierarchy in the representation of vocalizations and other complex sounds in the mammalian auditory system (Rauschecker, 1998). Recently, a new auditory region was discovered in the insular cortex of the primate that is highly selective for conspecifics vocalizations. Neurons in this region responded stronger to conspecific vocalizations than to environmental sounds, heterospecific vocalizations and manipulated versions of conspecific vocalizations (Remedios et al., 2009).

Learning and the mammalian auditory system

Understanding how the auditory system changes during learning is important for understanding the mechanisms of vocal recognition learning. While most of the studies that examine learning-related plasticity in the auditory system do not focus on animals that use vocal communication, the same plasticity mechanisms they uncover might be involved in vocal recognition learning. Numerous studies over the last few decades have shown that the responses of neurons in the auditory system are highly plastic (Weinberger, 2004). Some of the earliest studies of auditory plasticity found that classical fear conditioning led to

changes in core regions of the auditory cortex beyond AI. Conditioning led to both increases and decreases in the responses of single neurons to conditioned tones in the region AII (Diamond and Weinberger, 1984), and it was later shown that these effects were specific to the training frequency (Diamond and Weinberger, 1986). In AI, classical conditioning was also shown to produce changes in the receptive fields of single neurons. Following conditioning, the responses to the conditioned tone were increased, while responses to surrounding tones were decreased or unchanged. These changes caused shifts the receptive fields towards the training tone and often changed the neuron's best frequency (Bakin and Weinberger, 1990). Other forms of learning besides conditioning also induce plasticity in the auditory cortex. Discrimination training caused neurons to respond more strongly to training sounds (Edeline and Weinberger, 1993). While repeated presentations of a single tone in a habituation procedure caused neuronal responses to the training tone to decrease (Condon and Weinberger, 1991). Examining the receptive fields of large populations of neurons in AI showed how learning changed the representation of sounds on a larger scale. Systematically mapping the receptive fields of AI neurons in monkeys that were trained in tone discriminations demonstrated that training increased the overall area of the cortex that responds to the training tones. No changes in representation were found in control monkeys that heard the same sounds without performing a discrimination, indicating that the plasticity was associative (Recanzone et al., 1993). A recent study expanded on these findings to show that cognitive association was necessary for plasticity. Yoked monkeys that

received the same stimulus and reward stimulation without performing discriminations did not show the same changes in AI as monkeys that performed the task (Blake et al., 2006). Learning-related plasticity can also alter the representations of sound characteristics besides frequency. Training rats to use the repetition rate of sounds in tasks led to an improvement in the ability of AI neurons to follow repetitive sounds at high rates (Bao et al., 2004). Similarly training rats on an intensity recognition task changed the intensity tuning of neurons in AI and AII to increase the representation of the training intensity (Polley et al., 2006).

Recent studies have shown that learning-related plasticity in auditory cortex can occur rapidly. Fritz et al. (03) developed a technique to map changes in STRFs of neurons in the auditory cortex of awake ferrets performing behavioral tasks. When ferrets performed a tone detection task, STRFs of neurons in AI shifted the excitatory regions of their receptive fields towards the training tone. The techniques in this study allowed for the estimation that STRFs were changing on the timescale of minutes. When neurons were held for extended periods of time, it was shown that these STRF changes could last for hours after the task was completed (Fritz et al., 2003). The types of receptive field changes induced depended on the specifics of the task. Training ferrets on a frequency discrimination task instead of a tone detection task, enhanced the regions of the STRF near the target tones and decreased the regions near the reference tones (Fritz et al., 2005).

There is now a large literature showing that training increases the responses of single neurons or the size of the representation of training sounds in auditory

cortex (Weinberger, 2004). Several studies, however, have questioned the generality of these findings. Training guinea pigs on a discrimination task with one target tone and several reference tones led to decreases in the responses of AI neurons to the target tone and increases in responses to surrounding tones (Ohl and Scheich, 1996, 1997). Training cats on a frequency task where the reference tones were variable led to similar results (Witte and Kipke, 2005). These changes are thought to improve discrimination performance by increasing the contrast sensitivity of AI neurons (Ohl and Scheich, 2005). In other studies, no changes in the firing rates of AI neurons were observed following training. In one example, cats learned to perform a frequency discrimination task, but no changes were observed in tonotopic maps (Brown et al., 2004). These results, with those of Fritz et al. (05), indicate that even subtle differences in the design of learning tasks can lead to differences in plasticity in the auditory cortex (Ohl and Scheich, 2005).

Advances have also been made in the study of vocal recognition in mammals. An emerging system for the study of vocal recognition is the ultrasonic communication system in mice. When mouse pups are separated from their mothers, they produce ultrasonic isolation calls (Noirot, 1966; Sewell, 1968), which cause mothers to search for their lost pup (Haack et al., 1983; Sewell, 1970). Mothers respond to isolation calls and prefer them to other sounds, while female virgin mice do not (Ehret et al., 1987), indicating that mothers learn to recognize the communicative significance of pup calls (Ehret, 2005). The differences in the ability to recognize pup isolation calls between mothers and virgins are accompanied by

changes in the representation of pup calls in AI. Pup calls occur at a high repetition rate (5kHz). In mothers, AI neurons entrain to sound repetitions at the rate of pup calls, whereas AI neurons in virgin mice cannot entrain to sounds at high rates (Liu et al., 2006). This suggests that pup calls are better represented in mothers and indicate that mothers undergo plasticity in the representation of pup calls in the auditory cortex. Indeed, the responses to pup calls in AI neurons in mothers carry more information than responses in virgins (Liu and Schreiner, 2007). In addition, pup calls also elicit stronger suppression in mothers, which may increase sound contrast and aid detection (Galindo-Leon et al., 2009a). These results suggest that the changes in the representation of pup calls in the auditory cortex of mothers might underlie call recognition.

Mechanisms of sensory plasticity

Little is known about the mechanisms that underlie learning-related related plasticity in sensory systems. However, the mechanisms for sensory plasticity caused by manipulations of sensory exposure are better understood. The classic experiments of Wiesel and Hubel (63) demonstrated that sensory deprivation could cause plasticity in cortical representations. In these monocular deprivation experiments, closing one eye in developing cats led to an imbalance in the representation of the eyes in the visual cortex. Plasticity from monocular deprivation only occurred in a critical period during development (Wiesel and Hubel, 1963). Recent studies have shown that monocular deprivation can also cause

plasticity in adult animals, though not to the same degree as during development (Hofer et al., 2006; Sawtell et al., 2003). Monocular deprivation plasticity proceeds in two stages, an initial weakening of responses to inputs from the deprived eye and then a strengthening of responses to the spared eye (Frenkel and Bear, 2004). Before the critical period begins, monocular deprivation fails to produce plasticity. The critical period is triggered by the development of local inhibition in the visual cortex (Hensch et al., 1998; Huang et al., 1999). Experimentally enhancing GABA-A inhibition can prematurely trigger the start of the critical period (Fagiolini et al., 2004).

Long-term potentiation (LTP) and long-term depression (LTD) have been implicated in the plasticity of excitatory inputs in caused by altered sensory exposure. LTD is involved in the weakening of deprived sensory inputs and LTP is thought to be involved in the strengthening of spared sensory inputs (Feldman, 2009). For example, plucking all but one whisker in rats shifts the receptive fields of neurons in the primary somatosensory cortex (S1) towards the spared whisker while weakening the inputs from the plucked whiskers through LTD (Allen et al., 2003). Plasticity caused by changes in sensory exposure also involves inhibitory circuits. A recent experiment that induced plasticity through visual deprivation found strengthened inhibitory synapses onto excitatory neurons in V1, but no change in the strength of excitatory connections (Maffei et al., 2006). When whisker plucking shifts the receptive fields of S1 neurons, inhibition inactivates responses to the deprived whisker to enhance the representation of the spared whisker (Foeller

et al., 2005). In barn owls with altered visual input, inhibition controls the plasticity of the map of auditory space in the IC. During the early stages of learning, inhibition inactivates the new learned map, and preserves the normal map of space (Zheng and Knudsen, 2001). As learning progresses, inhibition switches to inactivate the normal map of space and enhance the representation of the new adaptive map (Zheng and Knudsen, 1999). Inhibition has also been implicated in learning-related plasticity. Following fear conditioning, the shifts in receptive fields in S1 are accompanied by increased levels of GABA and GABA receptors (Gierdalski et al., 2001; Lech et al., 2001). Similar results have been found in V1, showing that these effects generalize to other sensory systems (Liguz-Lecznar et al., 2009). In addition, fear conditioning increased the density and strength of inhibitory synapses in S1 (Jasinska et al., 2010; Tokarski et al., 2007). Inhibition also appears to be involved in non-associative forms of learning such as habituation. Repeated stimulation of a single whisker led to a reduction of responses to that whisker and was shown to occur with an increase in the density of inhibitory synapses in the region of S1 corresponding to the whisker (Knott et al., 2002). A functional role for inhibition in learning-related plasticity has yet to be demonstrated.

Inhibition and stimulus selectivity

In addition to plasticity, inhibition plays a general role in shaping the receptive fields of neurons in several sensory systems. Inhibition shapes the selectivity of neurons at several stages of the visual system. In V1, blocking

inhibition has been reported to reduce the surround region of center-surround receptive fields, and reduces direction and orientation selectivity (Sillito, 1975, 1977). Inhibition also contributes to the selectivity for direction in MT (Thiele et al., 2004) and for complex visual objects in IT cortex (Wang et al., 2000; Wang et al., 2002b). In IT, blocking inhibition expands receptive fields and causes neurons to respond to a wider range of complex objects (Wang et al., 2000; Wang et al., 2002b). Inhibition also shapes the receptive field of neurons throughout the auditory system. Blocking inhibition has been shown to reduce selectivity for sounds and expand receptive fields in IC (Klug et al., 2002; LeBeau et al., 2001; Xie et al., 2005). In AI, inhibition performs a similar function and increases the selectivity for frequency (Chang et al., 2005; Chen and Jen, 2000; Wang et al., 2002a). Inhibition has also been shown to contribute to the selectivity for complex sounds in field L and CM of chicks (Muller and Scheich, 1987). Despite the clear role for inhibition in shaping sensory receptive fields, it is unclear whether these mechanisms are also involved in the selectivity for vocalizations.

In the following chapters we describe our efforts to understand the neural mechanisms of vocal recognition learning in songbirds. We focus on NCM due to the evidence suggesting that NCM is involved in other types of song learning. In chapter 1, we examine how song recognition learning changes the responses of NCM neurons, to learn about the role of NCM in song recognition learning. In chapter 2, we investigate the role of local inhibition NCM in plasticity, to learn about the mechanisms underlying learning-related plasticity in NCM.

References

Adrethausberger, M., and Jenkins, P.F. (1988). COMPLEX ORGANIZATION OF THE WARBLING SONG IN THE EUROPEAN STARLING STURNUS VULGARIS. Behaviour *107*, 138-156.

Aitkin, L.M., and Webster, W.R. (1971). Tonotopic organization in the medial geniculate body of the cat. Brain Res *26*, 402-405.

Allen, C.B., Celikel, T., and Feldman, D.E. (2003). Long-term depression induced by sensory deprivation during cortical map plasticity in vivo. Nat Neurosci *6*, 291-299.

Amin, N., Gill, P.R., and Theunissen, F.E. (2010). The role of the zebra finch auditory thalamus in generating complex representations for natural sounds. J Neurophysiol.

Atencio, C.A., Sharpee, T.O., and Schreiner, C.E. (2009). Hierarchical computation in the canonical auditory cortical circuit. Proc Natl Acad Sci U S A *106*, 21894-21899.

Aubin, T., and Jouventin, P. (2002). Localisation of an acoustic signal in a noisy environment: the display call of the king penguin Aptenodytes patagonicus. J Exp Biol *205*, 3793-3798.

Bakin, J.S., and Weinberger, N.M. (1990). Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. Brain Res *536*, 271-286.

Ball, G.F., Sockman, K.W., Duffy, D.L., and Gentner, T.Q. (2006). A neuroethological approach to song behavior and perception in European starlings: Interrelationships among testosterone, neuroanatomy, immediate early gene expression, and immune function. In Advances in the Study of Behavior, Vol 36 (San Diego, Elsevier Academic Press Inc), pp. 59-121.

Bao, S., Chang, E.F., Woods, J., and Merzenich, M.M. (2004). Temporal plasticity in the primary auditory cortex induced by operant perceptual learning. Nat Neurosci *7*, 974-981.

Beecher, M.D., Campbell, S.E., and Burt, J.M. (1994). SONG PERCEPTION IN THE SONG SPARROW - BIRDS CLASSIFY BY SONG TYPE BUT NOT BY SINGER. Anim Behav *47*, 1343-1351.

Biederman-Thorson, M. (1970). Auditory responses of units in the ovoid nucleus and cerebrum (field L) of the ring dove. Brain Res *24*, 247-256.

Bigalke-Kunz, B., Rubsamen, R., and Dorrscheidt, G.J. (1987). Tonotopic organization and functional characterization of the auditory thalamus in a songbird, the European starling. J Comp Physiol A *161*, 255-265.

Blake, D.T., Heiser, M.A., Caywood, M., and Merzenich, M.M. (2006). Experience-dependent adult cortical plasticity requires cognitive association between sensation and reward. Neuron *52*, 371-381.

Bolhuis, J.J., and Gahr, M. (2006). Neural mechanisms of birdsong memory. Nat Rev Neurosci *7*, 347-357.

Bolhuis, J.J., Zijlstra, G.G., den Boer-Visser, A.M., and Van Der Zee, E.A. (2000). Localized neuronal activation in the zebra finch brain is related to the strength of song learning. Proc Natl Acad Sci U S A *97*, 2282-2285.

Bonke, D., Scheich, H., and Langner, G. (1979). RESPONSIVENESS OF UNITS IN THE AUDITORY NEOSTRIATUM OF THE GUINEA FOWL (NUMIDA-MELEAGRIS) TO SPECIES-SPECIFIC CALLS AND SYNTHETIC STIMULI .1. TONOTOPY AND FUNCTIONAL ZONES OF FIELD-L. Journal of Comparative Physiology *132*, 243-255.

Brenowitz, E.A., Margoliash, D., and Nordeen, K.W. (1997). An introduction to birdsong and the avian song system. J Neurobiol *33*, 495-500.

Brooks, R.J., and Falls, J.B. (1975). INDIVIDUAL RECOGNITION BY SONG IN WHITE-THROATED SPARROWS .3. SONG FEATURES USED IN INDIVIDUAL RECOGNITION. Can J Zool-Rev Can Zool *53*, 1749-1761.

Brown, M., Irvine, D.R., and Park, V.N. (2004). Perceptual learning on an auditory frequency discrimination task by cats: association with changes in primary auditory cortex. Cereb Cortex *14*, 952-965.

Chaiken, M., Bohner, J., and Marler, P. (1993). SONG ACQUISITION IN EUROPEAN STARLINGS, STURNUS-VULGARIS - A COMPARISON OF THE SONGS OF LIVE-TUTORED, TAPE-TUTORED, UNTUTORED, AND WILD-CAUGHT MALES. Anim Behav *46*, 1079-1090.

Chaiken, M., Bohner, J., and Marler, P. (1994). REPERTOIRE TURNOVER AND THE TIMING OF SONG ACQUISITION IN EUROPEAN STARLINGS. Behaviour *128*, 25-39.

Chang, E.F., Bao, S., Imaizumi, K., Schreiner, C.E., and Merzenich, M.M. (2005). Development of spectral and temporal response selectivity in the auditory cortex. Proc Natl Acad Sci U S A *102*, 16460-16465.

Chechik, G., Anderson, M.J., Bar-Yosef, O., Young, E.D., Tishby, N., and Nelken, I. (2006). Reduction of information redundancy in the ascending auditory pathway. Neuron *51*, 359-368.

Chen, Q.C., and Jen, P.H. (2000). Bicuculline application affects discharge patterns, rate-intensity functions, and frequency tuning characteristics of bat auditory cortical neurons. Hear Res *150*, 161-174.

Cheney, D.L., and Seyfarth, R.M. (1980). Vocal recognition in free-ranging vervet monkeys. Anim Behav *28*, 362-367.

Chew, S.J., Mello, C., Nottebohm, F., Jarvis, E., and Vicario, D.S. (1995). Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. Proc Natl Acad Sci U S A *92*, 3406-3410.

Chew, S.J., Vicario, D.S., and Nottebohm, F. (1996). A large-capacity memory system that recognizes the calls and songs of individual birds. Proc Natl Acad Sci U S A *93*, 1950-1955.

Clopton, B.M., and Winfield, J.A. (1973). Tonotopic organization in the inferior colliculus of the rat. Brain Res *56*, 355-358.

Condon, C.D., and Weinberger, N.M. (1991). Habituation produces frequency-specific plasticity of receptive fields in the auditory cortex. Behav Neurosci *105*, 416-430.

Cuthill, I., and Hindmarsh, A. (1985). INCREASE IN STARLING SONG ACTIVITY WITH REMOVAL OF MATE. Anim Behav *33*, 326-327.

Diamond, D.M., and Weinberger, N.M. (1984). Physiological plasticity of single neurons in auditory cortex of the cat during acquisition of the pupillary conditioned response: II. Secondary field (AII). Behav Neurosci *98*, 189-210.

Diamond, D.M., and Weinberger, N.M. (1986). Classical conditioning rapidly induces specific changes in frequency receptive fields of single neurons in secondary and ventral ectosylvian auditory cortical fields. Brain Res *372*, 357-360.

Doupe, A.J., and Kuhl, P.K. (1999). Birdsong and human speech: common themes and mechanisms. Annu Rev Neurosci *22*, 567-631.

Edeline, J.M., and Weinberger, N.M. (1993). Receptive field plasticity in the auditory cortex during frequency discrimination training: selective retuning independent of task difficulty. Behav Neurosci *107*, 82-103.

Eens, M. (1997). Understanding the complex song of the European starling: An integrated ethological approach. In Advances in the Study of Behavior, Vol 26 (San Diego, Elsevier Academic Press Inc), pp. 355-434.

Eens, M., and Pinxten, R. (1995). INTERSEXUAL CONFLICTS OVER COPULATIONS IN THE EUROPEAN STARLING - EVIDENCE FOR THE FEMALE MATE GUARDING HYPOTHESIS. Behavioral Ecology and Sociobiology *36*, 71-81.

Eens, M., Pinxten, R., and Verheyen, R.F. (1989). TEMPORAL AND SEQUENTIAL ORGANIZATION OF SONG BOUTS IN THE STARLING. Ardea *77*, 75-86.

Eens, M., Pinxten, R., and Verheyen, R.F. (1990). ON THE FUNCTION OF SINGING AND WING-WAVING IN THE EUROPEAN STARLING STURNUS-VULGARIS. Bird Stud *37*, 48-52.

Eens, M., Pinxten, R., and Verheyen, R.F. (1991). MALE SONG AS A CUE FOR MATE CHOICE IN THE EUROPEAN STARLING. Behaviour *116*, 210-238.

Eens, M., Pinxten, R., and Verheyen, R.F. (1992). SONG LEARNING IN CAPTIVE EUROPEAN STARLINGS, STURNUS-VULGARIS. Anim Behav *44*, 1131-1143.

Eens, M., Pinxten, R., and Verheyen, R.F. (1993). FUNCTION OF THE SONG AND SONG REPERTOIRE IN THE EUROPEAN STARLING (STURNUS-VULGARIS) - AN AVIARY EXPERIMENT. Behaviour *125*, 51-66.

Eens, M., Pinxten, R., and Verheyen, R.F. (1994). VARIATION IN SINGING ACTIVITY DURING THE BREEDING CYCLE OF THE EUROPEAN STARLING STURNUS-VULGARIS. Belgian J Zool *124*, 167-174.

Ehret, G. (2005). Infant rodent ultrasounds -- a gate to the understanding of sound communication. Behav Genet *35*, 19-29.

Ehret, G., Koch, M., Haack, B., and Markl, H. (1987). Sex and parental experience determine the onset of an instinctive behavior in mice. Naturwissenschaften *74*, 47.

Fagiolini, M., Fritschy, J.M., Low, K., Mohler, H., Rudolph, U., and Hensch, T.K. (2004). Specific GABAA circuits for visual cortical plasticity. Science *303*, 1681-1683.

Feare, C.J. (1984). The Starling (New York, Oxford Univ. Press).

Feldman, D.E. (2009). Synaptic mechanisms for plasticity in neocortex. Annu Rev Neurosci *32*, 33-55.

Foeller, E., Celikel, T., and Feldman, D.E. (2005). Inhibitory sharpening of receptive fields contributes to whisker map plasticity in rat somatosensory cortex. J Neurophysiol *94*, 4387-4400.

Fortune, E.S., and Margoliash, D. (1992). Cytoarchitectonic organization and morphology of cells of the field L complex in male zebra finches (Taenopygia guttata). J Comp Neurol *325*, 388-404.

Frenkel, M.Y., and Bear, M.F. (2004). How monocular deprivation shifts ocular dominance in visual cortex of young mice. Neuron *44*, 917-923.

Fritz, J., Shamma, S., Elhilali, M., and Klein, D. (2003). Rapid task-related plasticity of spectrotemporal receptive fields in primary auditory cortex. Nat Neurosci *6*, 1216-1223.

Fritz, J.B., Elhilali, M., and Shamma, S.A. (2005). Differential dynamic plasticity of A1 receptive fields during multiple spectral tasks. J Neurosci *25*, 7623-7635.

Frommolt, K.-H., Goltsman, M.E., and Macdonald, D.W. (2003). Barking foxes, Alopex lagopus: field experiments in individual recognition in a territorial mammal. Anim Behav *65*, 509-518.

Fuzessery, Z.M. (1994). Response selectivity for multiple dimensions of frequency sweeps in the pallid bat inferior colliculus. J Neurophysiol *72*, 1061-1079.

Fuzessery, Z.M., and Hall, J.C. (1996). Role of GABA in shaping frequency tuning and creating FM sweep selectivity in the inferior colliculus. J Neurophysiol *76*, 1059-1073.

Galindo-Leon, E.E., Lin, F.G., and Liu, R.C. (2009). Inhibitory plasticity in a lateral band improves cortical detection of natural vocalizations. Neuron *62*, 705-716.

Gentner, T.Q. (2008). Temporal scales of auditory objects underlying birdsong vocal recognition. J Acoust Soc Am *124*, 1350-1359.

Gentner, T.Q., and Hulse, S.H. (1998). Perceptual mechanisms for individual vocal recognition in European starlings, Sturnus vulgaris. Anim Behav *56*, 579-594.

Gentner, T.Q., and Hulse, S.H. (2000a). Female European starling preference and choice for variation in conspecific male song. Anim Behav *59*, 443-458.

Gentner, T.Q., and Hulse, S.H. (2000b). Perceptual classification based on the component structure of song in European starlings. J Acoust Soc Am *107*, 3369-3381.

Gentner, T.Q., Hulse, S.H., and Ball, G.F. (2004). Functional differences in forebrain auditory regions during learned vocal recognition in songbirds. J Comp Physiol A Neuroethol Sens Neural Behav Physiol *190*, 1001-1010.

Gentner, T.Q., and Margoliash, D. (2003). Neuronal populations and single cells representing learned auditory objects. Nature *424*, 669-674.

Gierdalski, M., Jablonska, B., Siucinska, E., Lech, M., Skibinska, A., and Kossut, M. (2001). Rapid regulation of GAD67 mRNA and protein level in cortical neurons after sensory learning. Cereb Cortex *11*, 806-815.

Gill, P., Woolley, S.M., Fremouw, T., and Theunissen, F.E. (2008). What's that sound? Auditory area CLM encodes stimulus surprise, not intensity or intensity changes. J Neurophysiol *99*, 2809-2820.

Gill, P., Zhang, J., Woolley, S.M., Fremouw, T., and Theunissen, F.E. (2006). Sound representation methods for spectro-temporal receptive field estimation. J Comput Neurosci *21*, 5-20.

Gobes, S.M., and Bolhuis, J.J. (2007). Birdsong memory: a neural dissociation between song recognition and production. Curr Biol *17*, 789-793.

Godard, R. (1991). Long-term memory of individual neighbours in a migratory songbird. Nature *350*, 228-229.

Goulden, R., Nation, P., and Read, J. (1990). How large can a receptive vocabulary be? Applied Linguistics *11*, 341-363.

Grace, J.A., Amin, N., Singh, N.C., and Theunissen, F.E. (2003). Selectivity for conspecific song in the zebra finch auditory forebrain. J Neurophysiol *89*, 472-487.

Haack, B., Markl, H., and Ehret, G. (1983). Sound Communication between parents and offspring. In The auditory psychobiology of the mouse, J.F. Willott, ed. (Springfield, Thomas), pp. 57-97.

Hackett, T.A., Stepniewska, I., and Kaas, J.H. (1998). Subdivisions of auditory cortex and ipsilateral cortical connections of the parabelt auditory cortex in macaque monkeys. J Comp Neurol *394*, 475-495.

Hensch, T.K., Fagiolini, M., Mataga, N., Stryker, M.P., Baekkeskov, S., and Kash, S.F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science *282*, 1504-1508.

Hind, J.E., Goldberg, J.M., Greenwood, D.D., and Rose, J.E. (1963). Some discharge characteristics of single neurons in the inferior colliculus of the cat. II. Timing of the discharges and observations on binaural stimulation. J Neurophysiol *26*, 321-341.

Hodos, W., and Butler, A.B. (1997). Evolution of sensory pathways in vertebrates. Brain Behav Evol *50*, 189-197.

Hofer, S.B., Mrsic-Flogel, T.D., Bonhoeffer, T., and Hubener, M. (2006). Prior experience enhances plasticity in adult visual cortex. Nat Neurosci *9*, 127-132.

Hsu, A., Woolley, S.M., Fremouw, T.E., and Theunissen, F.E. (2004). Modulation power and phase spectrum of natural sounds enhance neural encoding performed by single auditory neurons. J Neurosci *24*, 9201-9211.

Huang, Z.J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M.F., Maffei, L., and Tonegawa, S. (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell *98*, 739-755.

Insley, S.J. (2000). Long-term vocal recognition in the northern fur seal. Nature *406*, 404-405.

Jasinska, M., Siucinska, E., Cybulska-Klosowicz, A., Pyza, E., Furness, D.N., Kossut, M., and Glazewski, S. (2010). Rapid, learning-induced inhibitory synaptogenesis in murine barrel field. J Neurosci *30*, 1176-1184.

Jouventin, P., Aubin, T., and Lengagne, T. (1999). Finding a parent in a king penguin colony: the acoustic system of individual recognition. Anim Behav *57*, 1175-1183.

Kaas, J.H., Hackett, T.A., and Tramo, M.J. (1999). Auditory processing in primate cerebral cortex. Curr Opin Neurobiol *9*, 164-170.

Keller, G.B., and Hahnloser, R.H. (2009). Neural processing of auditory feedback during vocal practice in a songbird. Nature *457*, 187-190.

King, A.J., and Nelken, I. (2009). Unraveling the principles of auditory cortical processing: can we learn from the visual system? Nat Neurosci *12*, 698-701.

Klug, A., Bauer, E.E., Hanson, J.T., Hurley, L., Meitzen, J., and Pollak, G.D. (2002). Response selectivity for species-specific calls in the inferior colliculus of Mexican free-tailed bats is generated by inhibition. Journal of neurophysiology *88*, 1941-1954.

Knott, G.W., Quairiaux, C., Genoud, C., and Welker, E. (2002). Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. Neuron *34*, 265-273.

Kreiman, J., VanLancker-Sidtis, D., and Gerratt, B.R. (2005). Perception of Voice Quality. In The Handbook of Speech Perception, D.B. Pisoni, and R.E. Remez, eds. (Malden, MA, Blackwell Publishing), pp. 338-362.

Kroodsma, D.E., and Byers, B.E. (1991). The Function(s) of Bird Song. American Zoologist *31*, 318-328.

LeBeau, F.E., Malmierca, M.S., and Rees, A. (2001). Iontophoresis in vivo demonstrates a key role for GABA(A) and glycinergic inhibition in shaping frequency response areas in the inferior colliculus of guinea pig. J Neurosci *21*, 7303-7312.

Lech, M., Skibinska, A., and Kossut, M. (2001). Delayed upregulation of GABA(A) alpha1 receptor subunit mRNA in somatosensory cortex of mice following learning-dependent plasticity of cortical representations. Brain Res Mol Brain Res *96*, 82-86.

Lengagne, T., Aubin, T., Jouventin, P., and Lauga, J. (2000). Perceptual salience of individually distinctive features in the calls of adult king penguins. J Acoust Soc Am *107*, 508-516.

Leppelsack, H.J., and Vogt, M. (1976). RESPONSES OF AUDITORY NEURONS IN FOREBRAIN OF A SONGBIRD TO STIMULATION WITH SPECIES-SPECIFIC SOUNDS. Journal of Comparative Physiology *107*, 263-274.

Liguz-Lecznar, M., Waleszczyk, W.J., Zakrzewska, R., Skangiel-Kramska, J., and Kossut, M. (2009). Associative pairing involving monocular stimulation selectively mobilizes a subclass of GABAergic interneurons in the mouse visual cortex. J Comp Neurol *516*, 482-492.

Lind, H., Dabelsteen, T., and McGregor, P.K. (1996). Female great tits can identify mates by song. Anim Behav *52*, 667-671.

Liu, R.C., Linden, J.F., and Schreiner, C.E. (2006). Improved cortical entrainment to infant communication calls in mothers compared with virgin mice. Eur J Neurosci *23*, 3087-3097.

Liu, R.C., and Schreiner, C.E. (2007). Auditory cortical detection and discrimination correlates with communicative significance. PLoS Biol *5*, e173.

London, S.E., and Clayton, D.F. (2008). Functional identification of sensory mechanisms required for developmental song learning. Nat Neurosci *11*, 579-586.

Lu, T., Liang, L., and Wang, X. (2001). Temporal and rate representations of time-varying signals in the auditory cortex of awake primates. Nat Neurosci *4*, 1131-1138.

Maffei, A., Nataraj, K., Nelson, S.B., and Turrigiano, G.G. (2006). Potentiation of cortical inhibition by visual deprivation. Nature *443*, 81-84.

Marler, P. (1981). Birdsong: The acquisition of a learned motor skill. Trends in Neurosciences *4*, 88-94.

Marler, P., and Peters, S. (1987). A sensitive period for song acquisition in song sparrow, Melospiza melodia: a case of age-limited learning. Ethology *76*, 89-100.

McComb, K., Moss, C., Saylalel, S., and Baker, L. (2000). Unusually extensive networks of vocal recognition in African elephants. Anim Behav *59*, 1103-1109.

Meliza, C.D., Chi, Z., and Margoliash, D. (2010). Representations of conspecific song by starling secondary forebrain auditory neurons: toward a hierarchical framework. Journal of neurophysiology *103*, 1195-1208.

Mello, C., Nottebohm, F., and Clayton, D. (1995). Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene's response to that song in zebra finch telencephalon. J Neurosci *15*, 6919-6925.

Mello, C.V., Vicario, D.S., and Clayton, D.F. (1992). Song presentation induces gene expression in the songbird forebrain. Proc Natl Acad Sci U S A *89*, 6818-6822.

Merzenich, M.M., and Brugge, J.F. (1973). Representation of the cochlear partition of the superior temporal plane of the macaque monkey. Brain Res *50*, 275-296.

Merzenich, M.M., Kaas, J.H., and Roth, G.L. (1976). Auditory cortex in the grey squirrel: tonotopic organization and architectonic fields. J Comp Neurol *166*, 387-401.

Merzenich, M.M., and Reid, M.D. (1974). Representation of the cochlea within the inferior colliculus of the cat. Brain Res *77*, 397-415.

Miller, D.B. (1979a). The acoustic basis of mate recognition by female Zebra finches (Taeniopygia guttata). Anim Behav *27*, 376-380.

Miller, D.B. (1979b). LONG-TERM RECOGNITION OF FATHERS SONG BY FEMALE ZEBRA FINCHES. Nature *280*, 389-391.

Miller, L.M., Escabi, M.A., Read, H.L., and Schreiner, C.E. (2001). Functional convergence of response properties in the auditory thalamocortical system. Neuron *32*, 151-160.

Mountjoy, D.J., and Lemon, R.E. (1991). SONG AS AN ATTRACTANT FOR MALE AND FEMALE EUROPEAN STARLINGS, AND THE INFLUENCE OF SONG COMPLEXITY ON THEIR RESPONSE. Behavioral Ecology and Sociobiology *28*, 97-100.

Mountjoy, D.J., and Lemon, R.E. (1995). EXTENDED SONG LEARNING IN WILD EUROPEAN STARLINGS. Anim Behav *49*, 357-366.

Mountjoy, D.J., and Lemon, R.E. (1996). Female choice for complex song in the European starling: A field experiment. Behavioral Ecology and Sociobiology *38*, 65-71.

Müller, C.M., and Leppelsack, H.J. (1985). Feature extraction and tonotopic organization in the avian forebrain. Experimenatl Brain Research *59*, 587-599.

Muller, C.M., and Scheich, H. (1987). GABAergic inhibition increases the neuronal selectivity to natural sounds in the avian auditory forebrain. Brain Res *414*, 376-380.

Nagel, K.I., and Doupe, A.J. (2008). Organizing principles of spectro-temporal encoding in the avian primary auditory area field L. Neuron *58*, 938-955.

Narayan, R., Grana, G., and Sen, K. (2006). Distinct time scales in cortical discrimination of natural sounds in songbirds. J Neurophysiol *96*, 252-258.

Nelson, D.A. (1989). SONG FREQUENCY AS A CUE FOR RECOGNITION OF SPECIES AND INDIVIDUALS IN THE FIELD SPARROW (SPIZELLA-PUSILLA). J Comp Psychol *103*, 171-176.

Noirot, E. (1966). Ultra-sounds in young rodents. I. Changes with age in albino mice. Anim Behav *14*, 459-462.

O'Loghlen, A.L., and Beecher, M.D. (1997). Sexual preferences for mate song types in female song sparrows. Anim Behav *53*, 835-841.

Ohl, F.W., and Scheich, H. (1996). Differential frequency conditioning enhances spectral contrast sensitivity of units in auditory cortex (field Al) of the alert Mongolian gerbil. Eur J Neurosci *8*, 1001-1017.

Ohl, F.W., and Scheich, H. (1997). Learning-induced dynamic receptive field changes in primary auditory cortex of the unanaesthetized Mongolian gerbil. J Comp Physiol [A] *181*, 685-696.

Ohl, F.W., and Scheich, H. (2005). Learning-induced plasticity in animal and human auditory cortex. Curr Opin Neurobiol *15*, 470-477.

Phan, M.L., Pytte, C.L., and Vicario, D.S. (2006). Early auditory experience generates long-lasting memories that may subserve vocal learning in songbirds. Proc Natl Acad Sci U S A *103*, 1088-1093.

Polley, D.B., Read, H.L., Storace, D.A., and Merzenich, M.M. (2007). Multiparametric auditory receptive field organization across five cortical fields in the albino rat. J Neurophysiol *97*, 3621-3638.

Polley, D.B., Steinberg, E.E., and Merzenich, M.M. (2006). Perceptual learning directs auditory cortical map reorganization through top-down influences. J Neurosci *26*, 4970-4982.

Rauschecker, J.P. (1998). Cortical processing of complex sounds. Curr Opin Neurobiol *8*, 516-521.

Rauschecker, J.P., and Tian, B. (2000). Mechanisms and streams for processing of "what" and "where" in auditory cortex. Proc Natl Acad Sci U S A *97*, 11800-11806.

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

Recanzone, G.H., and Cohen, Y.E. (2010). Serial and parallel processing in the primate auditory cortex revisited. Behav Brain Res *206*, 1-7.

Recanzone, G.H., Schreiner, C.E., and Merzenich, M.M. (1993). Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. J Neurosci *13*, 87-103.

Remedios, R., Logothetis, N.K., and Kayser, C. (2009). An auditory region in the primate insular cortex responding preferentially to vocal communication sounds. J Neurosci *29*, 1034-1045.

Rendell, D., Rodman, P.S., and Edmond, R.E. (1996). Vocal recognition of individuals and kin in free-ranging rhesus monkeys. Anim Behav *51*, 1007-1015.

Ribeiro, S., Cecchi, G.A., Magnasco, M.O., and Mello, C.V. (1998). Toward a song code: evidence for a syllabic representation in the canary brain. Neuron *21*, 359-371.

Ryan, M.J., and Rand, A.S. (1993). Species Recognition and Sexual Selection as a Unitary Problem in Animal Communication. Evolution *47*, 647-657.

Sawtell, N.B., Frenkel, M.Y., Philpot, B.D., Nakazawa, K., Tonegawa, S., and Bear, M.F. (2003). NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. Neuron *38*, 977-985.

Sayigh, L.S., Tyack, P.L., Wells, R.S., and Scott, M.D. (1990). Signature whistles of free-ranging bottlenose dolphins Tursiops truncatus: stability and mother-offspring comparisons. Behavioral Ecology and Sociobiology *26*, 247-260.

Scheich, H. (1991). Auditory cortex: comparative aspects of maps and plasticity. Curr Opin Neurobiol *1*, 236-247.

Searcy, W.A. (1986). Sexual Selection and the evolution of song. Ann Rev Ecol Syst *17*, 507-533.

Semple, M.N., and Scott, B.H. (2003). Cortical mechanisms in hearing. Curr Opin Neurobiol *13*, 167-173.

Sen, K., Theunissen, F.E., and Doupe, A.J. (2001). Feature analysis of natural sounds in the songbird auditory forebrain. J Neurophysiol *86*, 1445-1458.

Sewell, G.D. (1968). ULTRASOUND IN RODENTS. Nature *217*, 682-&.

Sewell, G.D. (1970). ULTRASONIC COMMUNICATION IN RODENTS. Nature *227*, 410-&.

Sillito, A.M. (1975). The contribution of inhibitory mechanisms to the receptive field properties of neurones in the striate cortex of the cat. J Physiol *250*, 305-329.

Sillito, A.M. (1977). Inhibitory processes underlying the directional specificity of simple, complex and hypercomplex cells in the cat's visual cortex. J Physiol *271*, 699-720.

Snowdon, C.T., and Cleveland, J. (1980). Individual recognition of contact calls by pygmy marmosets. Anim Behav *28*, 717-727.

Stoddard, P.K. (1996). Vocal recognition of neighbors by territorial passerines. . In Ecology and Evolution of Acoustic Communication in Birds, D.E. Kroodsma, and E.H. Miller, eds. (Ithaca, Comstock/Cornell), pp. 356-374.

Stoddard, P.K., Beecher, M.D., Horning, C.L., and Campbell, S.E. (1991). Recognition of individual neighbors by song in the song sparrow, a species with song repertoires. Behavioral Ecology and Sociobiology *29*, 211-215.

Stripling, R., Volman, S.F., and Clayton, D.F. (1997). Response modulation in the zebra finch neostriatum: relationship to nuclear gene regulation. J Neurosci *17*, 3883-3893.

Symmes, D., Alexander, G.E., and Newman, J.D. (1980). Neural processing of vocalizations and artificial stimuli in the medial geniculate body of squirrel monkey. Hear Res *3*, 133-146.

Terpstra, N.J., Bolhuis, J.J., and den Boer-Visser, A.M. (2004). An analysis of the neural representation of birdsong memory. J Neurosci *24*, 4971-4977.

Theunissen, F.E., and Shaevitz, S.S. (2006). Auditory processing of vocal sounds in birds. Curr Opin Neurobiol *16*, 400-407.

Thiele, A., Distler, C., Korbmacher, H., and Hoffmann, K.P. (2004). Contribution of inhibitory mechanisms to direction selectivity and response normalization in macaque middle temporal area. Proc Natl Acad Sci U S A *101*, 9810-9815.

Thorpe, W.H. (1958). Further Studies on the Process of Song Learning in the Chaffinch. Nature *182*, 554-557.

Tian, B., and Rauschecker, J.P. (1998). Processing of frequency-modulated sounds in the cat's posterior auditory field. J Neurophysiol *79*, 2629-2642.

Tokarski, K., Urban-Ciecko, J., Kossut, M., and Hess, G. (2007). Sensory learning-induced enhancement of inhibitory synaptic transmission in the barrel cortex of the mouse. Eur J Neurosci *26*, 134-141.

Trillmich, F. (1981). Mutual Mother-Pup Recognition in Galapagos Fur Seals and Sea Lions: Cues Used and Functional Significance. Behavior *78*, 21-42.

Vates, G.E., Broome, B.M., Mello, C.V., and Nottebohm, F. (1996). Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. J Comp Neurol *366*, 613-642.

Wang, J., McFadden, S.L., Caspary, D., and Salvi, R. (2002a). Gamma-aminobutyric acid circuits shape response properties of auditory cortex neurons. Brain Res *944*, 219-231.

Wang, L., Narayan, R., Grana, G., Shamir, M., and Sen, K. (2007). Cortical discrimination of complex natural stimuli: can single neurons match behavior? J Neurosci *27*, 582-589.

Wang, X., and Kadia, S.C. (2001). Differential representation of species-specific primate vocalizations in the auditory cortices of marmoset and cat. J Neurophysiol *86*, 2616-2620.

Wang, X., Merzenich, M.M., Beitel, R., and Schreiner, C.E. (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.

Wang, Y., Fujita, I., and Murayama, Y. (2000). Neuronal mechanisms of selectivity for object features revealed by blocking inhibition in inferotemporal cortex. Nat Neurosci *3*, 807-813.

Wang, Y., Fujita, I., Tamura, H., and Murayama, Y. (2002b). Contribution of GABAergic inhibition to receptive field structures of monkey inferior temporal neurons. Cereb Cortex *12*, 62-74.

Weinberger, N.M. (2004). Specific long-term memory traces in primary auditory cortex. Nat Rev Neurosci *5*, 279-290.

Wiesel, T.N., and Hubel, D.H. (1963). Single-Cell Responses in Striate Cortex of Kittens Deprived of Vision in One Eye. J Neurophysiol *26*, 1003-1017.

Williams, H. (2004). Birdsong and singing behavior. Ann N Y Acad Sci *1016*, 1-30.

Winer, J.A., Miller, L.M., Lee, C.C., and Schreiner, C.E. (2005). Auditory thalamocortical transformation: structure and function. Trends Neurosci *28*, 255-263.

Winter, P., and Funkenstein, H.H. (1973). The effect of species-specific vocalization on the discharge of auditory cortical cells in the awake squirrel monkey. (Saimiri sciureus). Exp Brain Res *18*, 489-504.

Witherby, H.F., Jourdain, F.C.R., Ticehurst, N.F., and Tucker, B.W. (1943). The Handbook of British Birds (London, H. G. & G. Witherby Ltd.).

Witte, R.S., and Kipke, D.R. (2005). Enhanced contrast sensitivity in auditory cortex as cats learn to discriminate sound frequencies. Brain Res Cogn Brain Res *23*, 171-184.

Wollberg, Z., and Newman, J.D. (1972). Auditory cortex of squirrel monkey: response patterns of single cells to species-specific vocalizations. Science *175*, 212-214.

Woolley, S.M., and Casseday, J.H. (2004). Response properties of single neurons in the zebra finch auditory midbrain: response patterns, frequency coding, intensity coding, and spike latencies. J Neurophysiol *91*, 136-151.

Woolley, S.M., Gill, P.R., and Theunissen, F.E. (2006). Stimulus-dependent auditory tuning results in synchronous population coding of vocalizations in the songbird midbrain. J Neurosci *26*, 2499-2512.

Xie, R., Meitzen, J., and Pollak, G.D. (2005). Differing roles of inhibition in hierarchical processing of species-specific calls in auditory brainstem nuclei. Journal of neurophysiology *94*, 4019-4037.

Zheng, W., and Knudsen, E.I. (1999). Functional selection of adaptive auditory space map by GABAA-mediated inhibition. Science *284*, 962-965.

Zheng, W., and Knudsen, E.I. (2001). Gabaergic inhibition antagonizes adaptive adjustment of the owl's auditory space map during the initial phase of plasticity. J Neurosci *21*, 4356-4365.

II. Song recognition learning and stimulus-specific weakening of neural responses in the avian auditory forebrain

Abstract

Learning typically increases the strength of responses and the number of neurons that respond to training stimuli. Few studies have explored representational plasticity using natural stimuli, however, leaving unknown the changes that accompany learning under more realistic conditions. Here, we examine experience-dependent plasticity in European starlings, a songbird with rich acoustic communication signals tied to robust, natural recognition behaviors. We trained starlings to recognize conspecific songs, and recorded the extracellular spiking activity of single neurons in the caudomedial nidopallium (NCM), a secondary auditory forebrain region analogous to mammalian auditory cortex. Training induced a stimulus-specific weakening of the neural responses (lower spike rates) to the learned songs, while the population continued to respond robustly to unfamiliar songs. Additional experiments rule out stimulus-specific adaptation and general biases for novel stimuli as explanations of these effects. Instead, the results indicate that associative learning leads to single neuron responses in which both irrelevant and unfamiliar stimuli elicit more robust responses than behaviorally relevant natural stimuli. Detailed analyses of these effects at a finer temporal scale point to changes in the number of motifs eliciting excitatory responses above a neuron's spontaneous discharge rate. These results demonstrate a novel form of experience-dependent plasticity in the auditory forebrain that is tied to associative

learning and in which the overall strength of responses is inversely related to learned behavioral significance.

Introduction

The response characteristics of neurons can be modified by developmental manipulations (Hubel and Wiesel, 1965; Zhang et al., 2001, 2002), sensory deprivation (Robertson and Irvine, 1989; Wiesel and Hubel, 1963), and learning (Merzenich et al., 1984; Nudo et al., 1996). A common finding in many regions of the brain is that learning enlarges the representations of learned stimuli. In the primary auditory cortex (AI) learning typically causes a shift in the receptive fields of single neurons toward training sounds, resulting in an increased number of neurons responding strongly to the training sounds (Bakin and Weinberger, 1990; Fritz et al., 2003; Polley et al., 2006; Recanzone et al., 1993; Rutkowski and Weinberger, 2005; Weinberger, 2004). Both simple stimulus exposure and non-contingent pairing of stimulus and reward fail to induce tonotopic changes (Blake et al., 2006; Recanzone et al., 1993). Instead, the experience-dependent tonotopic expansion in AI is understood to be mediated by associative learning mechanisms (Weinberger, 1995).

The foregoing studies demonstrate that learning modifies broad-scale changes in the tonotopic organization of AI. Nonetheless, it remains unclear how experience-dependent plasticity contributes to the processing of complex natural stimuli under the demands of ecologically relevant behaviors. Natural acoustic signals typically vary along multiple spectral and temporal dimensions, and power

at single spectral bands is seldom behaviorally meaningful. In animals where auditory learning is an adaptive species-typical behavior, qualitatively different types of neural plasticity may be involved (e.g. Galindo-Leon et al., 2009b).

Songbirds provide an opportunity to examine sensory plasticity in a neural system where behavioral relevance is tied to complex natural sounds. The songs of individual songbirds, including those of European starlings (*Sturnus vulgaris*), are composed of unique spectro-temporal features with continuous energy across multiple frequencies (see examples in Figures 2.3 and 2.4). These signals are critical in several adaptive behaviors (Kroodsma and Miller, 1996), and the recognition of individual conspecific songs is common among all songbird species studied (Stoddard, 1996a).

Here, we examine experience-dependent plasticity within the context of individual vocal recognition in European starlings. We focus on the caudomedial nidopallium (NCM), an auditory forebrain region analogous to secondary auditory cortex in mammals (Farries, 2004). Neurons in NCM have complex response properties (Müller and Leppelsack, 1985; Stripling et al., 1997) that can change with experience (Chew et al., 1995; Phan et al., 2006; Stripling et al., 1997) and are involved in developmental vocal learning (Bolhuis et al., 2000; Gobes and Bolhuis, 2007; Phan et al., 2006; Terpstra et al., 2004). NCM is connected directly to Field L, the primary thalamo-recipient zone in the avian auditory forebrain, and to the caudomedial mesopallium (CMM), another secondary auditory forebrain region (Fig. 2.1A). Neurons in these regions respond more strongly to conspecific song than

to other complex stimuli (Chew et al., 1996; Stripling et al., 1997; Theunissen et al., 2004; Theunissen and Shaevitz, 2006), and CMM neurons show an increased selectivity for the behaviorally relevant components of learned songs (motifs) following recognition training (Gentner and Margoliash, 2003). NCM is likely part of a forebrain network involved in processing conspecific song (Gentner et al., 2004; Pinaud and Terleph, 2008)

The present study describes an unexpected form of experience-dependent plasticity in NCM following song recognition training. Unlike CMM (or most studies of mammalian A1), single NCM neurons, particularly in the ventral region, respond more strongly to unfamiliar songs than to learned songs. We show that this plasticity is tied to associative learning, not stimulus exposure or novelty. These song-level changes can be explained by a decrease in the number of motifs from learned songs that elicit an excitatory response from neurons in NCM.

Methods

Subjects. For this study we caught 12 adult (9 male, 3 female) wild European starlings (*Sturnus vulgaris*) in southern California. Both sexes readily learn to recognize the songs of individual conspecifics (Gentner and Hulse, 1998; Gentner et al., 2000) and CMM neurons in both sexes undergo experience-dependent plasticity in auditory responsiveness (Gentner and Margoliash, 2003). Prior to training and testing, the starlings were housed in large, mixed-sex, flight aviaries with free access to food and water. Throughout captivity and testing, light-dark cycles were

synchronized to natural photoperiods. Subjects were unfamiliar with all song stimuli used in this study at the start of training. All procedures were conducted in accordance with the University of California, San Diego IACUC guidelines, and adhere to the APS "Guiding Principles in the Care and Use of Animals".

Recognition Training. We trained nine subjects (6 males, 3 females) to recognize 2-3 conspecific songs from two individuals (4-6 songs total) using an established Go/No-go operant procedure (Gentner et al., 2006; Gentner and Margoliash, 2003). We removed each starling from the aviary and isolated it in a sound attenuation chamber (Acoustic Systems, Austin TX). Each chamber was equipped with an operant panel containing a response port and a food hopper (Figure 2.1B). Experimental contingencies controlled access to the food hopper. Water was freely available. The starlings remained in their chambers 24 hours a day during training and we provided all their food as part of the song recognition training. Each starling learned to use the operant panel through a series of successive shaping procedures. We monitored peck responses and controlled the stimulus presentation, food hopper and lights with custom software. We maintained natural photoperiods and the starlings performed trials freely from dawn to dusk. At dusk the computer turned off the house light and operant panel.

Subjects initiated a trial by pecking their beak into the response port. This triggered the presentation of a song from a speaker mounted inside the testing chamber and behind the operant panel. On each trial, the computer selected the

song randomly (with replacement) from the set of all training songs for that given subject. After the song completed, the starling had a to either (1) peck the response port again within 2 s if the song belonged to one singer (Go trials), or (2) withhold a peck to the response port if the song was from the other singer (No-go trials). While no punishment was given for attempting to respond before the song finished playing, responses made during the song were not counted. We reinforced responses to the port on Go trials by allowing the subject access to the food hopper for 2 s. Responses to the port on No-go trials initiated a short time-out (10-60 seconds) during which the house light was extinguished and food was not available. We did not reinforce correctly withholding responses on No-go trials or failing to respond on Go trials.

We created song stimuli by sampling 10-s episodes of continuous singing from large recorded libraries of starling song bouts. We chose song libraries recorded from 6 different adult male starlings that were captured in Maryland, ensuring the songs were unfamiliar to the subjects at the start of this study. For the training songs, we chose 2-3 songs from one male and 2-3 songs from another male. Song stimuli from the same male were taken from non-overlapping segments of the original source song. Some song stimuli from the same male share a few similar, but not identical motifs. For each subject, we saved 5-9 songs from different singers for later use as unfamiliar songs during electrophysiological testing. We counterbalanced the assignment of different singer's songs across subjects as

training and unfamiliar. Each song served as a training stimulus for 24.2 % of neurons and as an unfamiliar stimulus for 37.6% of neurons in our sample.

Recognition/Passive Exposure Training: To distinguish the effects of song recognition learning from song exposure, we trained three additional starlings (all male), using a modified version of the training procedure described above. We taught each starling to use the operant panel using normal shaping procedures. Throughout song recognition training we alternated 1-hour blocks of song recognition and passive song exposure. Each starling began the day with a training block in which they learned to recognize two songs from one male and two songs from another male. As above, each starling controlled the initiation of a trial and the same Go/No-go procedures were used. After an hour, a passive block began when we turned off the operant panel and dimmed the house lights. During each passive block we played four songs to the subject. We selected the passive and the training songs from different males, and always played the same set of four songs in each passive block. We yoked each passive song to a training song, such that the songs in each passive block were presented the same number of times, in the same order, and with the same inter-stimulus intervals as the corresponding training songs in the previous block. This regimen matches song exposure between blocks, but removes all operant contingencies from the passive blocks.

After a passive block completed, a new training block began when we turned on the main house light and operant panel. We continued the sequence of a training

block followed by a passive block until dusk. For each subject we reserved four songs (two each from two males) for use as unfamiliar stimuli in subsequent electrophysiological testing. We counterbalanced the assignment of songs as training, passive and unfamiliar such that each song was used once for the training, passive and unfamiliar conditions. We trained each starling until electrophysiological testing. Each starling's final trials were completed approximately twelve hours before the electrophysiological experiment began.

Electrophysiology. We recorded extracellular single neuron responses to songs in the caudomedial nidopallium (NCM). We affixed a small steel pin stereotaxically to the skull with dental acrylic. We attached the pin on the day of electrophysiological testing with the starling under 20% urethane anesthesia (7-8 ml/kg; administered in 3 IM injections over 90-120 minutes) or in the days preceding the electrophysiological testing with the starling under isoflurane anesthesia. For electrophysiological recording, we placed the subject in a cloth jacket and secured the attached pin to a stereotaxic apparatus inside a sound attenuation chamber. We lowered custom-made, high impedance, glass coated Pt-Ir microelectrodes into a small craniotomy dorsal to NCM. We used Spike2 (CED, Cambridge UK) to present song stimuli, record extracellular waveforms and sort single neuron spike waveforms offline. Recordings were considered single units only in cases where the signal-to-noise ratio was high, and the sorted waveforms were clearly separate from other spikes (See Figures 2.3 and 2.4 for examples).

In our initial experiments a starling's stimulus set consisted of the 4-6 songs used during recognition training (from two individuals) and 5-9 unfamiliar songs (from 1-4 individuals). For each starling trained in the song recognition/song exposure procedure, the stimulus set consisted of four songs used in recognition training, four songs heard passively and four unfamiliar songs. We matched the intensity of all songs to 68db peak RMS and presented them free-field. To search for auditory responsive units, we played all songs in a starling's stimulus set. We searched for neurons from dorsal to ventral, and typically made more than one penetration per starling. We presented blocks of 5 repetitions of each song to each recording site. In a block, we played songs in a randomized order with a 4 second inter-stimulus interval. Once a block was completed, we searched for a new site. We played the same songs at each recording site and collected responses to a minimum of five stimulus repetitions. Sites were confirmed as being driven by the auditory stimuli if at least one stimulus caused a mean firing rate greater than 1 standard deviation above the mean spontaneous firing rate.

In total we recorded 119 single neurons from 12 starlings – roughly 10 neurons per starling (mean = 9.9, range = 6 – 15 neurons). 92 neurons were recorded from males and 27 neurons were recorded from females. We observed no significant differences in the results when the data were split by sex (Two-way ANOVA main effect of sex $F_{1,234} = 0.00$, $p = 0.9965$; main effect of familiarity $F_{1,234} = 6.77$, $p = 0.0098$; interaction between familiarity and sex $F_{1,234} = 1.14$, $p = 0.2867$,

Figure 2.S1, Supplementary Material), and report results below for data pooled from both sexes.

Histology. Following the recording session, we injected the starling with a lethal dose of Nembutal, perfused it trans-cardially with 10% neutral-buffered formalin, extracted the brain, and post-fixed it in neutral-buffered formalin. After several days we transferred the brain to 30% sucrose PBS for cryoprotection. We sectioned the brains and stained for Nissl. We confirmed the position of each recording site in NCM by locating its position relative to small electrolytic lesions made following recording (Figure 2.2).

Data Analysis. To quantify behavioral performance we calculated d-prime (d') values over blocks of 100 trials as: d' = Z(hit rate) – Z(false alarm rate) ,
where Z is the z-score, 'hit rate' is the proportion of responses on Go trials and 'false alarm rate' is the proportion of responses on No-go trials. A d' value of 0 indicates chance recognition and d' increases with recognition performance.

We exported spike times from Spike2 into MATLAB (Mathworks, Natick MA) for all analyses. We calculated the firing rate to each song as the number of spikes elicited during the song divided by the song length. We measured the spontaneous firing rate for a given neuron by taking the mean firing rate over the two seconds prior to the onset of each song stimulus.

We used a bias measure, adapted from previous studies (Janata and Margoliash, 1999; Solis and Doupe, 1997), to examine a preference for learned or unfamiliar songs in single neurons. The bias measure uses the ratio of mean raw firing rates evoked by learned and unfamiliar song stimuli, calculated as:

$$bias = \frac{\left(\overline{R}_U - \overline{R}_L\right)}{\left(\overline{R}_U + \overline{R}_L\right)},$$

where $\overline{R}_U$ is the mean firing rate to unfamiliar songs and $\overline{R}_L$ is the mean firing rate to learned songs for a single neuron. Bias ranges from -1 for a neuron that responds only to the learned songs to +1 for a neuron that responds only to the unfamiliar songs. Bias is 0 for a neuron that responds equally to learned and unfamiliar songs. To determine whether a bias value was significantly higher or lower than chance we compared it with a distribution of simulated bias values. To simulate bias values we shuffled the firing rates on each stimulus repetition randomly among the song stimuli. We then calculated bias values as above using these shuffled rates. This was repeated 1000 times and a bias value was considered significant if it was either higher or lower than 95% of the simulated bias values. We also obtained similar results when we compared real bias values to a distribution of simulated bias values generated by drawing random firing rates from a normal distribution that matched the empirical mean and standard deviation of a given neuron's firing rates to the song stimuli.

To enable the comparison of firing rates across neurons with widely varying response rates, we converted each neuron's firing rates to z-scores as:

$$z_i = \frac{(r_i - \bar{r})}{\sigma},$$

where r_i is the firing rate to the *i*-th stimulus and $\bar{r}$ is the mean firing rate over all stimuli presented to the neuron and σ is the standard deviation of the firing rate for all stimuli presented to the neuron. For a given neuron, z_i is the z-score normalized firing rate evoked by a given stimulus.

To normalize the firing rates to motifs we calculated response strength, *RS*, as follows:

$$RS = \frac{FR_i - FR_{spon}}{\sigma_{FR}}$$

where FR_i is the mean firing rate associated with the *i*-th motif, *FR* is the set of rates for all motifs, s is the standard deviation, and FR_{spon} is the mean spontaneous firing rate, calculated over the 2 seconds prior to the onset of each stimulus presentation. RS is identical to converting responses to z-scores, except that the resulting distribution is centered on the spontaneous response rate rather than the mean response rate over all stimuli.

Unless otherwise noted, we report the mean and standard error to describe the central tendency and variability in each measure. Because our data often did not meet the assumptions of a normal distribution we used the Wilcoxon signed-rank, Wilcoxon rank-sum test, and the Friedman test to examine differences between groups. The Friedman test was used as a nonparametric equivalent of a repeated measures ANOVA. Where appropriate, we used two-way ANOVAs to examine the effects of multiple variables. All comparisons were two-tailed ($\alpha = 0.05$).

Results

Song Recognition Learning

To examine the neural mechanisms of individual vocal recognition, we taught starlings to recognize the songs of conspecifics. We trained nine adult starlings to classify two to three songs of one starling and 2-3 songs of another starling using a Go/No-go operant procedure (Fig. 2.1B). The starlings learned to respond to the songs of one singer (Go songs), and withhold responses to the songs of the other singer (No-go songs). Each starling acquired all food from the operant apparatus, making the learned songs behaviorally important. The starlings quickly learned the recognition task (Fig. 2.1C), requiring 1.0 ± 0.2 days (968 ± 94 trials, range = 600-1600) to classify the Go and No-go songs accurately (d' ≥ 1 for a 100-trial block; see methods). The starlings continued to train for several weeks, completing 674 ± 31 trials per day. We trained the starlings for numerous trials to ensure that they had extensive experience with the songs. At the time of electrophysiological testing, the starlings had trained for 63.44 ± 11.10 days (range = 23-118 days, 170-766 blocks of 100 trials) and recognition accuracy had increased to high levels (mean d' = 3.6 ± 0.4, over the 10 final 100-trial blocks of training, corresponds to 89.56 ± 2.45% Correct; see Table 2.S1 for additional information).

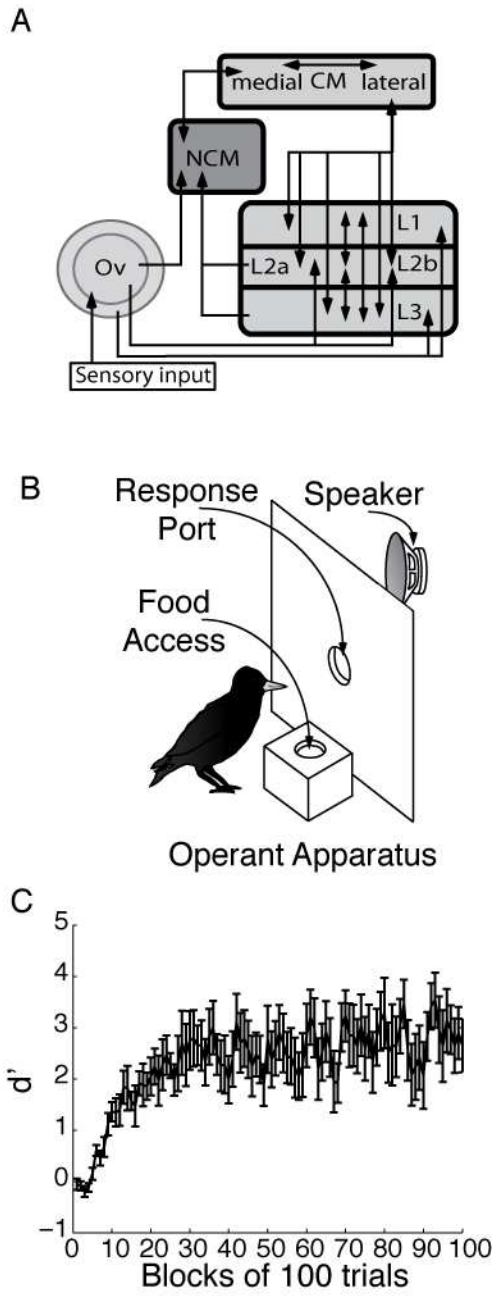


Figure 2.1. Auditory Diagram and song recognition training. (A) Schematic of the songbird auditory forebrain. Ov = Nucleus Ovoidalis, L = field L, NCM = Caudomedial Nidopallium, CM = Caudal Mesopallium. (B) Schematic of the operant panel used for song recognition learning. (C) Acquisition curve showing the mean (± sem) performance (d-prime) over first 100-blocks of song recognition training for nine starlings, 100 trials per block.

Preference for Unfamiliar Songs in NCM

Following song recognition training, we anesthetized the starlings with urethane and recorded extracellular electrophysiological activity from 93 well-isolated single neurons in NCM (Fig. 2.2). To each neuron, we presented the 4-6 songs learned during recognition training and 5-9 songs (sung by 1 to 4 conspecific males) that the subject had never heard before. We refer to these unheard songs as "unfamiliar". Many single neurons responded more strongly to the unfamiliar songs than to the learned songs. Figures 2.3 and 2.4 show two sample NCM neurons that prefer (i.e. respond with a higher mean firing rate to) unfamiliar songs. These examples illustrate the range of responses over which a preference for unfamiliar songs can be observed. Note that the neuron in Figure 2.3 responds to all song stimuli that were presented, but responds more strongly on average to the unfamiliar songs than the learned songs. The mean firing rate of this neuron to all unfamiliar songs was 15.72 ± 2.55 and to all learned songs was 12.12 ± 1.71 spikes/sec. In contrast, the neuron in Figure 2.4 responds to only a few songs and responds strongly to a single unfamiliar song. The mean firing rate of this neuron to all unfamiliar songs was 1.86 ± 1.57 and to all learned songs was 0.01 ± 0.01 spikes/sec. To quantify each neuron's response we calculated a bias measure using a normalized ratio of the mean firing rates for learned and unfamiliar songs (see methods). Bias values were calculated using the mean firing rate over whole songs, and we found no variation in bias values throughout the length of the song (Supplementary Material). Bias values could range from -1 for a neuron that

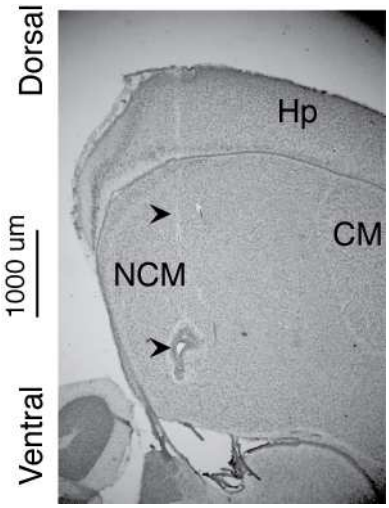


Figure 2.2. Recording location. Para-sagittal section of a starling brain showing an electrode tract and small fiducial electrolytic lesion in NCM. The arrowheads mark the electrode tract and the lesion. CM = Caudal Mesopallium, Hp = Hippocampus, NCM = Caudomedial Nidopallium.

Figure 2.3. Preference for unfamiliar songs in single neurons. (A) Spectrogram, peristimulus time histogram (PSTH) and raster plot for responses of a single neuron to five repetitions of the three learned and (B) three unfamiliar songs that elicited the strongest mean firing rates from this neuron. Spikes are binned in 20ms bins for the PSTH. (C) Sample trace showing raw voltage recorded during the first repetition of the bottom song. Zero marks the time of stimulus onset. (D) Overlay of spike waveforms with the mean (gray line). (E) Distribution of inter-spike intervals. Capped at 100 msec to show values near zero. (F) Zoomed in raster, PSTH, and spectrogram for the three motifs underlined in the bottom song in (B) The separate lines show the start and stop of the different motifs. The mean spontaneous firing rate of this neuron was 5.46 spikes/sec. This neuron was recorded at a depth of 3020 um.

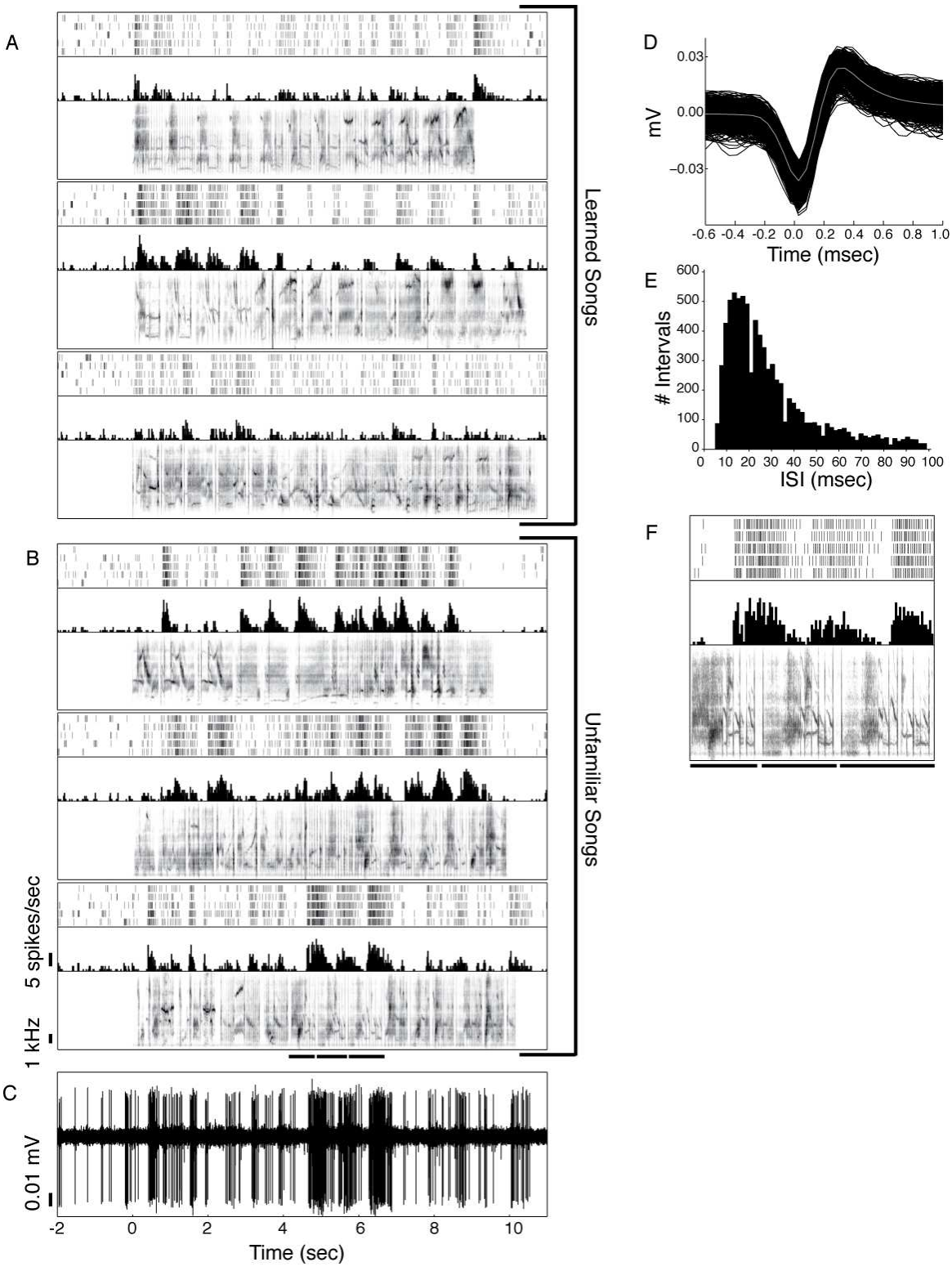
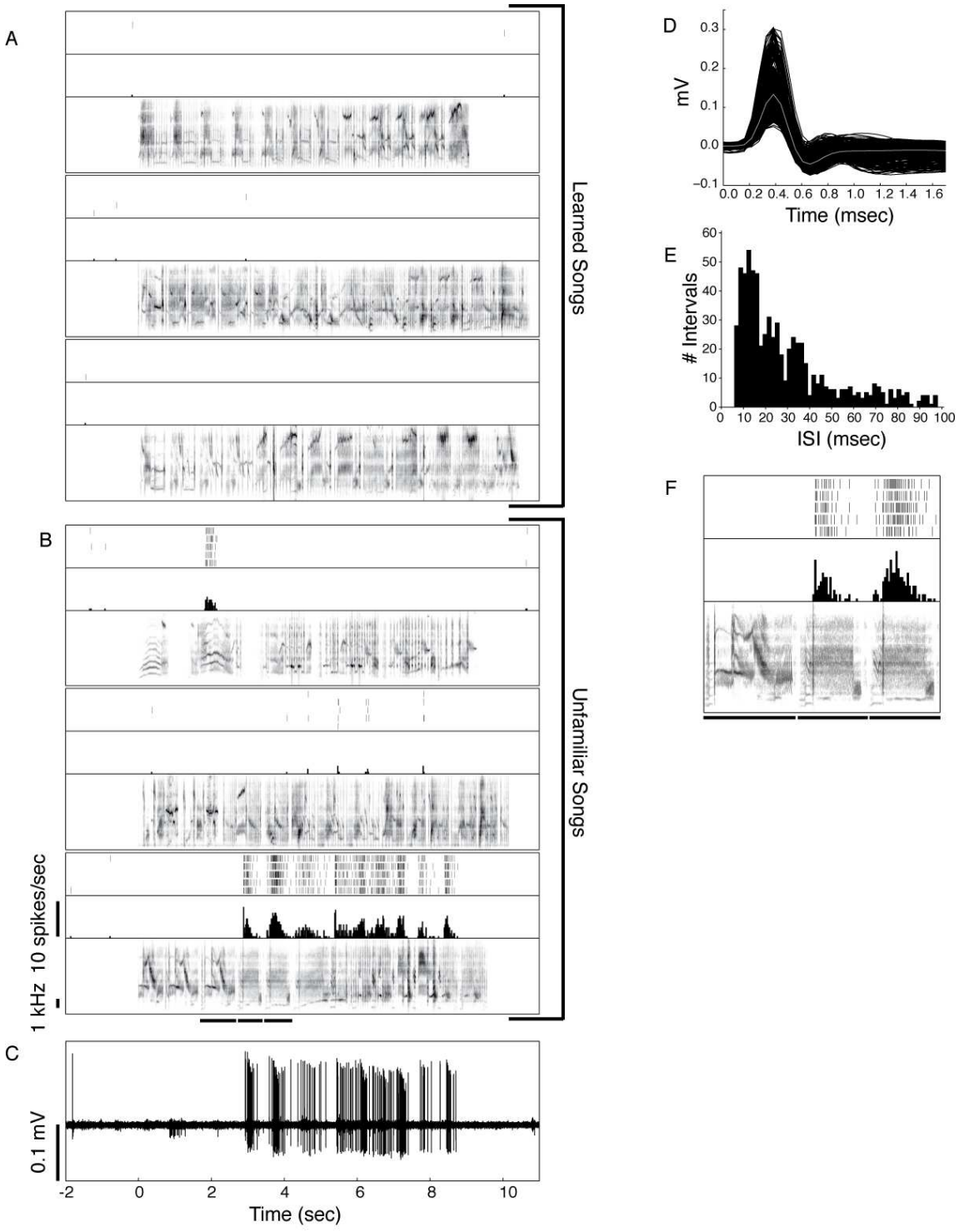

A
Learned Songs
B
Unfamiliar Songs
5 spikes/sec
1 kHz
C
0.01 mV
-2
0
2
4
6
8
10
Time (sec)
D
0.03
0.00
−0.03
mV
-0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8 1.0
Time (msec)
E
600
500
400
300
200
100
0
Intervals
0 10 20 30 40 50 60 70 80 90 100
ISI (msec)
F

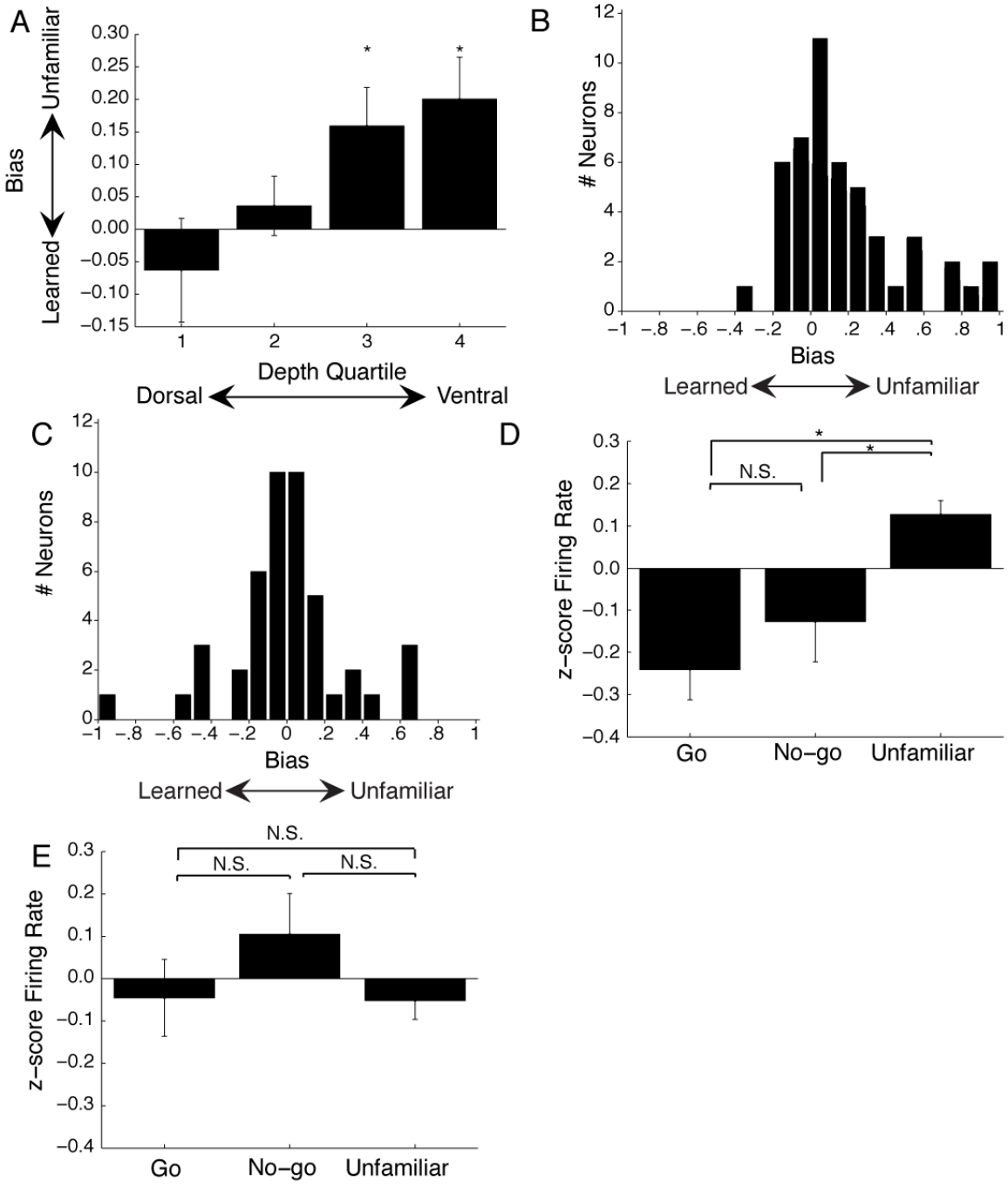
Figure 2.4. Preference for unfamiliar songs in single neurons. As in Figure 3 for a different NCM neuron. The mean spontaneous firing rate of this neuron was 0.13 spikes/sec. This neuron was recorded at a depth of 2920 um. In (D) there is some variability in spike height caused by an improvement in isolation during recording as spike height increased.


A
Learned Songs
B
Unfamiliar Songs
1 kHz
10 spikes/sec
C
0.1 mV
-2
0
2
4
6
8
10
Time (sec)
D
mV
0.3
0.2
0.1
0.0
-0.1
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6
Time (msec)
E
Intervals
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100
ISI (msec)
F

responds only to learned songs, to 1 for a neuron that responds only to unfamiliar songs. The neuron in Figure 2.3 has a bias value of 0.13 and the neuron in Figure 2.4 has a bias value of 0.99. The mean bias across all neurons (n = 93) was 0.088 ± 0.033, which corresponds to roughly a 15% increase in the firing rates elicited by unfamiliar compared to learned songs. A significant majority of single NCM neurons responded more strongly to unfamiliar than learned songs (56/93 neurons; X^2 = 3.88, $p < 0.05$; bias > 0), leading to a mean bias for the population that was significantly greater than zero (One sample Wilcoxon signed-rank test, p = 0.0144). Therefore, a larger number of NCM neurons are driven more strongly by unfamiliar songs than by songs that the starlings have learned are behaviorally relevant.

NCM is a large nucleus and based on connectivity patterns with other auditory regions (Vates et al., 1996) and immediate early gene expression patterns (Chew et al., 1995; Gentner et al., 2004; Ribeiro et al., 1998), previous studies have suggested that different sub-regions of NCM may be involved in different types of auditory processing. To determine if plasticity accompanying song recognition is concentrated in a sub-region of NCM, we evenly divided our sample of single units into quartiles along the dorsal-ventral axis of each electrode penetration (quartile 1: 1090-1870 n=21; quartile 2: 1871-2580 n=24; quartile 3: 2581-3065 n=24; quartile 4: 3066-4091 um n=24). We find significant variability in the bias values of neurons along this dorsal-ventral axis (Kruskal-Wallis, p = 0.0402; Fig. 2.5A). In the two dorsal quartiles, the mean bias values were not significantly different from zero, (quartile 1: mean = -0.063 ± 0.080; quartile 2: mean = 0.046 ± 0.046; One-sample

Figure 2.5. Preference for unfamiliar songs in NCM neurons. (A) Bar graph showing the bias values for neurons from different depth quartiles. The range of the quartiles from dorsal to ventral was 1090-1870 (n=21), 1871-2580 (n=24), 2581-3065 (n=24), and 3066-4091 um (n=24), measured from the surface of the brain. Asterisks (*) mark bias values significantly different from zero. (B) Distribution of bias values for 48 ventral NCM neurons. Bias values > 0 indicate neurons that responded higher to unfamiliar songs, whereas bias values < 0 indicate neurons that responded higher to learned songs (see methods). (C) Distribution of bias values for 45 dorsal NCM neurons. (D) Bar graph showing the z-scores of the firing rates to the Go, No-go and unfamiliar songs for 48 ventral NCM neurons. (E) Bar graph showing the z-scores of the firing rates to the Go, No-go and unfamiliar songs for 45 dorsal NCM neurons. Asterisks (*) denote $p < 0.05$. N.S. denotes $p > 0.05$. Error bars show standard error.


A
Bias
Unfamiliar
Learned
0.30
0.25
0.20
0.15
0.10
0.05
0.00
−0.05
−0.10
−0.15
*
*
1
2
3
4
Depth Quartile
Dorsal
Ventral
B
Neurons
12
10
8
6
4
2
0
−1 −.8 −.6 −.4 −.2 0 .2 .4 .6 .8 1
Bias
Learned
Unfamiliar
C
Neurons
12
10
8
6
4
2
0
−1 −.8 −.6 −.4 −.2 0 .2 .4 .6 .8 1
Bias
Learned
Unfamiliar
D
z−score Firing Rate
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
*
*
N.S.
Go
No−go
Unfamiliar
E
z−score Firing Rate
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
N.S.
N.S.
N.S.
Go
No−go
Unfamiliar

Wilcoxon signed-rank test, p = 0.4549 and p= 0.6071 respectively). In the two ventral quartiles, however, mean bias values were significantly greater than zero (quartile 3: mean = 0.159 ± 0.060; quartile 4: mean = 0.200 ± 0.065; One-sample Wilcoxon signed-rank test, p = 0.0140 and p = 0.0119 respectively). In subsequent analyses where depth is included, we collapse the neurons from the two dorsal quartiles and refer to them as "dorsal NCM" and the neurons from the two ventral quartiles and refer to them as "ventral NCM". The distribution of bias values for neurons in ventral and dorsal NCM are shown in Figures 2.5B and 2.5C respectively. The large mean bias values of neurons in the two ventral quartiles arise from an increase in the fraction of neurons that show a significant preference for unfamiliar over learned songs in this sub-region. In ventral NCM, 24/48 neurons have bias values that are significantly different than those expected by chance (methods). A significant majority of these neurons (20/24) have bias values > 0, while few (4/24) have bias values < 0 ($X^2 = 10.667$, df = 1 $p < 0.005$). In dorsal NCM, 20/45 neurons have significant bias values. In contrast to ventral NCM, roughly half of these neurons (9/20) have bias values > 0, and half (11/20) have bias values < 0 ($X^2 = 0.200$, df = 1, $p > 0.500$).

The reduced firing rates to learned songs induced by recognition training are also observed in the mean firing rates elicited by the various song stimuli. To facilitate comparisons across neurons with widely varying evoked spike rates (range: 0.12 – 31.97 spikes/sec), for each neuron, we converted the mean rates associated with each song to z-scores (methods). Among neurons located within the

two ventral quartiles (n = 48), the mean normalized firing rates evoked by the learned songs were significantly weaker than those evoked by the unfamiliar songs (learned: -0.184 ± 0.055 units of SD, unfamiliar: 0.127 ± 0.033; Wilcoxon signed-rank test, p = 0.0005). But, among neurons located within the two dorsal quartiles (n = 45), there was no difference in the mean normalized firing rates evoked by learned and unfamiliar songs (learned: 0.030 ± 0.056 in units of SD, unfamiliar: -0.052 ± 0.044; Wilcoxon signed-rank test, p = 0.5091). Pooling response data from all the NCM neurons in our sample (n = 93), the difference in normalized firing rates evoked by learned (-0.081 ± 0.040, in units of SD) and unfamiliar songs (0.040 ± 0.029) remains statistically significant (Wilcoxon signed-rank, p = 0.0391). NCM displays an overall preference to respond more strongly to unfamiliar than learned songs, which is greatly magnified in the ventral region.

We also examined whether the differences in reinforcement during training had any effect on the strength of the mean evoked response in either the dorsal or ventral portion of NCM. It did not. For this analysis, we divided the responses to learned songs into Go and No-go classes (i.e. those songs associated with food reinforcement and those associated with no reinforcement, respectively). Consistent with the results already reported, neurons in the two ventral-most quartiles showed a significant overall difference between responses to the three classes of song: Go, No-go and unfamiliar (Friedman test, p = 0.0173; Fig. 2.5D). For these neurons, the mean normalized firing rates evoked by the Go songs (-0.241 ± 0.072 units of SD) and by the No-go songs (-0.128 ± 0.095) were significantly weaker than those

evoked by the unfamiliar songs (0.127 ± 0.033; for comparison with unfamiliar songs, Wilcoxon signed-rank test, p = 0.0240 for No-go songs, p = 0.0005 for Go songs), but did not significantly differ from one another (Wilcoxon signed-rank test, p = 0.4152). Among neurons in the two dorsal-most quartiles, we observed no significant differences between the strength of the evoked responses to the different classes of song stimuli (Go: -0.045 ± 0.091 units of SD, No-go: 0.105 ± 0.096, Unfamiliar: -0.052 ± 0.044; Friedman test, p = 0.9780; Fig. 2.5E). The experience-dependent decrease of firing rates associated with learned songs is strongest in the ventral portion of NCM and is observable across both classes of learned songs (i.e. Go and No-go).

We note that the weakened responses to learned songs cannot be explained by simple differences in initial spectro-temporal tuning properties of NCM neurons. By design, the song stimuli were balanced across the subjects and neurons so that songs used during recognition training for one starling served as unfamiliar songs for others (see methods). Instead, the response profiles of individual NCM neurons are modified by each animal's behavioral interaction with the training songs. We next examine how the specific characteristics of the behavioral training modify the responses of NCM neurons to songs.

NCM responses are shaped by song learning not song exposure

Repeated presentations of the same song decrease both electrophysiological and immediate early gene (IEG) responses in NCM (Chew et al., 1995; Mello et al.,

1995). In principle, this adaptation, which is driven by song exposure, could account for the decreased responses to learned songs observed in the present study. To dissociate the effects of song recognition learning and song exposure, we trained a second group of starlings (N = 3) to recognize songs as described in the preceding sections while concurrently exposing them to a different set of songs without explicit behavioral consequences. We termed the latter songs "passive" songs (Fig. 2.6A; see methods). Even with the concurrent passive song exposure, starlings quickly learned to recognize the songs associated with operant contingencies (mean = 1133 ± 285 trials to reach a d' > 1, range = 800 – 1700 trials; Fig. 2.6B). To ensure extensive experience with all the stimuli, including the passive songs, we allowed the starlings to perform numerous trials over several weeks (range = 56-208 days, 97-1267 blocks of 100 trials). At the time of electrophysiological testing, recognition accuracy had increased to high levels (mean d' = 3.7 ± 0.7, over the 10 final 100-trial blocks of training, this corresponds to 92.93 ± 4.80% Correct).

Following the song recognition/passive exposure procedure, we anesthetized each subject with urethane and recorded extracellular activity from a total of 26 well-isolated NCM neurons in response to the behaviorally relevant learned songs, the passive songs, and an equal number of unfamiliar starling songs. If the decreased response to the learned songs was caused by simple exposure, then the responses to learned and passive songs should both be significantly weaker than those to unfamiliar songs. This was not the case. To normalize responses between neurons, we converted the firing rates of each neuron to z-scores. The passive

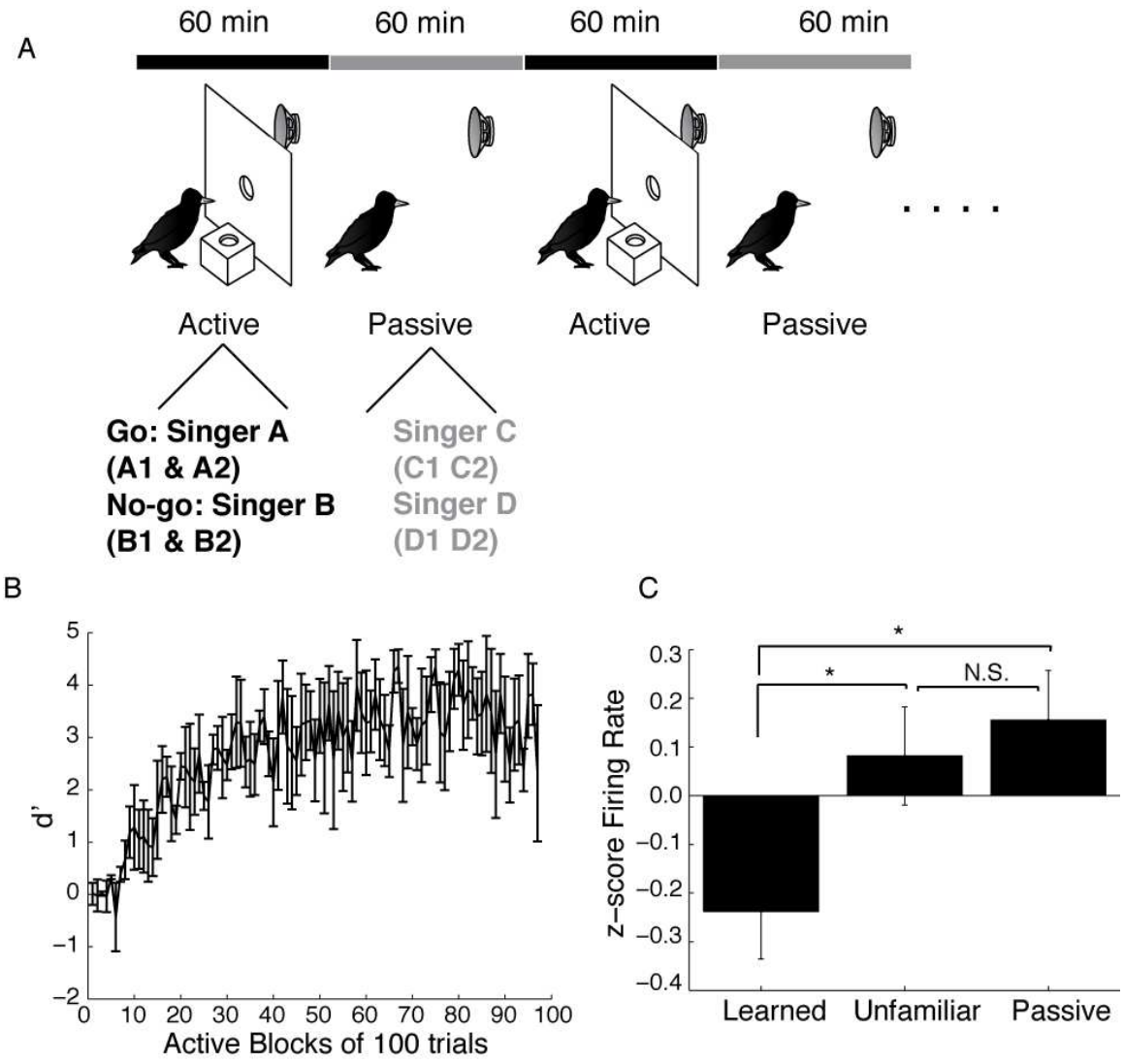


Figure 2.6. Song exposure does not cause weakened responses to learned songs in NCM. (A) Diagram showing modified recognition-training procedure where the starlings alternated between one-hour training and passive listening blocks. (B) Mean acquisition curve for the song recognition learned in the training block for three starlings. d' values were calculated over blocks of 100 trials. Data is shown for the first 97 blocks, not the entirety of each starling's training. Error bars show standard error. (C) Bar graph showing the z-scores of the firing rates to learned, unfamiliar and passively heard songs for 26 NCM neurons. Asterisks (*) denote $p < 0.05$. N.S. denotes $p > 0.05$.Error bars show standard error.

songs elicited very strong responses. The mean normalized firing rate to the learned songs was -0.238 ± 0.098 (units of SD), to the unfamiliar songs was 0.082 ± 0.101, and to the passive songs was 0.156 ± 0.101; responses varied significantly between the three song classes (Friedman test, $p = 0.0111$; Fig. 2.6C). Most importantly, the responses evoked by the learned songs were significantly weaker than those evoked by both the passive songs and the unfamiliar songs (Tukey's LSD, $p < 0.05$, both post-hoc comparisons). Although quantitatively stronger, the responses to the passive songs were not significantly different than responses to the unfamiliar songs (Tukey's LSD, $p > 0.50$). These results replicate the original learning effects between learned and unfamiliar songs, and rule out simple exposure as an explanation. Instead, NCM neurons respond robustly to both unfamiliar songs and to familiar songs made irrelevant by repeated exposure in the absence of behavioral contingencies. In contrast, these same neurons respond weakly to familiar songs with learned behavioral significance.

We also examined whether the weakened responses to learned songs in NCM could be the result of firing rate adaptation during electrophysiological recording. In NCM, adaptation occurs rapidly with the largest changes in spike rate occurring in the first few stimulus presentations (Stripling et al., 1997). Because we presented the same song stimuli at each recording site in a starling, we looked for changes in firing rates in the first neuron recorded in each starling (n=12, all subjects from all experiments). Figure 2.7 shows the changes in firing rate for each neuron over five song repetitions. Overall there was no significant change in firing rate with

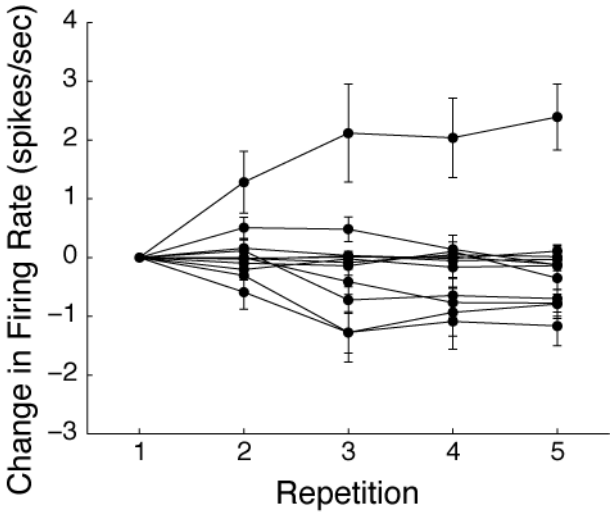


Figure 2.7. No evidence for firing rate adaptation during recording experiments. Change in firing rate across five repetitions in the first neuron recorded in each starling. For visualization, the firing rates of each neuron have the mean firing rate on the first repetition subtracted. Each point is the mean firing rate across all song stimuli.

repetition (Two-way ANOVA ,effect of repetition $F_{4,695} = 0.05$, $p = 0.9961$). Importantly, there was no interaction between repetition and familiarity, indicating that firing rates did not change significantly with repeated presentations of either learned or unfamiliar songs (Two-way ANOVA, interaction between repetition and familiarity $F_{8,695} = 0.02$, $p = 0.9976$). While the failure to find a significant effect of repetition does not rule out the occurrence of adaptation during our electrophysiological experiments, we find no evidence that it has occurred and it cannot explain the observed difference in responses to learned and unfamiliar songs. Taken together, these results and others (Supplementary Material) rule out any single mechanism of plasticity that relies only on stimulus exposure, such as adaptation or habituation. The results of the song recognition/song exposure experiment, where decreased responses are seen only for songs that are paired with reinforcement demonstrate that instead, the plasticity in NCM responses accompanying song recognition learning likely occurs through mechanisms that are tied to associative learning.

Response weakening to learned songs increases with training

While we did not make multiple electrophysiological recordings from subjects throughout training, there was a wide range in the total number of trials each starling performed. Starlings performed between 97 and 1267 blocks of 100 trials during training. This allowed us to examine whether the decrease in the magnitude of responses to learned songs varied with different amounts of training.

Neurons from starlings that performed more blocks tended to have larger bias values, indicating a greater preference for unfamiliar songs over learned songs. The mean bias value from each starling was significantly correlated with the total number of blocks the starling performed (data pooled from 12 starlings used in both experiments described above, Pearson Correlation, $r = 0.7247$ $p = 0.0077$). We note that starlings with short training lengths did not show a strengthening of responses to learned songs. Instead, the mean bias values for starlings with short training lengths were above, but near zero, indicating a weak preference for unfamiliar songs. For starlings that performed less than 300 100-trial blocks ($N = 3$), the mean bias value was 0.0836 ± 0.0765. Some studies have shown that learning leads to an initial expansion of the representation of learned stimuli followed by a contraction (Molina-Luna et al., 2008; Yotsumoto et al., 2008), however this does not appear to be occurring in NCM. While the preference to respond stronger to unfamiliar songs increased with training in NCM, there was not an initial expansion of the representation of learned songs.

Song-level training differences reflect motif-level response variability

Starling songs are composed of stereotyped acoustic units called motifs (See examples of motifs in Figures 2.3 and 2.4). The motif structure of songs plays an important role in recognition behavior and in the responses of neurons in the auditory forebrain region CMM (Gentner, 2008; Gentner and Margoliash, 2003). Variability in the neural responses to motifs was also observed in NCM. As shown in

Figures 2.3 and 2.4, some motifs elicited robust responses while others elicited very little or no response. To understand the basis of the decreased response to learned songs on a finer timescale, we divided songs into their component motifs and analyzed the responses to each motif separately. The decreased responses to learned songs could be explained by any combination of: (1) a decrease in the number of motifs that elicit excitatory responses from each neuron, (2) an increase in the number of motifs that elicit suppressive responses from each neuron, (3) a decrease in the magnitude of excitatory response to motifs or (4) an increase in the magnitude of suppressive responses to motifs. We focused on the subset of NCM neurons that respond most weakly to learned songs by limiting the following analyses to ventral NCM (n = 61 neurons, combined from both experiments above). We obtain qualitatively similar results when all neurons are included (Supplementary Material).

For each neuron, we calculated the mean firing rate to each motif from learned and unfamiliar songs. We considered a response more than 1 SD above the mean spontaneous (i.e. non-driven) firing rate as excitation and more than 1 SD below as suppression. Overall, the spontaneous rates tended to be low (mean spontaneous firing rate = 1.86 ± 0.42 spikes/sec; see Figures 2.3 and 2.4 for examples), and changing the thresholds for a significant response above or below the spontaneous firing rate yields similar results (Table 2.S2). At the 1SD threshold, all neurons (61/61) showed excitation for at least one motif, and most neurons

(48/61) showed suppression for at least one motif. Accordingly, 48/61 neurons showed both excitation and suppression for at least one motif.

We first examined whether the song-level differences in the firing rates evoked by learned and unfamiliar songs may come from differences in the fraction of learned and unfamiliar motifs that elicit an excitatory or suppressive response. For each neuron (n = 61), we calculated the percentage of motifs from learned and unfamiliar songs that elicited a response > 1 SD above the mean spontaneous firing rate. Overall, neurons gave excitatory responses to significantly fewer motifs from learned songs than from unfamiliar songs (Fig. 2.8A). A mean of 41.31 ± 3.20 percent of unfamiliar motifs and 35.48 ± 3.61 percent of learned motifs elicited an excitatory response above spontaneous firing rates (Wilcoxon signed-rank test, $p = 0.0043$). Suppressive responses were more equally distributed. There was a small difference in the percentage of motifs from unfamiliar and learned songs that evoked a decrease in response rate more that 1 SD below spontaneous firing rates, though this difference was not significant. A mean of 32.13 ± 3.61 percent of unfamiliar motifs and 34.25 ± 3.93 percent of learned motifs elicited a suppressive response from each neuron (Wilcoxon signed-rank test, $p = 0.2815$; Fig. 2.8B). Following learning, motifs from learned songs are less likely to elicit excitatory responses from ventral NCM neurons than are motifs from unfamiliar songs. Consistent with these results, in ventral NCM the bias value in individual neurons (computed for responses over whole songs) is negatively correlated with the percentage of motifs from learned songs causing excitation ($r = -0.56$, $p < 0.0001$).

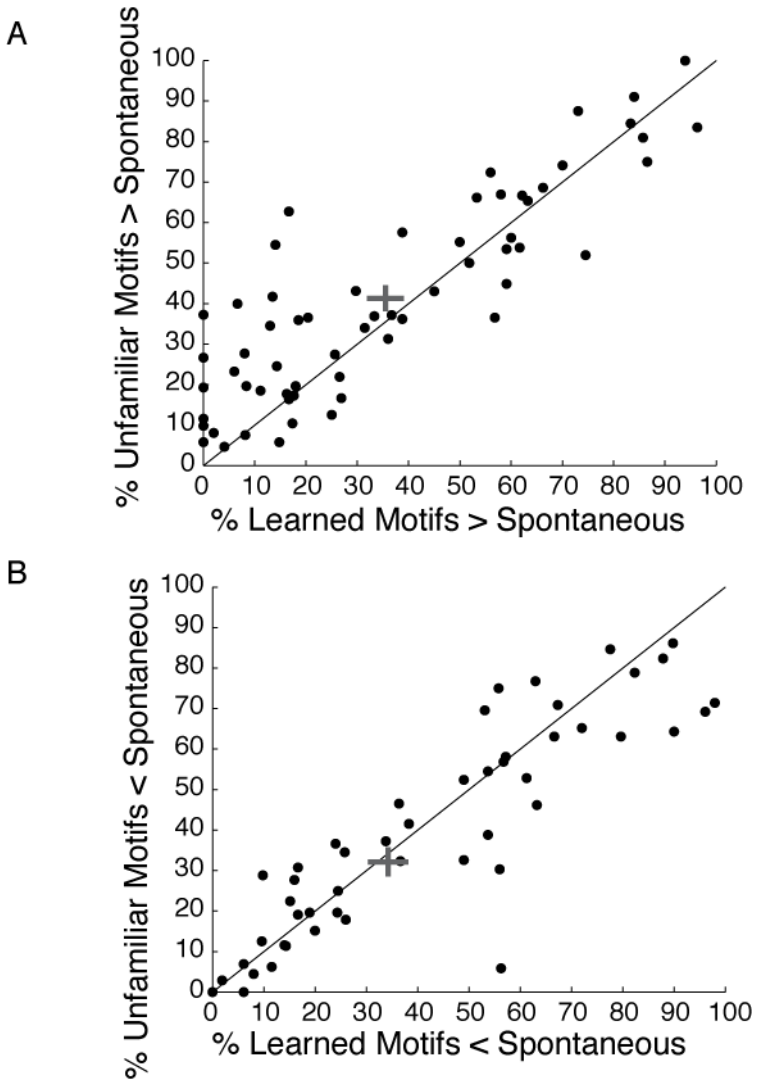


Figure 2.8. Motif-level contributions to song-level effects. (A,B) Scatter plots showing the relationship between the percentages of motifs from learned and unfamiliar songs that elicited significant (A) excitatory or (B) suppressive responses in each neuron. Significant excitatory and suppressive responses are defined as any response 1 SD above or below the spontaneous rate, respectively (see text). Each circle corresponds to one neuron. The gray cross in each plot shows the mean and standard errors.

Bias is not significantly correlated, however, with the percentage of motifs from learned songs causing a suppressive response or the percentage of unfamiliar motifs causing a suppressive or excitatory response ($r = 0.19$ $p = 0.1419$, $r = 0.02$; $p = 0.8987$, $r = -0.24$, $p = 0.0662$ respectively).

We next examined whether the decreased responses to learned songs could also be explained by a difference in the magnitude of the excitatory or suppressive firing rates to motifs from learned and unfamiliar songs. For each neuron we calculated the mean firing rate for all learned and unfamiliar motifs. To facilitate comparison between neurons with widely varying rates, we normalized the firing rates for each motif presented to a given neuron using a response strength measure, similar to a z-score, in which zero marked the spontaneous firing rate for that neuron (methods). To examine excitatory responses, we included all ventral NCM neurons in our sample that showed a strong increase in firing rate (1 SD or more above the spontaneous firing rate) for one or more motifs from both a learned and unfamiliar song ($n = 52$). We observed no difference in the strength of the excitatory responses to motifs in learned and unfamiliar songs. The mean excitatory response strength evoked by motifs in learned songs was 1.22 ± 0.08 (in units of standard deviation) and in unfamiliar songs was 1.32 ± 0.06, which are not significantly different (Wilcoxon signed-rank test, $p = 0.1451$). We examined suppressive responses in a similar way, including all ventral NCM neurons in our sample that showed a strong decrease in firing rate (1 SD or more below the spontaneous firing rate) to one or more motifs from both a learned and unfamiliar song ($n = 44$). Again,

we observed no difference in the strength of suppressive responses to motifs in the learned and unfamiliar songs. The mean suppressive response strength evoked by motifs in learned songs was -0.44 ± 0.07 and in unfamiliar songs was -0.43 ± 0.07, and these are not significantly different (Wilcoxon signed-rank test, $p = 0.3305$). Across the population of ventral NCM neurons, the magnitude of both the excitatory and suppressive responses to single motifs do not appear to be different for motifs from learned or unfamiliar songs. Instead, the observed differences in the song-level responses are mostly driven by a decrease in the proportion of motifs from learned compared to unfamiliar songs that elicit excitatory responses. We note that the decrease in responsiveness to learned motifs is relatively small given the magnitude of the overall song-level effects. This suggests that changes in the frequency and magnitude of suppression, and in the magnitude of excitatory responses, are likely also involved in some neurons, but not in a manner consistent enough to allow detection across our population of ventral NCM neurons.

Discussion

Our results provide evidence that recognition learning can weaken the sensory representation of acoustically complex, behaviorally relevant auditory stimuli. We find that training a starling to recognize sets of conspecific songs leads to a significant decrease in the responses to learned compared to unfamiliar songs in single NCM neurons, particularly those neurons in ventral NCM. This stimulus-specific response weakening cannot be explained by either stimulus-specific

adaptation or by a general bias for novel stimuli. Songs presented during recognition training, but without explicit behavioral contingencies, elicit robust responses similar to unfamiliar songs. Rather, the experience-dependent plasticity in NCM that we observe here is likely associative, coding both stimulus exposure and behavioral relevance.

While the decrease in firing rates to learned songs was observed across neurons throughout NCM, the effects were stronger in neurons recorded from more ventral parts of the region. The difference in the representations of ventral and dorsal NCM may arise from variation in the connectivity with other regions of the auditory system. While CMM projects widely throughout NCM, projections from field L (L2a and L3) are much more dense in ventral NCM (Vates et al., 1996). Converging inputs from field L, the region receiving the strongest thalamic input, could provide one possible source for the greater degree of plasticity in ventral NCM.

The experience-dependent weakening of responses to learned songs reported here diverges from canonical studies of plasticity in mammalian primary auditory cortex. Sensory learning is typically tied to expansion of tonotopic representations in AI, revealed through an increase in the number of neurons giving excitatory responses or an increase in firing rates to learned frequencies (Bakin and Weinberger, 1990; Edeline and Weinberger, 1993; Fritz et al., 2003; Gao and Suga, 2000; Polley et al., 2006; Recanzone et al., 1993; Rutkowski and Weinberger, 2005; Weinberger, 2004) or other low-level, stimulus response characteristics (Bao et al.,

2004). We examined learning-induced plasticity several synapses afferent to the primary thalamo-recipient zone (Vates et al., 1996), in neurons with complex receptive fields (Stripling et al., 1997) and response properties not captured by simple, linear, tone-evoked frequency tuning functions (Müller and Leppelsack, 1985; Ribeiro et al., 1998). We found that recognition learning leads to a significant decrease in the magnitude of responses to the learned compared to unfamiliar songs.

Decreased responses to training stimuli have been observed, albeit less commonly, following habituation (Bao et al., 2003; Condon and Weinberger, 1991) and backward conditioning tasks (Bao et al., 2003), but in both cases the training sounds were not behaviorally important. Response decreases in the context of associative behavior are also uncommon in the literature, and are accompanied by an enhancement of responses to sounds near the frequency of the training stimuli. This presumably enhances response signal-to-noise ratios by improving spectral (Ohl and Scheich, 1996; Witte and Kipke, 2005) or other contrast sensitivities (Beitel et al., 2003). It is difficult to project a similar framework onto our own data, as its unclear what an enhancement in contrast sensitivity means in the context of spectro-temporally complex signals, unless the contrast is instantiated at a higher level of representation, i.e. between complex auditory object composed of many features rather than between single tones. Under this scenario, the contrast may be between motifs or perhaps whole songs. In this sense, our results may be similar to the response suppression for learned sounds, found using simpler auditory stimuli

(Ohl and Scheich, 1996; Witte and Kipke, 2005). In any case, our results extend these earlier findings to show that learning can lead to similar response suppression in the auditory system for spectro-temporally complex natural sounds.

Other results also challenge the simple hypothesis that learning always enhances auditory responses. Recent results indicate that learning induced response increases depend on the learning strategy employed by the animal (Bieszczad and Weinberger, 2009). In mice, after mothers naturally learn the behavioral relevance of pup calls, both the timing and the strength of inhibition in AI is significantly altered in spectral bands surrounding the frequency of the pup calls (Galindo-Leon et al., 2009b), showing that a natural form of learning (and/or the use of natural stimuli) can altering inhibitory processing. Understanding the full range of response changes induced by learning requires model systems in which complex natural signals can be tied to adaptive behaviors. In the present task, we gain direct control over a natural auditory recognition behavior that uses spectro-temporally complex signals and our results add to the diversity of plastic changes observed following learning. In visual and motor cortex, learning initially leads to an expansion of the representation of training stimuli followed by a contraction once learning performance plateaus (Molina-Luna et al., 2008; Yotsumoto et al., 2008). The present results provide no evidence for an initial strengthening of responses to learned songs in NCM. However, it is possible that we did not examine NCM at early enough stages of learning.

Our results describe a novel form of experience–dependent plasticity in NCM. Previous studies measuring IEG expression and electrophysiological activity in NCM report that both response measures decrease over time as a single song is repeatedly presented (Chew et al., 1995; Mello et al., 1995; Stripling et al., 1997). These effects are often described as stimulus-specific habituation or adaptation (Dong and Clayton, 2009; Pinaud and Terleph, 2008). In contrast, the decreased response to learned songs observed in the present experiments is not caused by song exposure. We find no evidence in our experiments for response adaptation over the course of repeated stimulus exposure (Fig. 2.7). The difference between our results and those of previous studies may reflect any number of methodological differences, including the parameters for stimulus presentation. In our study, the starlings controlled stimulus presentation rates during initial exposure/training, making the interval between song presentations variable, sometimes lengthy (often several seconds), and likely coincident with shifts in attention. Studies that report response adaptation typically present songs at short, fixed intervals (usually every 2 sec). In addition the starling songs used in our study were much longer (~10 sec) than the zebra finch songs used in previous studies (~2 sec). These factors alone make comparisons between previous studies of NCM and the present results difficult. In fact, response adaptation has yet to be investigated in the NCM of starlings. It is possible that it does not occur in songbirds such as starlings with more complex and variable songs. While our results clearly demonstrate that associative learning mechanisms are critical in shaping NCM response

characteristics over the course of song recognition, they do not rule out a role for non-associative mechanisms in shaping NCM responses. Indeed, given that NCM is also important for storing the memory of a songbird's tutor song (Bolhuis et al., 2000; Gobes and Bolhuis, 2007; Phan et al., 2006), it may be that multiple forms or mechanisms of plasticity are at work in this region. Future studies are required to understand how different types of NCM plasticity might be involved in song perception and vocal learning.

The responses in ventral NCM may be seen as more "selective" for learned than unfamiliar motifs, in that fewer of the motifs in learned songs evoke strong responses. The responses in NCM are qualitatively different from the "selective" responses observed in the reciprocally connected area CMM under similar song recognition learning and testing conditions. As in ventral NCM, neurons in CMM respond to small sets of motifs within the training songs. But unlike NCM, motifs in unfamiliar songs evoke very weak responses from neurons in CMM, leading to a strong preference for learned compared to unfamiliar songs (Gentner and Margoliash, 2003). Additionally, NCM and CMM may differ in how plasticity generalizes across different classes of behaviorally relevant songs. In CMM, Go songs, which were paired with food reward, elicited even stronger responses than No-go songs, those never paired with reward. In NCM, there was no significant difference in the responses elicited by Go or No-go songs, however there was a trend for Go songs to elicit weaker responses than No-go songs. While CMM appears to be more sensitive to variation in reinforcement, the difference in how CMM and NCM

generalize response changes across Go and No-go songs may be quantitative rather than qualitative. The mechanisms that underlie the markedly different responses to unfamiliar songs in these adjacent regions are not clear. The response profile in CMM may be seen as the result of a classic, feed-forward sensory hierarchy that selects for increasingly complex features. Using a similar model to understand NCM is more problematic, however, as its unclear how NCM neurons could be driven by a small set of acoustic features in familiar motifs and a much larger set of features heard in unfamiliar motifs. Instead, we hypothesize that the apparent selectivity in the evoked response of NCM neurons to learned motifs arises from selective suppression of specific motifs in the learned songs. CMM is a potential source of such selective suppression. It is not yet known how the responses of neurons in NCM and CMM relate in real-time during song recognition. A large proportion of the neurons in both NCM and CMM are inhibitory (Pinaud and Mello, 2007; Pinaud et al., 2004), and a large number of these inhibitory neurons show IEG activation that is directly tied to song experience (Pinaud et al., 2004). Earlier work suggests that NCM and CMM, or subsets of IEG positive neurons within these regions, are differentially activated during the acquisition and recall stages of song recognition (Gentner et al., 2004). Future work is required to understand the role of the bidirectional pathway between NCM and CMM, and its relationship to behavior.

Our results tie the response characteristics of NCM neurons directly to associative learning, but the function of the weakened responses to learned songs is not clear. The selective representation of learned songs in NCM shares several

similarities with observations in the primate ventral visual stream following object recognition learning (e.g. Gross et al., 1972; Logothetis, 1998; Tanaka, 1996). There, behavioral improvement in visual object recognition is reflected in the activity of inferior temporal and lateral prefrontal cortical neurons by an increase in stimulus selectivity among familiar images (both passively presented and trained) compared to novel images, and an overall decrease in firing rates for familiar compared to novel images (Freedman et al., 2006; c.f. Kobatake et al., 1998; Rainer and Miller, 2000). The selective representation of familiar visual objects in these areas is thought to provide a concentrated and sparse representation of behaviorally important objects that is resistant to noise (Freedman et al., 2006; Rainer and Miller, 2000). Similar advantages may be obtained in the songbird auditory system through associative learning. It may be that because ventral NCM neurons are driven by fewer motifs in learned songs, spike rate variability over of the course of a learned song becomes more informative than over a similar run of unfamiliar song. It remains to be seen whether the motifs from learned songs that continue to drive NCM neurons after recognition learning are also the most behaviorally relevant for song recognition. In any case, similarities in the neural mechanisms that underlie the recognition of natural visual objects and complex acoustic signals may represent coding strategies for natural stimuli that are heavily conserved, or efficient enough to evolve multiple times.

The representation of songs we observed in NCM could perform additional functions. The increased firing rates for unfamiliar song stimuli in NCM may provide

a mechanism for novel (and familiar but behaviorally irrelevant) information to integrate into the auditory system should behavioral relevance change. In addition, the kind of responses observed in NCM could also act as a sensory prediction error to the recognition system, providing a signal when the acoustics of input signals diverge from representations that have acquired strong behavioral relevance.

Supplementary Material

Weakened response is consistent throughout learned songs

The songs we taught the starlings to recognize were relatively long stimuli (~10 sec) and based on the strategy employed by the starlings, different portions of the song could be more relevant to behavior. To investigate differences in plasticity throughout responses to song, we examined whether bias values varied across the length of the songs. We divided the response to each song in to one-second bins and then calculated bias values using the mean firing rate to learned and unfamiliar songs across each bin. Since each song varied in total length, bias values were not calculated for bins beyond the shortest length (9 sec). While there was a trend for bias values from bins at the final parts of responses to be higher, there was no significant difference overall (Friedman test, $p = 0.051$; mean ± sem: 0-1 sec: 0.10 ± 0.05, 1-2 sec: 0.09 ± 0.05, 2-3 sec: 0.13 ± 0.05, 3-4 sec: 0.10 ± 0.06, 4-5 sec: 0.10 ± 0.05, 5-6 sec: 0.08 ± 0.05, 6-7 sec: 0.13 ± 0.05, 7-8 sec: 0.20 ± 0.05, 8-9 sec: 0.25 ± 0.05) To examine this in more detail, we also compared the bias values calculated over the first and last portions of the song. This allowed us to compare the bias for

the beginning and end of the responses without truncating a portion at the end. Because the songs varied in total length, the absolute time over which the last portion of the response is taken varies for different songs. This prevents any comparisons to the bias values for the end of the songs in the analysis above. To vary the amount of song considered, we calculated bias values across the first and last one, two and four seconds. Again, we found no difference in bias values calculated over the beginning or end of the song. For the first and last one second of song the mean bias values were 0.10 ± 0.05 and 0.07 ± 0.05 respectively (Wilcoxon signed-rank test, $p = 0.8805$). For the first and last two seconds of song the mean bias values were 0.08 ± 0.05 and 0.13 ± 0.05 respectively (Wilcoxon signed-rank test, $p = 0.3460$). For the first and last four seconds of song the mean bias values were 0.09 ± 0.04 and 0.13 ± 0.05 respectively (Wilcoxon signed-rank test, $p = 0.3460$). We find no evidence that the weakened response to learned compared to unfamiliar songs varies throughout the duration of songs. Instead, the weakened response to learned songs compared to unfamiliar songs is consistent across the entire responses to song.

Changes in firing rates during experiment

We also looked for evidence of adaptation by examining changes in the mean firing rates over the course of an electrophysiological experiment. We grouped the first neuron recorded from each starling (n = 12) and the last neuron recorded from each starling (n = 12) and compared the mean firing rates. We found no significant

differences in the mean firing rates. The mean firing rate in the first neurons was 3.424 ± 0.847 (spikes/sec) and in the last neurons was 2.225 ± 0.617 (Wilcoxon signed-rank test p = 0.2661). Finding a significant difference in the firing rates between the first and last recorded neurons would not be proof that firing rate adaptation has occurred, because several factors could lead to a difference in firing rates. Nevertheless, the lack of a significant difference is consistent with evidence suggesting that adaptation has not occurred.

Motif-level analysis for all NCM neurons

We obtained similar results when we investigated response variability at the motif level for dorsal and ventral NCM neurons combined (n = 119). Excitatory responses were elicited from more motifs from unfamiliar songs than from learned songs. A mean of 40.94 ± 2.25 percent of unfamiliar motifs and 36.12 ± 2.40 percent of learned motifs elicited an excitatory response above spontaneous firing rates, which is significantly different (Wilcoxon signed-rank test, p = 0.0010). As in ventral NCM, suppressive responses were elicited from slightly more motifs from learned than unfamiliar songs, though when all neurons are considered together this difference reaches significance. A mean of 30.06 ± 2.43 percent of unfamiliar motifs and 33.30 ± 2.65 percent of learned motifs elicited a suppressive response from each neuron (Wilcoxon signed-rank test, p = 0.0038). When all NCM neurons are considered together, there is no difference in the strength of excitatory or suppressive responses elicited by motifs. Here, as in the main text we use a

normalized measure of firing rate to individual motifs (see methods). The mean excitatory response strength evoked by motifs in learned songs was 1.27 ± 0.06 (in units of standard deviation) and in unfamiliar songs was 1.30 ± 0.04, which are not significantly different (Wilcoxon signed-rank test, $p = 0.1171$). The mean suppressive response strength evoked by motifs in learned songs was -0.42 ± 0.06 (in units of standard deviation) and in unfamiliar songs was -0.42 ± 0.06, which are also not significantly different (Wilcoxon signed-rank test, $p = 0.9560$). Similar to ventral NCM when considered in isolation, the weakened response to learned songs across NCM as a whole is driven most strongly by differences in the fraction of motifs eliciting excitatory responses.

Consistent motif-level effects for different response thresholds

Table 2.S2 gives the statistics for the motif-level effects in ventral NCM when the threshold for measuring significant responses above and below the spontaneous firing rate is varied. In the top part of the table the mean excitatory and suppressive firing rates in response to motifs from learned and unfamiliar songs are given. Only neurons that gave a significant response to motifs from *both* learned and unfamiliar songs are included in the analysis, causing the total number of neurons to decrease as the threshold for significant suppression or excitation becomes stricter and is moved further from the spontaneous firing rate. As can be seen in Table 2.S2, the results are similar overall when the thresholds for responses above and below spontaneous activity are varied.

Plasticity is consistent for male and female starlings

To investigate the effects of sex on the plasticity in NCM, we examined neurons recorded from male and female starlings separately. We compared the mean normalized firing rate in response to learned and unfamiliar songs for neurons from males and females. We found no significant interaction between familiarity and sex indicating that neurons recorded from both males and females showed a similar weakened response to learned songs compared to unfamiliar songs (Figure 2.S1, Two-way ANOVA, effect of Familiarity $F_{1,234} = 6.77$, $p = 0.0098$; effect of sex $F_{1,234} = 0$, $p = 0.9965$; interaction between familiarity and sex $F_{1,234} = 1.14$, $p = 0.2867$). In addition we calculated bias values separately for neurons from males and females. Again we found no significant difference (Figure 2.S2, Wilcoxon Rank-sum comparing bias values, $p = 0.3396$). While it is true that the effects appear stronger in the males than females, it is less clear that this difference is qualitative. Because qualitatively similar effects are observed in both males and females we think it is most appropriate to include all the data together in subsequent analyses.

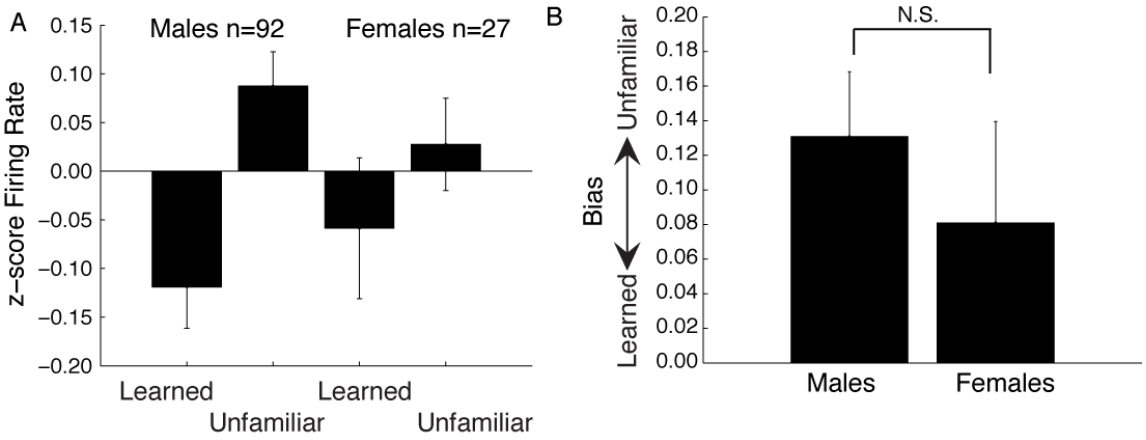


Figure 2.S1. Comparison of NCM plasticity for Male and Female Starlings. (A) Bar graph showing the z-scores of the firing rates to learned and unfamiliar songs for 92 NCM neurons recorded from male starlings and 27 neurons recorded from female starlings. (B) Bar graph showing the bias values separately for neurons recorded from males and females. N.S. denotes $p > 0.05$. Error bars show the standard error.

Table 2.S1. Behavioral performance for last 1000 trials.
The top nine starlings were in the initial song recognition experiment. The bottom three starlings were in the second song recognition/passive exposure experiment.

Starling ID	Hit Rate	False Alarm Rate	d'
274	0.98	0.12	3.62
275	0.97	0.02	4.56
283	0.89	0.08	3.32
371	0.78	0.16	2.04
386	0.99	0.14	4.05
387	0.80	0.02	4.32
404	0.98	0.17	3.85
405	0.80	0.28	1.67
408	0.87	0.02	4.67
310	0.79	0.12	2.27
318	0.99	0.18	4.70
322	0.98	0.05	4.13

Table 2.S2. Different threshold for spontaneous activity in ventral NCM motif analysis. SDs is the threshold in standard deviations of the spontaneous firing rate. Values are mean±sem. P value is result of a Wilcoxon signed-rank test. # Neurons is the number of neurons included in the analysis.

		SDs	Learned	Unfamiliar	p Value	# Neurons
Mean motif firing rate	Excitatory	0.5	1.09±0.07	1.23±0.05	0.059	54
		1.0	1.22±0.08	1.32±0.06	0.145	52
		1.5	1.29±0.08	1.40±0.06	0.202	52
		2.0	1.38±0.08	1.48±0.06	0.298	51
		2.5	1.46±0.08	1.57±0.06	0.196	51
		3.0	1.58±0.10	1.66±0.07	0.426	51
	Suppressive	0.5	-0.35±0.06	-0.35±0.06	0.439	54
		1.0	-0.44±0.07	-0.43±0.07	0.331	44
		1.5	-0.56±0.09	-0.54±0.09	0.090	30
		2.0	-0.60±0.11	-0.58±0.11	0.046	25
		2.5	-0.72±0.14	-0.70±0.14	0.027	20
		3.0	-0.84±0.18	-0.82±0.17	0.135	16
% Motifs with response	Excitatory	0.5	38.33±3.64	44.05±3.27	0.005	61
		1.0	35.48±3.61	41.31±3.20	0.004	61
		1.5	32.96±3.48	38.33±3.12	0.003	61
		2.0	30.61±3.40	35.80±3.06	0.002	61
		2.5	29.05±3.36	33.24±2.99	0.009	61
		3.0	27.37±3.30	31.20±2.95	0.013	61
	Suppressive	0.5	50.63±3.90	46.06±3.58	0.0349	61
		1.0	34.25±3.93	32.13±3.61	0.2815	61
		1.5	18.06±3.29	17.72±3.18	0.8710	61
		2.0	15.17±3.12	14.48±2.96	0.5614	61
		2.5	9.70±2.39	9.18±2.31	0.8192	61
		3.0	6.44±1.99	6.49±1.99	0.7439	61

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References

Bakin, J.S., and Weinberger, N.M. (1990). Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. Brain Res *536*, 271-286.

Bao, S., Chan, V.T., Zhang, L.I., and Merzenich, M.M. (2003). Suppression of cortical representation through backward conditioning. Proc Natl Acad Sci U S A *100*, 1405-1408.

Bao, S., Chang, E.F., Woods, J., and Merzenich, M.M. (2004). Temporal plasticity in the primary auditory cortex induced by operant perceptual learning. Nat Neurosci *7*, 974-981.

Beitel, R.E., Schreiner, C.E., Cheung, S.W., Wang, X., and Merzenich, M.M. (2003). Reward-dependent plasticity in the primary auditory cortex of adult monkeys trained to discriminate temporally modulated signals. Proc Natl Acad Sci U S A *100*, 11070-11075.

Bieszczad, K.M., and Weinberger, N.M. (2009). Learning strategy trumps motivational level in determining learning-induced auditory cortical plasticity. Neurobiol Learn Mem.

Blake, D.T., Heiser, M.A., Caywood, M., and Merzenich, M.M. (2006). Experience-dependent adult cortical plasticity requires cognitive association between sensation and reward. Neuron *52*, 371-381.

Bolhuis, J.J., Zijlstra, G.G., den Boer-Visser, A.M., and Van Der Zee, E.A. (2000). Localized neuronal activation in the zebra finch brain is related to the strength of song learning. Proc Natl Acad Sci U S A *97*, 2282-2285.

Chew, S.J., Mello, C., Nottebohm, F., Jarvis, E., and Vicario, D.S. (1995). Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. Proc Natl Acad Sci U S A *92*, 3406-3410.

Chew, S.J., Vicario, D.S., and Nottebohm, F. (1996). A large-capacity memory system that recognizes the calls and songs of individual birds. Proc Natl Acad Sci U S A *93*, 1950-1955.

Condon, C.D., and Weinberger, N.M. (1991). Habituation produces frequency-specific plasticity of receptive fields in the auditory cortex. Behav Neurosci *105*, 416-430.

Dong, S., and Clayton, D.F. (2009). Habituation in songbirds. Neurobiol Learn Mem *92*, 183-188.

Edeline, J.M., and Weinberger, N.M. (1993). Receptive field plasticity in the auditory cortex during frequency discrimination training: selective retuning independent of task difficulty. Behav Neurosci *107*, 82-103.

Farries, M.A. (2004). The avian song system in comparative perspective. Ann N Y Acad Sci *1016*, 61-76.

Freedman, D.J., Riesenhuber, M., Poggio, T., and Miller, E.K. (2006). Experience-dependent sharpening of visual shape selectivity in inferior temporal cortex. Cereb Cortex *16*, 1631-1644.

Fritz, J., Shamma, S., Elhilali, M., and Klein, D. (2003). Rapid task-related plasticity of spectrotemporal receptive fields in primary auditory cortex. Nat Neurosci *6*, 1216-1223.

Galindo-Leon, E.E., Lin, F.G., and Liu, R.C. (2009). Inhibitory Plasticity in a Lateral Band Improves Cortical Detection of Natural Vocalizations. Neuron *62*, 705-716.

Gao, E., and Suga, N. (2000). Experience-dependent plasticity in the auditory cortex and the inferior colliculus of bats: role of the corticofugal system. Proc Natl Acad Sci U S A *97*, 8081-8086.

Gentner, T.Q. (2008). Temporal scales of auditory objects underlying birdsong vocal recognition. J Acoust Soc Am *124*, 1350-1359.

Gentner, T.Q., Fenn, K.M., Margoliash, D., and Nusbaum, H.C. (2006). Recursive syntactic pattern learning by songbirds. Nature *440*, 1204-1207.

Gentner, T.Q., and Hulse, S.H. (1998). Perceptual mechanisms for individual vocal recognition in European starlings, Sturnus vulgaris. Anim Behav *56*, 579-594.

Gentner, T.Q., Hulse, S.H., and Ball, G.F. (2004). Functional differences in forebrain auditory regions during learned vocal recognition in songbirds. J Comp Physiol A Neuroethol Sens Neural Behav Physiol *190*, 1001-1010.

Gentner, T.Q., Hulse, S.H., Bentley, G.E., and Ball, G.F. (2000). Individual vocal recognition and the effect of partial lesions to HVc on discrimination, learning, and categorization of conspecific song in adult songbirds. J Neurobiol *42*, 117-133.

Gentner, T.Q., and Margoliash, D. (2003). Neuronal populations and single cells representing learned auditory objects. Nature *424*, 669-674.

Gobes, S.M., and Bolhuis, J.J. (2007). Birdsong memory: a neural dissociation between song recognition and production. Curr Biol *17*, 789-793.

Gross, C.G., Rocha-Miranda, C.E., and Bender, D.B. (1972). Visual propoerties of neurons in the inferotemporal cortex of the macaque. Journal of Neurophysiology *35*, 96-111.

Hubel, D.H., and Wiesel, T.N. (1965). Binocular interaction in striate cortex of kittens reared with artificial squint. J Neurophysiol *28*, 1041-1059.

Janata, P., and Margoliash, D. (1999). Gradual emergence of song selectivity in sensorimotor structures of the male zebra finch song system. J Neurosci *19*, 5108-5118.

Kobatake, E., Wang, G., and Tanaka, K. (1998). Effects of shape-discrimination training on the selectivity of inferotemporal cells in adult monkeys. J Neurophysiol *80*, 324-330.

Kroodsma, D.E., and Miller, E.H. (1996). Ecology and evolution of acoustic communication in birds (Ithaca, N.Y., Comstock Publishing Associates a division of Cornell University Press).

Logothetis, N. (1998). Object vision and visual awareness. Curr Opin Neurobiol *8*, 536-544.

Mello, C., Nottebohm, F., and Clayton, D. (1995). Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene's response to that song in zebra finch telencephalon. J Neurosci *15*, 6919-6925.

Merzenich, M.M., Nelson, R.J., Stryker, M.P., Cynader, M.S., Schoppmann, A., and Zook, J.M. (1984). Somatosensory cortical map changes following digit amputation in adult monkeys. J Comp Neurol *224*, 591-605.

Molina-Luna, K., Hertler, B., Buitrago, M.M., and Luft, A.R. (2008). Motor learning transiently changes cortical somatotopy. Neuroimage *40*, 1748-1754.

Müller, C.M., and Leppelsack, H.J. (1985). Feature extraction and tonotopic organization in the avian forebrain. Experimenatl Brain Research *59*, 587-599.

Nudo, R.J., Milliken, G.W., Jenkins, W.M., and Merzenich, M.M. (1996). Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. J Neurosci *16*, 785-807.

Ohl, F.W., and Scheich, H. (1996). Differential frequency conditioning enhances spectral contrast sensitivity of units in auditory cortex (field Al) of the alert Mongolian gerbil. Eur J Neurosci *8*, 1001-1017.

Phan, M.L., Pytte, C.L., and Vicario, D.S. (2006). Early auditory experience generates long-lasting memories that may subserve vocal learning in songbirds. Proc Natl Acad Sci U S A *103*, 1088-1093.

Pinaud, R., and Mello, C.V. (2007). GABA immunoreactivity in auditory and song control brain areas of zebra finches. J Chem Neuroanat *34*, 1-21.

Pinaud, R., and Terleph, T.A. (2008). A songbird forebrain area potentially involved in auditory discrimination and memory formation. J Biosci *33*, 145-155.

Pinaud, R., Velho, T.A., Jeong, J.K., Tremere, L.A., Leao, R.M., von Gersdorff, H., and Mello, C.V. (2004). GABAergic neurons participate in the brain's response to birdsong auditory stimulation. Eur J Neurosci *20*, 1318-1330.

Polley, D.B., Steinberg, E.E., and Merzenich, M.M. (2006). Perceptual learning directs auditory cortical map reorganization through top-down influences. J Neurosci *26*, 4970-4982.

Rainer, G., and Miller, E.K. (2000). Effects of visual experience on the representation of objects in the prefrontal cortex. Neuron *27*, 179-189.

Recanzone, G.H., Schreiner, C.E., and Merzenich, M.M. (1993). Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. J Neurosci *13*, 87-103.

Ribeiro, S., Cecchi, G.A., Magnasco, M.O., and Mello, C.V. (1998). Toward a song code: evidence for a syllabic representation in the canary brain. Neuron *21*, 359-371.

Robertson, D., and Irvine, D.R. (1989). Plasticity of frequency organization in auditory cortex of guinea pigs with partial unilateral deafness. J Comp Neurol *282*, 456-471.

Rutkowski, R.G., and Weinberger, N.M. (2005). Encoding of learned importance of sound by magnitude of representational area in primary auditory cortex. Proc Natl Acad Sci U S A *102*, 13664-13669.

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

Stoddard, P.K. (1996). Vocal recognition of neighbors by territorial passerines. In Ecology and evolution of acoustic communication in birds, D.E. Kroodsma, and E.H. Miller, eds. (Ithaca, Comstock/Cornell), pp. 356-374.

Stripling, R., Volman, S.F., and Clayton, D.F. (1997). Response modulation in the zebra finch neostriatum: relationship to nuclear gene regulation. J Neurosci *17*, 3883-3893.

Tanaka, K. (1996). Inferotemporal cortex and object vision. Annu Rev Neurosci *19*, 109-139.

Terpstra, N.J., Bolhuis, J.J., and den Boer-Visser, A.M. (2004). An analysis of the neural representation of birdsong memory. J Neurosci *24*, 4971-4977.

Theunissen, F.E., Amin, N., Shaevitz, S.S., Woolley, S.M., Fremouw, T., and Hauber, M.E. (2004). Song selectivity in the song system and in the auditory forebrain. Ann N Y Acad Sci *1016*, 222-245.

Theunissen, F.E., and Shaevitz, S.S. (2006). Auditory processing of vocal sounds in birds. Curr Opin Neurobiol *16*, 400-407.

Vates, G.E., Broome, B.M., Mello, C.V., and Nottebohm, F. (1996). Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. J Comp Neurol *366*, 613-642.

Weinberger, N.M. (1995). Dynamic regulation of receptive fields and maps in the adult sensory cortex. Annu Rev Neurosci *18*, 129-158.

Weinberger, N.M. (2004). Specific long-term memory traces in primary auditory cortex. Nat Rev Neurosci *5*, 279-290.

Wiesel, T.N., and Hubel, D.H. (1963). Single-Cell Responses in Striate Cortex of Kittens Deprived of Vision in One Eye. J Neurophysiol *26*, 1003-1017.

Witte, R.S., and Kipke, D.R. (2005). Enhanced contrast sensitivity in auditory cortex as cats learn to discriminate sound frequencies. Brain Res Cogn Brain Res *23*, 171-184.

Yotsumoto, Y., Watanabe, T., and Sasaki, Y. (2008). Different dynamics of performance and brain activation in the time course of perceptual learning. Neuron *57*, 827-833.

Zhang, L.I., Bao, S., and Merzenich, M.M. (2001). Persistent and specific influences of early acoustic environments on primary auditory cortex. Nat Neurosci *4*, 1123-1130.

Zhang, L.I., Bao, S., and Merzenich, M.M. (2002). Disruption of primary auditory cortex by synchronous auditory inputs during a critical period. Proc Natl Acad Sci U S A *99*, 2309-2314.

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III. Local inhibition inactivates learning-related plasticity in the songbird auditory forebrain

Abstract

Inhibition is involved in the plasticity of sensory representations from altered sensory input during development. Yet the function of inhibition in plasticity caused by learning is not well understood. We examined the role of local inhibition in learning-related plasticity in the adult songbird caudomedial nidopallium (NCM), a forebrain region analogous to secondary auditory cortices in mammals. Conspecific song recognition learning suppresses responses to learned songs in ventral NCM, while neurons in dorsal NCM respond similarly to learned and unfamiliar songs. Blocking local GABA-A increased the preference for unfamiliar songs in ventral NCM and uncovered a strong preference for learned songs in dorsal NCM. Disrupting inhibition decreased selectivity and unmasked responses to specific song features, which contributed to the preference for learned songs in dorsal NCM. Blocking inhibition also increased spectro-temporal receptive field nonlinearities. These results demonstrate novel roles for local inhibition including the inactivation of learning-related plasticity.

Introduction

Sensory representations in the brain are plastic both in development (Hensch, 2005) and adulthood (Buonomano and Merzenich, 1998; Weinberger, 1995). During development and adolescence, altered sensory input such as deprivation or continuous stimulus exposure modifies circuits in sensory areas (Wiesel and Hubel, 1965; Zhang et al., 2001). Inhibition is involved in the plasticity of sensory systems from altered sensory input (Feldman, 2009). Maturation of inhibition initiates the critical period for monocular deprivation plasticity in the visual cortex (Hensch et al., 1998) and responses to the deprived eye are weakened through strengthening of inhibitory synapses (Maffei et al., 2006). Sensory deprivation in the whisker system leads to shifts in receptive fields of neurons in the primary somatosensory cortex away from deprived inputs through increased inhibition (Foeller et al., 2005). Inhibition is also important for plasticity in the auditory space map of the inferior colliculus caused by altered visual experience (Zheng and Knudsen, 1999).

Little is known about the function of GABAergic inhibition in learning-related plasticity of sensory systems (Feldman, 2009). In adulthood, learning can dramatically shape the representations of sensory information in the brain (Diamond and Weinberger, 1984; Jenkins et al., 1990; Recanzone et al., 1993). In the auditory system, learning modifies the receptive fields of single neurons and changes the representations of sounds across neuronal populations (Bakin and Weinberger, 1990; Bao et al., 2003; Bieszczad and Weinberger, 2009; Fritz et al.,

2003; Galindo-Leon et al., 2009; Ohl and Scheich, 1996; Recanzone et al., 1993). In the songbird, auditory experience changes the representations of conspecific songs in regions of the auditory forebrain analogous to secondary auditory cortices in mammals (Gentner and Margoliash, 2003; Thompson and Gentner, 2010). In the ventral region of the caudomedial nidopallium (NCM), song recognition learning causes neurons to respond weaker to learned songs than to unfamiliar songs (Thompson and Gentner, 2010). Several studies have suggested a role for GABAergic inhibition in learning-related plasticity. Fear conditioning increases the levels of GABA and GABA receptors in primary sensory cortices (Gierdalski et al., 2001; Lech et al., 2001; Liguz-Lecznar et al., 2009). Fear conditioning in the whisker system also increases the density of inhibitory synapses and strengthens GABAergic transmission in the primary somatosensory cortex (Jasinska et al., 2010; Tokarski et al., 2007). Previous work has shown that NCM contains a large proportion of inhibitory neurons and that blocking inhibition can disrupt the temporal profile of auditory responses (Pinaud et al., 2008; Pinaud et al., 2004).

We examined the functional role of local inhibition in sensory representations that are changed with learning. We manipulated local inhibition in NCM of European starlings that were trained to recognize conspecific songs. Blocking inhibition increased the firing rates of NCM sites both at rest and in response to song. In ventral NCM, blockade of inhibition enhanced the preference for unfamiliar songs that is normally weakened by inhibition. Blocking inhibition in dorsal NCM uncovered a latent preference for learned songs that is completely

inactivated when inhibition is intact. Blocking inhibition also reduced the selectivity for song features and unmasked responses to segments of songs that evoked no response when inhibition was intact. In dorsal NCM, the frequency of these unmasked responses was higher during learned songs than unfamiliar songs, indicating that this is a way learning changes the representation of songs. In addition to changing the representation of learned and unfamiliar songs, blocking inhibition increased the nonlinearities of spectro-temporal receptive fields.

Methods

Subjects. In these experiments we used 10 adult European starlings (*Sturnus vulgaris*) that were caught in southern California. Both sexes were included in these studies, as previous studies have showed that similar experience-dependent changes accompany song recognition learning in both males and females (Gentner and Margoliash, 2003; Thompson and Gentner, 2010). Starlings were housed in a large, mixed-sex, flight aviary with free access to food and water until behavioral training began. Light-dark cycles were synchronized to natural photoperiods in the aviary and during behavioral training. Prior to behavioral training, all song stimuli were unfamiliar to the subjects. All procedures were conducted in accordance with the University of California, San Diego IACUC guidelines and the APS "Guiding Principles in the Care and Use of Animals."

Behavioral Training. We trained starlings to recognize the songs of conspecifics using operant conditioning techniques, described in detail previously (Gentner and Margoliash, 2003; Thompson and Gentner, 2010). Briefly, starlings were isolated in a sound attenuation chamber (Acoustic Systems, Austin TX) equipped with an operant panel and food hopper (Figure 3.1A). Starlings learned to use the operant panel and food hopper through a series of successive shaping procedures. Custom software was used to monitor peck responses, control the food hopper, lights and stimulus presentation.

Each starling learned to recognize two songs from two different conspecific individuals (four total). Starlings performed trials freely throughout the course of a day. A trial was initiated when a starling pecked the response port, triggering the presentation of a song from a speaker mounted inside the chamber. Songs were chosen randomly (with replacement) from the training songs. After the song, the starling had to either peck the response port within 2 seconds for the songs of one individual (Go trials) or withhold a peck response for the songs of the other individual (No-Go trials). Correct peck responses on Go trials were reinforced with 2 seconds of access to the food hopper. Incorrect peck responses on No-go trials were reinforced with a short time-out period (10-60 seconds) where the lights turned off and food was not available. Starlings learned the song recognition task quickly, were allowed to train for several weeks and reached high levels of performance (Figure 3.1B). We did not control for the overall time spent training or the total number of trials performed.

We created song stimuli by taking 5 second segments of continuous singing from longer song bouts. We used songs from four different adult male starlings (8 total) that were captured and recorded in Maryland, ensuring that the songs were initially unfamiliar to the subjects in this study. For each subject we used four songs during recognition training and saved four additional songs to be used as unfamiliar stimuli during electrophysiological testing. In initial experiments (4 subjects), we used longer segments of songs ranging from 8-11 seconds. We found no differences in the results between starlings trained with shorter or longer songs. We counterbalanced the assignment of songs across individuals such that each song was used as a learned and unfamiliar song the same number of times.

Electrophysiology/Microiontophoresis. In the days preceding electrophysiological recording, we anesthetized the starlings with isoflurane and attached a small pin stereotaxically to the surface of the skull. On the day of recording, we anesthetized the starlings with 20% urethane (7-8 ml/kg; in 3-4 IM injections over 2-4 hours). We placed the starling in a cloth jacket and secured the attached pin to a stereotaxic apparatus inside a sound attenuation chamber. We used Spike 2 software (CED, Cambridge UK) to play song stimuli, record extracellular activity and sort spike waveforms offline. We recorded neural activity with multi-barreled glass pipettes containing a carbon fiber electrode and six attached barrels for drug microiontophoresis (Kation Scientific, Minneapolis MN). We made a small craniotomy dorsal to NCM and removed the dura to insert the

electrodes. In each experiment we filled pipettes with NaCl for capacitance compensation (0.9%), and the GABA-A receptor antagonist Gabazine (3mM, Sigma Aldrich). In a subset of experiments we also filled pipettes with GABA (1 M, Sigma Aldrich), and the GABA-B receptor antagonist Saclofen (20mM, Sigma Aldrich). We used microiontophoresis (NPI, Tamm Germany) to control the application of drugs. We used retaining currents from -5 to -10 nA and injection currents from 25 to 50 nA.

To search for responsive sites, we played the four songs the starling learned to recognize and four songs that were unfamiliar. Unfamiliar songs were songs that the starlings had never heard before the electrophysiological experiment. Once an active site was located, we recorded responses to the four learned and four unfamiliar songs. We used the same set of eight songs at each recording site. At each site we began by recording a baseline block of responses with inhibition intact to each song stimulus. All blocks contained 10 repetitions of each song presented pseudo-randomly with a 4 second inter-stimulus interval. After this initial block, we turned on the microiontophoresis for the pipette containing gabazine. We waited one minute for the drug to wash in and then began a block of responses with the gabazine. Following this block we turned off the microiontophoresis and waited one minute for the drug to dissipate then began a recovery block. In experiments where we also recorded the activity with GABA or saclofen we also recorded blocks of 10 repetitions and waited one minute for the drugs to wash in and dissipate between blocks. In initial experiments (4 subjects) we did not wait one minute for drug onset

or offset. We found no major differences in the data collected from these experiments. We matched the intensity of songs to 68db peak RMS and presented them free-field. At the end of the recording experiment we made a small electrolytic lesion.

Histology. After the recording experiment we injected the starlings with a lethal dose of Nembutal. The starlings were then decapitated and the heads were fixed in 10% neutral-buffered formalin. After several days we transferred the brains to 30% sucrose PBS for cryoprotection. We sectioned the brain into 50 um sections and stained for Nissl. We confirmed that each site was located in NCM by comparing its position relative to the electrolytic lesion.

Data Analysis. We sorted recordings into single and multi-unit sites using Spike2. A site was considered a single unit only if there was a high signal to noise ratio and the waveform was clearly distinct from other spikes. We sorted the single units using template matching. We ensured that single units had no inter-spike-interval violations (<2msec). We used the same sort for the various microiontophoresis conditions. The majority of our data did not meet the criteria for a single unit and were considered multi-unit sites. These sites were typically made up of several waveforms that could not be confidently separated. For multi-unit sites we set a spike threshold that was above the noise floor and included most spikes. We used the same threshold for spikes collected in the various

microiontophoresis conditions. We exported spike times to MATLAB (Mathworks, Natick MA) for all analysis.

We calculated the spontaneous firing rate of a site as the mean rate of spikes in the 2 sec period before the onset of each song stimulus. We calculated the mean firing rate to a song stimulus as the mean number of spikes during the song divided by the song duration.

To examine the variability in the change in the firing rate to songs with inhibition intact and with inhibition blocked, we calculated the firing rate at each 50msec bin and calculated the standard deviation and coefficient of variation for the distributions of firing rates at each site

We identified regions of the song where responses were uncovered with inhibition blocked. We split the response with inhibition intact into 10msec bins and considered a bin as not responsive if the firing rate during the bin was not greater than the spontaneous activity. We defined a threshold for spontaneous activity as twice the maximum firing rate during a single 2sec period preceding song presentation. Bins in the response with inhibition intact that had firing rates below this measure were considered unresponsive. We then looked for bins that were unresponsive with inhibition intact but responsive with inhibition blocked. For responses with inhibition blocked, we calculated a new threshold for spontaneous activity using the spontaneous firing rate with inhibition blocked. To identify individual peaks with uncovered responses, we counted nonconsecutive 10msec bins that became responsive with inhibition blocked. We report the frequency of

peaks with uncovered responses as the number of peaks that were unresponsive with inhibition intact that then became responsive in the gabazine condition, divided by the total duration of the site's responses.

To measure the stimulus selectivity we broke songs into their motifs (~1 sec) and calculated the firing rates to each motif with inhibition intact and with inhibition blocked. We used a well-established measure of selectivity that has been called lifetime sparseness (Rolls and Tovee, 1995; Tolhurst et al., 2009; Vinje and Gallant, 2000; Vinje and Gallant, 2002), which we calculated for each site as:

$$\text{Selectivity} = \frac{1-\left(\frac{\left(\sum r_i\right)^2}{n\left(\sum\left(r_i^2\right)\right)}\right)}{1-\left(\frac{1}{n}\right)},$$

where r_i is the firing rate to the *i*th motif and *n* is the total number of motifs. Selectivity values range from 0 for dense codes where a site responds equally to all motifs to 1 for sparse codes where a site responds to a single motif.

To model receptive fields we used the maximally informative dimension technique (Sharpee et al., 2004; Sharpee et al., 2006). This technique estimates the spectro-temporal filter and static nonlinearity for each site. The filter shows the stimulus feature that accounts for the most information between the stimulus and neural response. The nonlinearity shows the probability of eliciting a spike as the similarity between the filter and nonlinearity varies.

Results

Using microiontophoresis, we examined the effects of altering GABAergic inhibition in NCM of starlings that had been trained to recognize conspecific songs (Fig 3.1A,B). Blocking GABA-A receptors caused a reversible increase in the mean firing rate to song stimuli (Fig 3.2A). Blockade of GABA-A receptors caused a large increase in driven firing rates throughout the ventral (Fig 3.2B,C) and dorsal (Fig 3.2D,E) regions of NCM. Blocking local GABA-A mediated inhibition also caused a large increase in the spontaneous firing rates for both dorsal and ventral sites (Fig 3.S1).

GABAergic inhibition inactivates experience-dependent plasticity in NCM

After conspecific song recognition learning, neurons in the ventral region of NCM respond stronger to songs that are unfamiliar than to songs that starlings have learned to recognize (Thompson and Gentner, 2010). We hypothesized that the preference for unfamiliar songs is caused by increased local inhibition during the responses to learned songs and predicted that blocking inhibition would reduce this preference. The opposite was true. Blocking inhibition strengthened the preference for unfamiliar songs in ventral NCM (Fig 3.3A). We compared the responses of NCM sites to learned and unfamiliar songs when GABA-A mediated inhibition is intact and when it is blocked. To normalize for the large variability in the firing rates of individual sites, we converted the firing rates of each site to z-scores. As in our previous study (Thompson and Gentner, 2010), learned songs elicited weaker firing

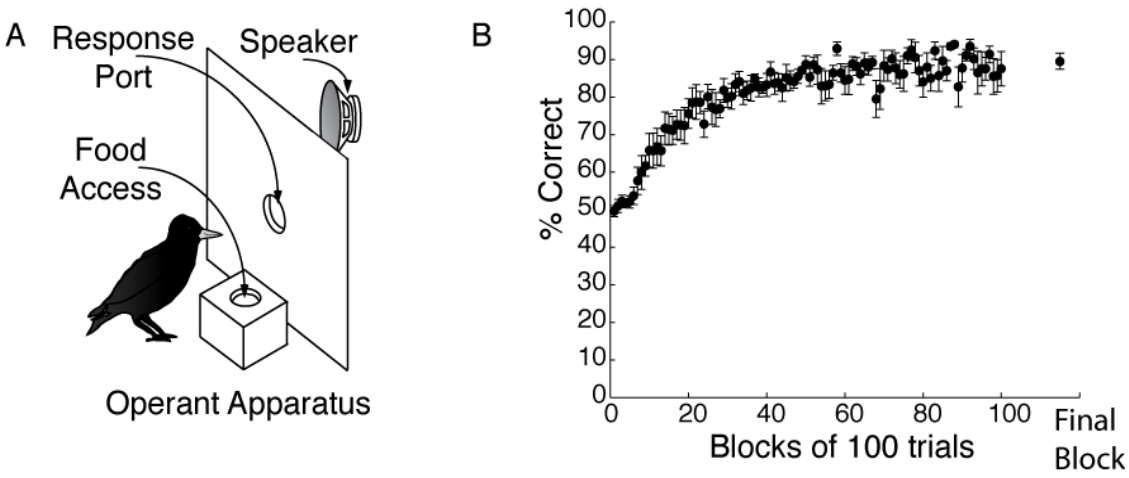


Figure 3.1. Song Recognition Training. (A) Diagram of the operant panel used for recognition training. (B) Acquisition curve showing the performance of the starlings on each 100-trial block of the Go/No-go song recognition task. The overall amount of training varied between subjects and the performance on the final block of training is shown. The mean performance for all starlings on the final block was 89.5 ± 2.2 % correct. Starlings trained for a total of 70.3 ± 27.6 days and performed 346.3 ± 151.8 blocks of 100 trials.

Figure 3.2. Blocking GABA-A inhibition increases firing rates. (A) Response of a sample multi-unit NCM site to a starling song stimulus (top), with inhibition intact, with inhibition blocked and in the recovery condition. Each response shows a raster plot and psth. In the raster plot, each row is the response on a single stimulus repetition and each tick is a spike. The psth shows the response across all repetitions. The top response is with inhibition intact (black psth), the middle response is with inhibition blocked (red psth) and the bottom response shows the recovery condition (green psth). The mean firing rate for each condition is given in the top right corner of each psth. The vertical scale bar along the psth marks 20 spikes/sec, the vertical scale bar along the spectrogram marks 4 kHz and the horizontal scale bar marks 1 sec. (B) Change in driven firing rate with inhibition blocked for ventral sites. Each line shows the change in firing for an individual site. For ventral sites, songs elicited a mean (± sem) of 41.29 ± 8.44 spikes/sec with inhibition intact, 57.04 ± 10.35 spikes/sec with GABA-A receptors blocked and 43.11 ± 8.60 spikes/sec in the recovery condition (Repeated Measures ANOVA $F = 28.8$, $p = 4.31 \times 10\text{-}10$, $n = 40$). (C) Z-score of driven firing rates to song for ventral sites with inhibition intact, with inhibition blocked and recovery conditions. To normalize for the variability in firing rates (firing rates with inhibition intact, ventral: 0.01 – 211.27 spikes/sec; dorsal: 0.09 – 35.73 spikes/sec) , we converted the firing rates of each site to z-scores. In ventral NCM, songs elicited a mean (± sem) z-score of -0.45 ± 0.06 with inhibition intact, 0.88 ± 0.06 with inhibition blocked and -0.43 ± 0.03 in the recovery condition (Repeated Measures ANOVA $F = 152.3$, $p < 1.0 \times 10\text{-}10$). (D)Same as in B) for dorsal sites. For dorsal sites, songs elicited a mean (± sem) of 8.24 ± 1.05 spikes/sec with inhibition intact, 13.06 ± 1.43 spikes/sec with GABA-A receptors blocked and 8.66 ± 1.10 spikes/sec in the recovery condition (Repeated Measures ANOVA $F = 44.1$, $p = 4.89 \times 10\text{-}15$, $n = 60$). (E) Same as in C) for Dorsal Sites. In dorsal NCM, songs elicited a mean (± sem) z-score of -0.34 ± 0.05 with inhibition intact, 0.70 ± 0.06 with inhibition blocked and -0.35 ± 0.03 in the recovery condition (Repeated Measures ANOVA $F = 104.1$, $p < 1.0 \times 10\text{-}10$).

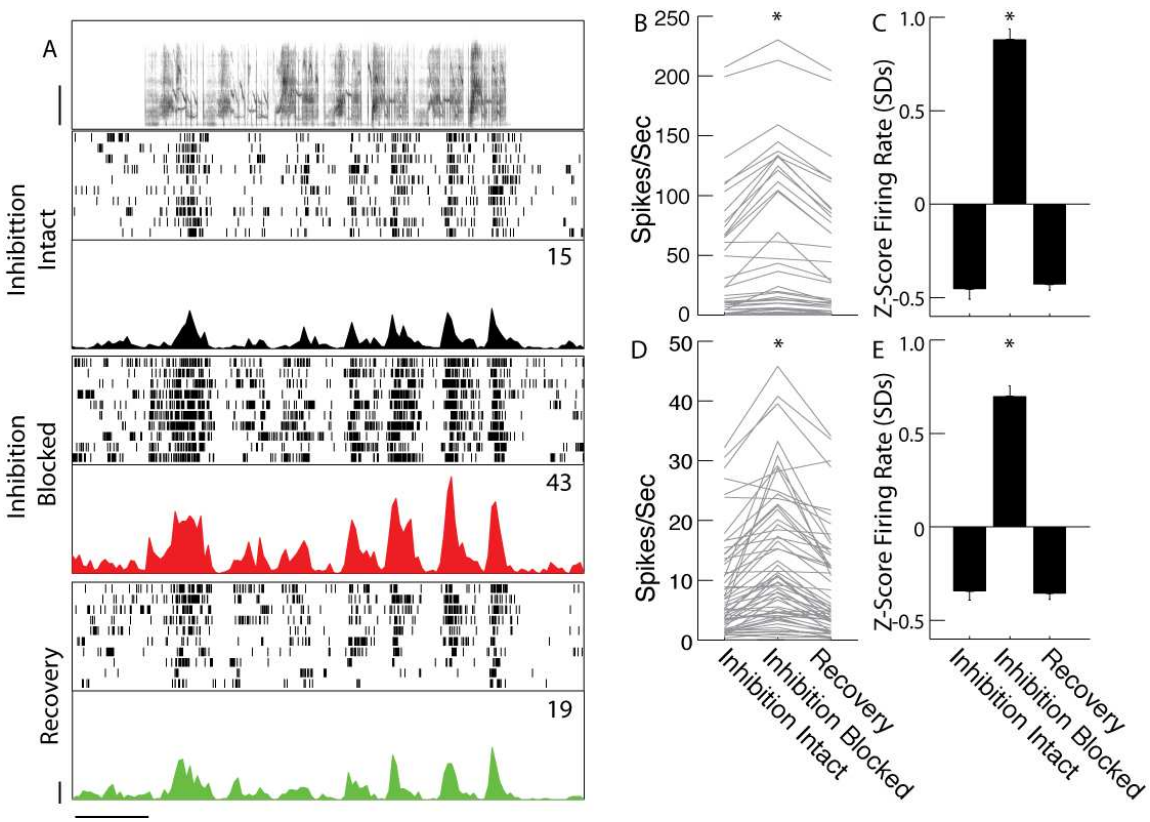
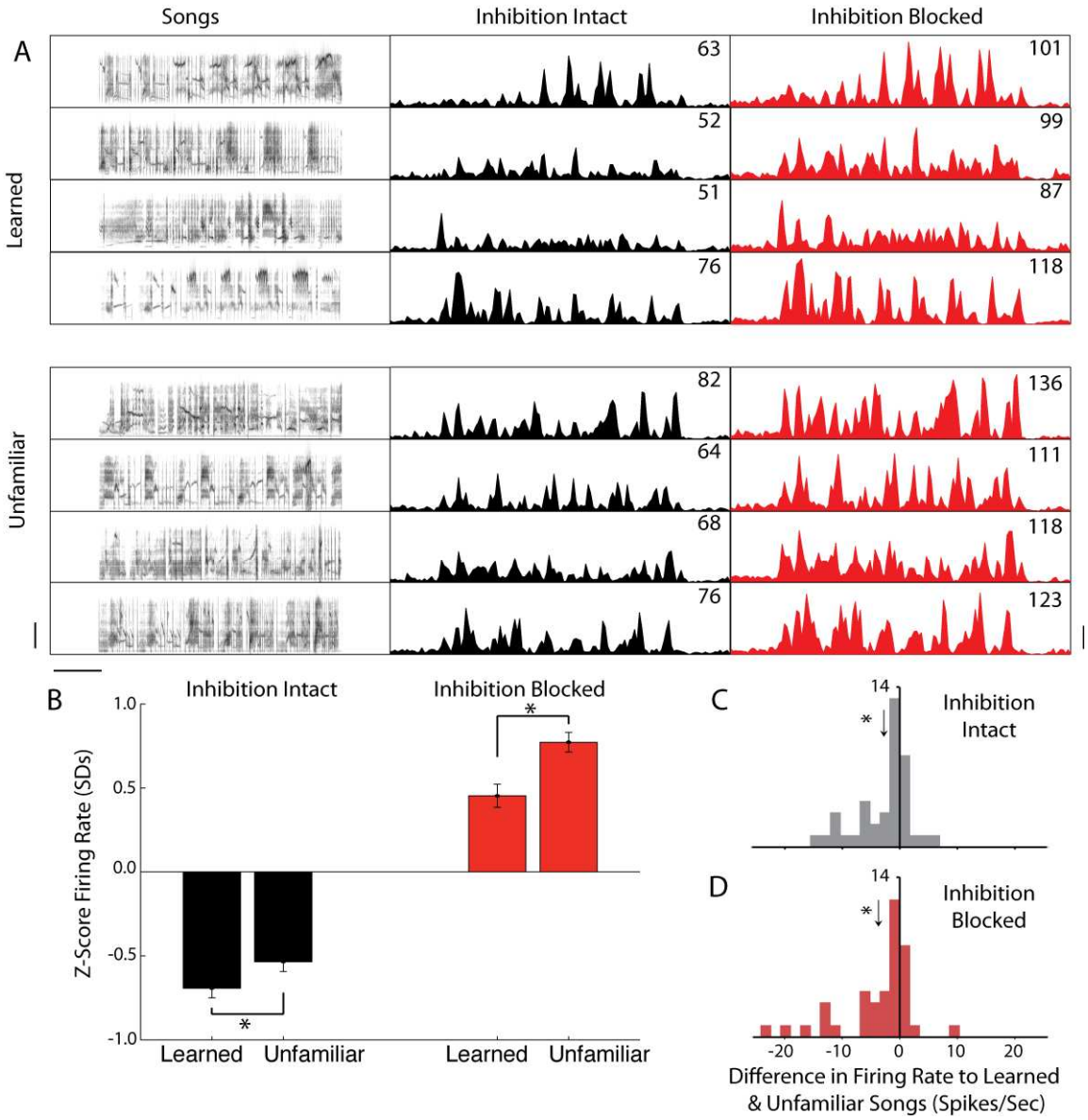

A
Inhibittion Intact
15
Inhibition Blocked
43
Recovery
19
B
Spikes/Sec
250
200
150
100
50
0
*
C
Z-Score Firing Rate (SDs)
1.0
0.5
0
-0.5
*
D
Spikes/Sec
50
40
30
20
10
0
*
E
Z-Score Firing Rate (SDs)
1.0
0.5
0
-0.5
*
Inhibition Intact
Inhibition Blocked
Recovery
Inhibition Intact
Inhibition Blocked
Recovery

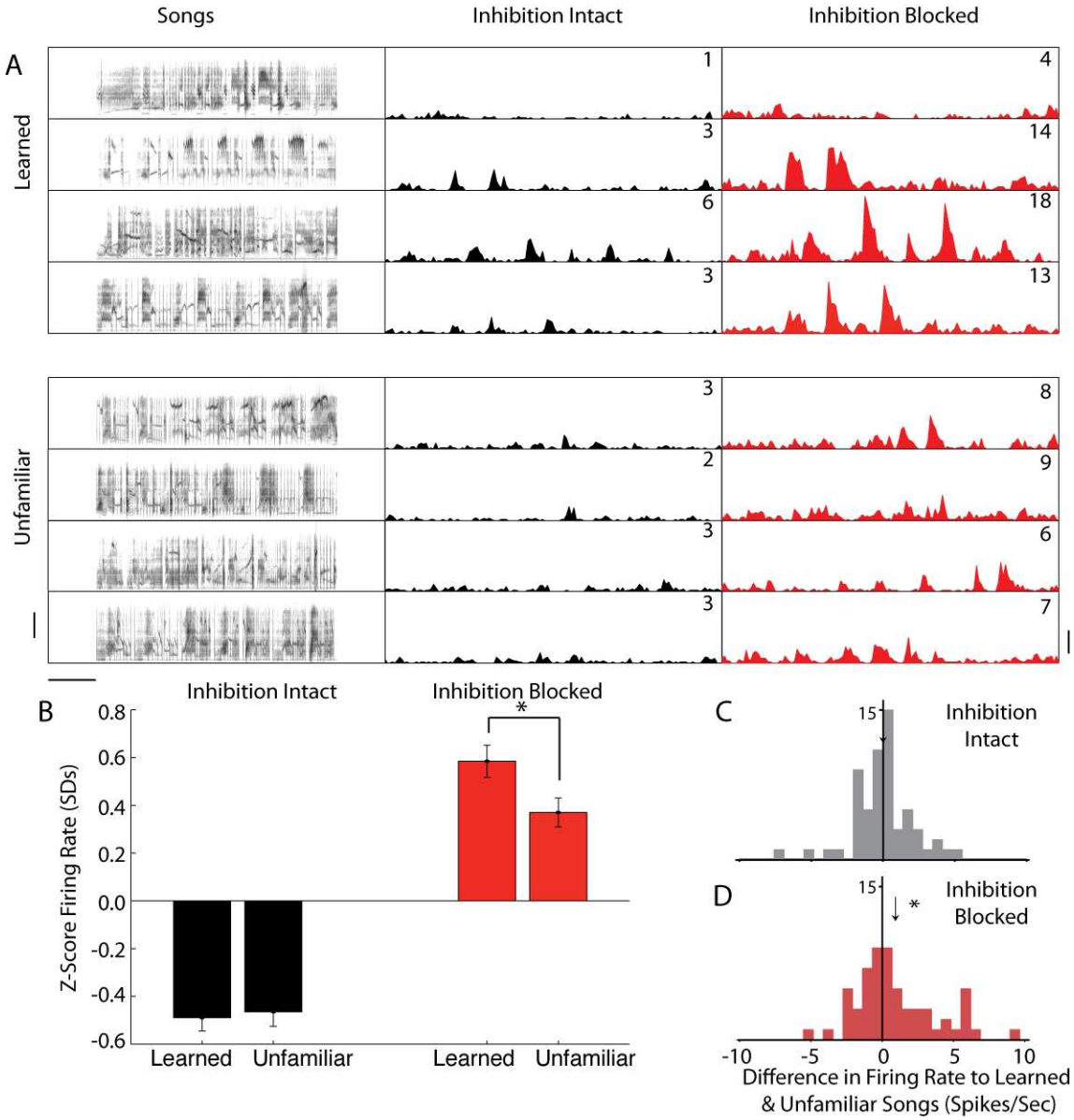
Figure 3.3. Blocking Inhibition Enhances Plasticity in Ventral NCM. (A) Sample Site in Ventral NCM where blocking inhibition enhanced the preference for unfamiliar songs. The psths of the responses with inhibition intact (black) and with inhibition blocked (red) to each learned and unfamiliar song (left). With inhibition intact, the firing rate to learned songs was 60.44 ± 5.72 spikes/sec and to unfamiliar songs was 72.64 ± 4.03 spikes/sec. When inhibition was blocked the firing rate to learned songs was 101.43 ± 6.39 spikes/sec and to unfamiliar songs was 121.72 ± 5.24 spikes/sec. The vertical scale bar along the psth marks 40 spikes/sec, the vertical scale bar along the spectrogram marks 4 kHz and the horizontal scale bar marks 1 sec. Values shown in the corner of each PSTH are the mean firing rates. (B) Z-scores of firing rates to learned and unfamiliar songs with inhibition intact and with inhibition blocked. There is a significant interaction between learning and drug condition indicating that blocking inhibition increases the magnitude of the difference in firing rate between learned and unfamiliar songs. (C) Distribution of the difference in firing rates between learned and unfamiliar songs with inhibition intact. (D) As in C), with inhibition blocked. * indicate a significant difference between means.


Songs
Inhibition Intact
Inhibition Blocked
A
Learned
Unfamiliar
63
52
51
76
101
99
87
118
82
64
68
76
136
111
118
123
B
Inhibition Intact
Inhibition Blocked
Z-Score Firing Rate (SDs)
1.0
0.5
0.0
-0.5
-1.0
Learned
Unfamiliar
Learned
Unfamiliar
C
14
Inhibition Intact
D
14
Inhibition Blocked
-20
-10
0
10
20
Difference in Firing Rate to Learned & Unfamiliar Songs (Spikes/Sec)

rates than unfamiliar songs with inhibition intact (Fig 3.3B). With inhibition intact, the z-score of the firing rate was -0.69 ± 0.06 to the learned songs and -0.53 ± 0.06 to the unfamiliar songs. When inhibition was blocked, the magnitude of the difference in the firing rates to the learned and unfamiliar songs increased (Fig 3.3B). With inhibition blocked, the z-score of the firing rate was 0.45 ± 0.07 to the learned songs and 0.77 ± 0.06 to the unfamiliar songs (Two-Way Repeated Measures ANOVA, Effect of Learning $F = 12.35$ $P = 0.0011$, Effect of Blocking Inhibition $F = 169.03$ $P = 8.9 \times 10^{-16}$, Interaction $F = 4.38$ $P = 0.0429$). Despite the large variance in firing rates, we obtained similar results when the raw firing rates were used in this analysis (Fig 3.3C,D). These results demonstrate that song recognition learning decreases the excitatory drive to learned songs in ventral NCM, but that increased levels of inhibition to unfamiliar songs weaken these changes.

Since there is no difference in the responses to learned and unfamiliar songs in dorsal NCM (Thompson and Gentner, 2010), we hypothesized that disrupting inhibition would have no major effects on the representation of learned and unfamiliar songs. Surprisingly, blocking inhibition uncovered a preference for learned songs in dorsal NCM (Fig 3.4A). As for ventral NCM, to normalize for the large variability in the firing rates of individual sites, we converted the firing rates of each site to z-scores. As expected, with inhibition intact, the firing rates to learned and unfamiliar songs were similar (Fig 3.4B). With inhibition intact, the z-score of the firing rate was -0.49 ± 0.06 to the learned songs and -0.47 ± 0.06 to the unfamiliar songs. When inhibition was blocked, the firing rates to learned songs

Figure 3.4. Blocking Inhibition uncovers a preference for learned songs in dorsal NCM. (A) Sample Site in Dorsal NCM where blocking inhibition uncovered the preference for unfamiliar songs. The psths of the responses with inhibition intact (black) and with inhibition blocked (red) to each learned and unfamiliar song (left). With inhibition intact, the firing rate to learned songs was 3.44 ± 0.93 spikes/sec and to unfamiliar songs was 2.79 ± 0.25 spikes/sec. When inhibition was blocked the firing rate to learned songs was 12.05 ± 3.03 spikes/sec and to unfamiliar songs was 7.45 ± 0.65 spikes/sec. The vertical scale bar along the psth marks 40 spikes/sec, the vertical scale bar along the spectrogram marks 4 kHz and the horizontal scale bar marks 1 sec. Values shown in the corner of each PSTH are the mean firing rates. (B) Z-scores of firing rates to learned and unfamiliar songs with inhibition intact and with inhibition blocked. There is a significant interaction between learning and drug condition indicating that blocking inhibition increases the magnitude of the difference in firing rate between learned and unfamiliar songs. (C) Distribution of the difference in firing rate between learned and unfamiliar songs in the baseline condition. (D) As in C), with inhibition blocked. * indicate a significant difference between means.

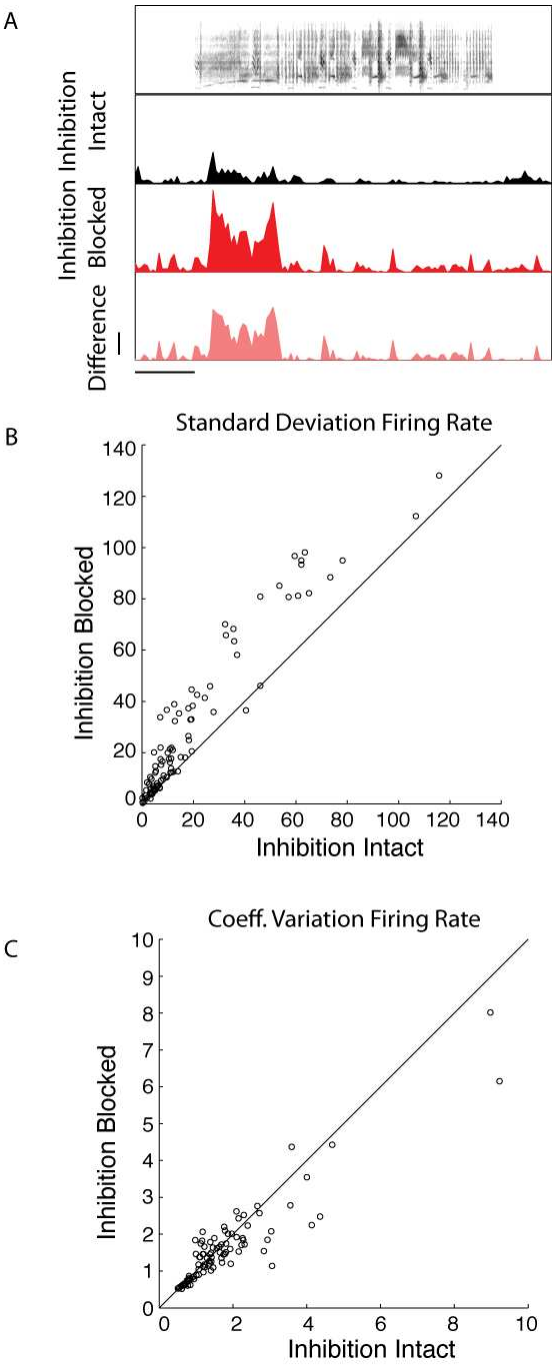

Songs
Inhibition Intact
Inhibition Blocked
A
Learned
Unfamiliar
1
4
3
14
6
18
3
13
3
8
2
9
3
6
3
7
B
Inhibition Intact
Inhibition Blocked
Z-Score Firing Rate (SDs)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
*
Learned
Unfamiliar
Learned
Unfamiliar
C
15
Inhibition Intact
D
15
Inhibition Blocked
*
-10
-5
0
5
10
Difference in Firing Rate to Learned & Unfamiliar Songs (Spikes/Sec)

were much stronger than the firing rates to unfamiliar songs (Fig 3.4B). With inhibition blocked, the z-score of the firing rate was 0.59 ± 0.07 to the learned songs and 0.37 ± 0.06 to the unfamiliar songs (Two-Way Repeated Measures ANOVA, Effect of Learning $F = 1.58$ $P = 0.2144$, Effect of Blocking Inhibition $F = 106.42$ $P = 7.9 \times 10^{-15}$, Interaction $F = 21.14$ $P = 2.3 \times 10^{-5}$). We obtained similar results when the raw firing rates were used in this analysis (Fig 3.4C,D; Two-Way Repeated Measures ANOVA, Effect of Learning $F = 2.46$ $P = 0.1224$, Effect of Blocking Inhibition $F = 42.79$ $P = 1.6 \times 10^{-8}$, Interaction $F = 17.51$ $P = 9.7 \times 10^{-5}$). Blocking local inhibition in dorsal NCM uncovers a strong preference to respond to learned songs over unfamiliar songs. These results demonstrate that song recognition learning increases the excitatory drive to learned songs in dorsal NCM, but that increased levels of inhibition inactivate these changes.

Variable release from inhibition during song responses

When inhibition is blocked, the change in firing rate could be constant across the duration of song stimuli or variable during different parts of songs. Figure 3.5A shows a sample site where the change in firing rate with inhibition blocked is variable throughout the response to a song. We used two measures to examine the variability in the release from inhibition. We divided the responses with inhibition intact and blocked into 50 msec bins and calculated the firing rate at each bin. To

Figure 3.5. Variable Release from Inhibition throughout songs. (A) Psths of responses to a song (spectogram) with inhibition intact (black) and with inhibition blocked (red). The bottom psth (pink) is the difference psth showing the release from inhibition. The vertical scale bar along the psth marks 40 spikes/sec, the vertical scale bar along the spectrogram marks 4 kHz and the horizontal scale bar marks 1 sec. (B) The standard deviation of the firing rates in 50 msec bins with inhibition intact and with inhibition blocked. (C) The coefficient of variation of the firing rates in 50 msec bins with inhibition intact and with inhibition blocked. Three outliers are omitted from this plot for visualization.


A
Inhibition Intact
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10

determine whether the change in firing rate is a constant additive increase, we compared the standard deviation in the firing rates. If the magnitude of the increase in firing rate with inhibition blocked were constant across the site's response, the standard deviation of the firing rates would remain the same with inhibition intact and with inhibition blocked. To determine whether the change in firing rate is a constant multiplicative increase, such as a doubling, we compared coefficient of variation (CV) in the firing rates. If the firing rate with inhibition blocked were multiplied by a constant factor to the firing rate with inhibition intact, the coefficient of variation would remain the same with inhibition intact and with inhibition blocked. Blocking inhibition caused a variable increase in firing rate for sites from both ventral and dorsal NCM. Blocking inhibition increased the standard deviation of the binned firing rates, indicating that the additive increase in firing rate varied across a neuron's response (Fig 3.5B). The standard deviation of the binned firing rates was 18.56 ± 2.30 with inhibition intact and increased to 28.24 ± 3.03 with inhibition blocked (Paired t-test $p = 3.98 \times 10^{-14}$). Blocking inhibition both increased and decreased the coefficient of variation in the firing rates for different sites (Fig 3.5C). Overall there was a significant decrease in the CV with inhibition blocked. The CV was 2.20 ± 0.30 with inhibition intact and decreased to 1.65 ± 0.12 with inhibition blocked (Paired t-test $p = 0.0264$). Decreases in CV indicate that the mean of the firing rates increased more than the standard deviation, but changes in the CV in either direction show that the multiplicative increase in firing rate varied across a neuron's response. The increase in firing rate with inhibition blocked is not a

constant additive or multiplicative increase to the firing rate with inhibition intact; rather it is larger and smaller at different portions of the song.

Inhibition shapes selectivity for complex sounds

In several sensory systems, local inhibition increases the selectivity for specific sensory features and when inhibition is blocked selectivity decreases (Ingham and McAlpine, 2005; Muller and Scheich, 1987; Wang et al., 2000). In songbirds, neurons in NCM respond preferentially to conspecific vocalizations and individual neurons are selective for specific feature of conspecific songs (Chew et al., 1996; George et al., 2008; Müller and Leppelsack, 1985; Ribeiro et al., 1998; Stripling et al., 1997; Thompson and Gentner, 2010). We examined whether the selectivity for complex features of conspecific songs in NCM is also caused by local inhibition. Starling songs are strings of unique acoustic units called motifs. To investigate selectivity we divided songs into their motifs (Fig 3.6A) and calculated the firing rates to each motif with inhibition intact and blocked (Fig 3.6B). We used a well-established measure of selectivity that has characterized the responses of visual neurons to complex objects (Rolls and Tovee, 1995; Vinje and Gallant, 2000; Vinje and Gallant, 2002). Selectivity values range from 0 for sites that respond to all song motifs to 1 for sites that respond to only a single motif. In NCM, blocking inhibition significantly reduced selectivity (Fig 3.6C). The selectivity of NCM sites was 0.39 ± 0.03 with inhibition intact and decreased to 0.32 ± 0.02 when inhibition

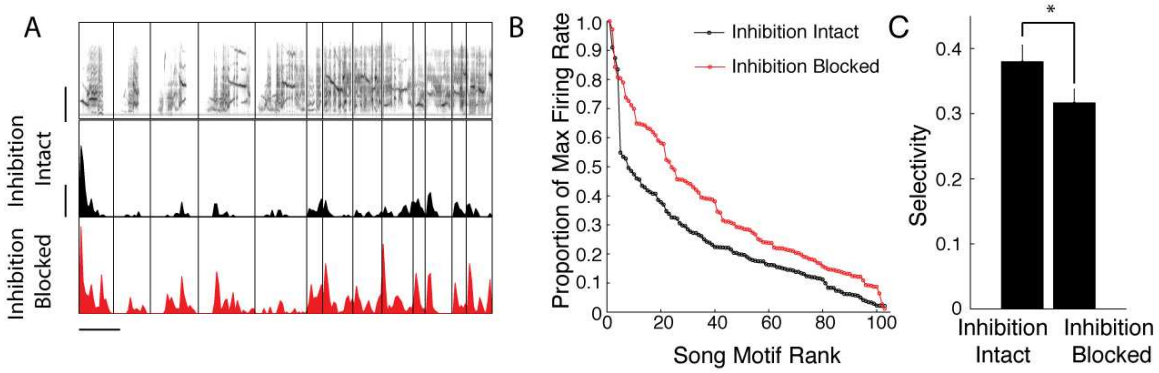

Figure 3.6. Blocking inhibition decreases selectivity. (A) Firing rates of a sample site with inhibition intact (black psth) and with inhibition blocked (red psth) to individual song motifs (spectrogram). The vertical lines mark the locations of the individual motifs. The vertical scale bar along the psth indicates 40 spikes/sec, the vertical scale bar along the spectrogram marks 4 kHz and the horizontal scale bar marks 1 sec. (B) Normalized firing rates to song motifs with inhibition intact and with inhibition blocked for the sample site shown in A). Firing rates are normalized to the maximum motif firing rate in each condition. Song motifs are rank ordered by firing rate for each condition. The selectivity for this site was 0.39 with inhibition intact and 0.30 when inhibition was blocked. (C) Selectivity for song motifs with inhibition intact and with inhibition blocked. Values were calculated using the firing rates to song motifs for each site. * indicates a significant change in means.

was blocked (t-test $p = 2.02 \times 10^{-6}$). Because mostly multi-unit sites were used in this analysis, the actual selectivity values for single neurons are likely higher. Nonetheless, in NCM, the selectivity for features of conspecific song is in part controlled by local inhibition. Consistent with the decreases in selectivity, at many sites blocking inhibition unmasked responses to portions of songs that normally evoked no response when inhibition was intact. Figure 3.7A shows a multi unit site where blocking inhibition unmasked peaks in the response during several segments of the song. Similar unmasked peaks were observed in single neurons (Fig 3.7B), showing that they are not caused by the recruitment of additional neurons to the multi unit response. Note that the unmasked peaks are robust and time-locked to the stimulus. We identified the portions of songs where the firing rate was not above the spontaneous rate with inhibition intact and asked how often these turned into responses above the spontaneous rate with inhibition blocked. The mean frequency of unmasked responses in ventral NCM was 0.44 ± 0.08 peaks/sec and in dorsal NCM was 0.50 ± 0.04 peaks/sec. Inhibition changes not only the magnitude of responses in NCM, but

also changes the spike pattern of the responses to songs by masking spikes to certain portions of song.

We examined whether peaks were unmasked more often during learned or unfamiliar songs. In ventral NCM, unmasked peaks occurred at a similar frequency in responses to learned and unfamiliar songs (Fig 3.7C). The mean frequency of uncovered peaks to learned songs was 0.45 ± 0.08 peaks/sec and to unfamiliar

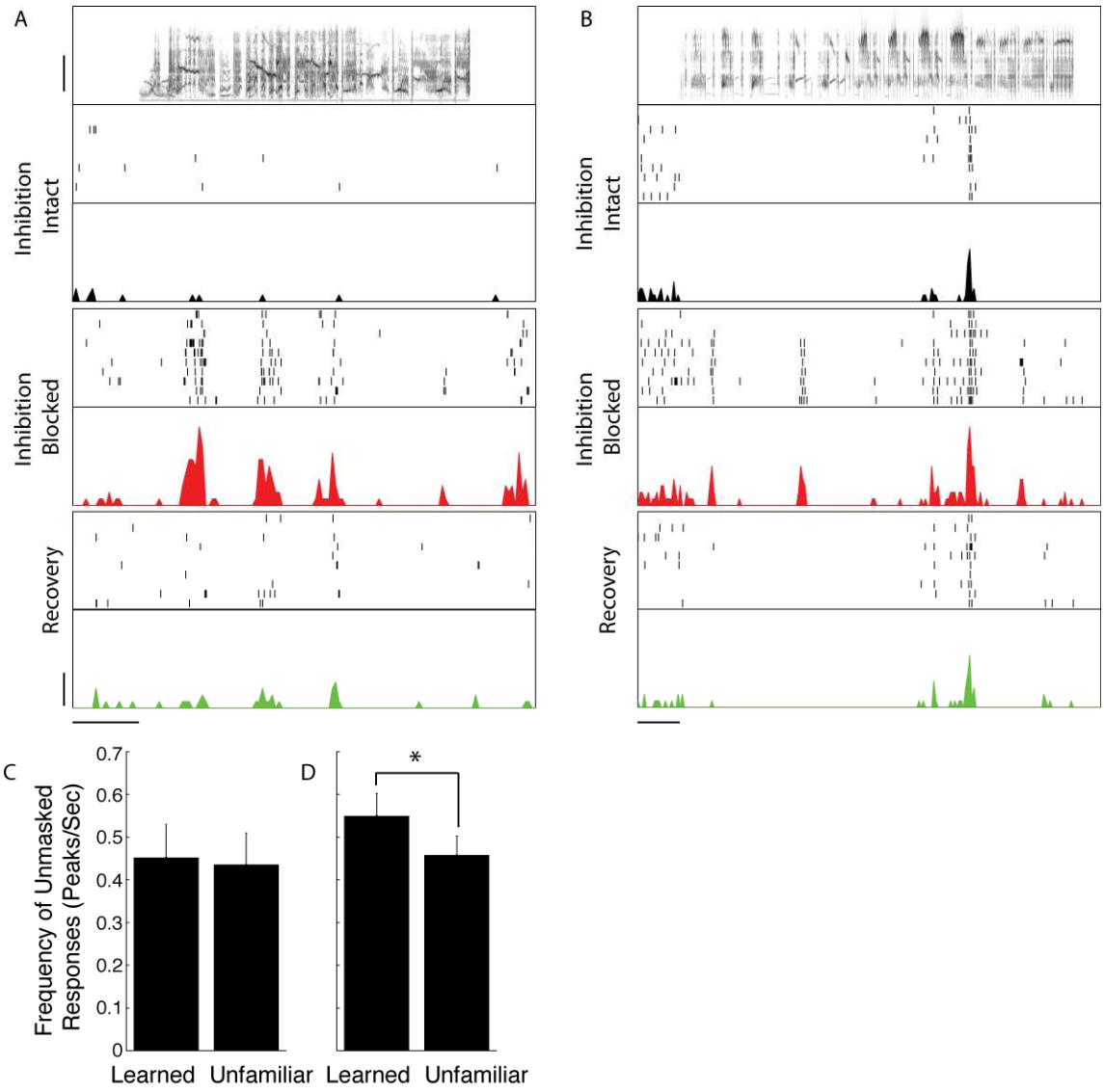

Figure 3.7. Blocking inhibition uncovers robust responses. (A) Sample multi-unit site where blocking inhibition uncovered several peaks of responses. The top PSTH (black) and raster plot show the response with inhibition intact, the middle PSTH (red) and raster plot show the response with inhibition blocked and the bottom PSTH (green) and raster plot show the response in the recovery condition. (B) As in A) for a single unit site. The vertical scale bar along the psth marks 10 spikes/sec, the vertical scale bar along the spectrogram marks 4 kHz and the horizontal scale bars mark 1 sec. (C) Frequency of unmasked peaks in learned and unfamiliar songs for ventral NCM. (D) As in C) for dorsal NCM. * indicate a significant difference in means.

songs was 0.44 ± 0.08 peaks/sec (Paired t-test p = 0.5226). In contrast, in dorsal NCM, uncovered peaks were found more often in responses to learned songs than in responses to unfamiliar songs (Fig 3.7D). The mean frequency of uncovered peaks to learned songs was 0.55 ± 0.05 peaks/sec and to unfamiliar songs was 0.46 ± 0.05 peaks/sec (Paired t-test p = 0.0041). When different measures were used to determine when a response has been unmasked, the values of the frequency were increased or decreased, but in all cases the frequency was significantly higher during learned songs. In dorsal NCM, the inactivation of the preference for learned songs is in part caused by responses during learned songs that are masked by local inhibition

Inhibition increases spectro-temporal receptive fields nonlinearities

The variable change in firing rate and decrease in selectivity when inhibition is blocked, suggest that blocking inhibition changes receptive fields in NCM. Most previous studies that reported changes in receptive fields from blocking inhibition based their claims on changes in selectivity. Few studies have used receptive fields models and examined changes while blocking local inhibition. We investigated whether blocking inhibition changed the spectro-temporal receptive fields of NCM sites. Using the maximally informative dimension technique (MID) we modeled each site's spectro-temporal filter and static nonlinearity with inhibition intact and with inhibition blocked (Atencio et al., 2008; Sharpee et al., 2004; Sharpee et al., 2006). The MID technique searches for the filter and nonlinearity that maximizes the

mutual information between the stimuli and the neuronal responses. The filter shows the stimulus feature that accounts for the most information between the stimulus and the neural response. The nonlinearity shows the probability of eliciting a spike as the similarity between the filter and the stimulus varies. Figure 3.8A shows the filter and nonlinearity for a sample site with inhibition intact and blocked. When inhibition was blocked, there was little change in the shape of the filter, but the nonlinearity was shifted upwards. Figure 3.8B shows a sample response and prediction for this site. With inhibition intact, the magnitude of the predicted response is lower than the actual response, but the MID accurately predicts which portions of song elicit a response. With inhibition blocked, the increase in the nonlinearity of the MID predicts an increase in firing that is consistent with the increase in the actual response. These effects were consistent across NCM. Overall blocking inhibition caused little change in the shape of the filter (Fig 3.8C). The shape of the filter was strongly correlated when inhibition was intact and blocked ($R = 0.72 \pm 0.02$). This falls into the range of normal variability in MIDs, and was similar to the correlation between the filter when inhibition is intact and during recovery ($R = 0.71 \pm 0.03$). Blocking inhibition also had little effect on the shape of the nonlinearity (Fig 3.8D). The nonlinearity with inhibition intact was strongly correlated with the nonlinearity with inhibition blocked ($R=0.85 \pm 0.02$), which was similar to the correlation between the nonlinearity with inhibition intact and during recovery ($R = 0.83 \pm 0.02$). Although there were no consistent changes in the shapes of the filters or nonlinearity, there was a significant increase in the peak of the

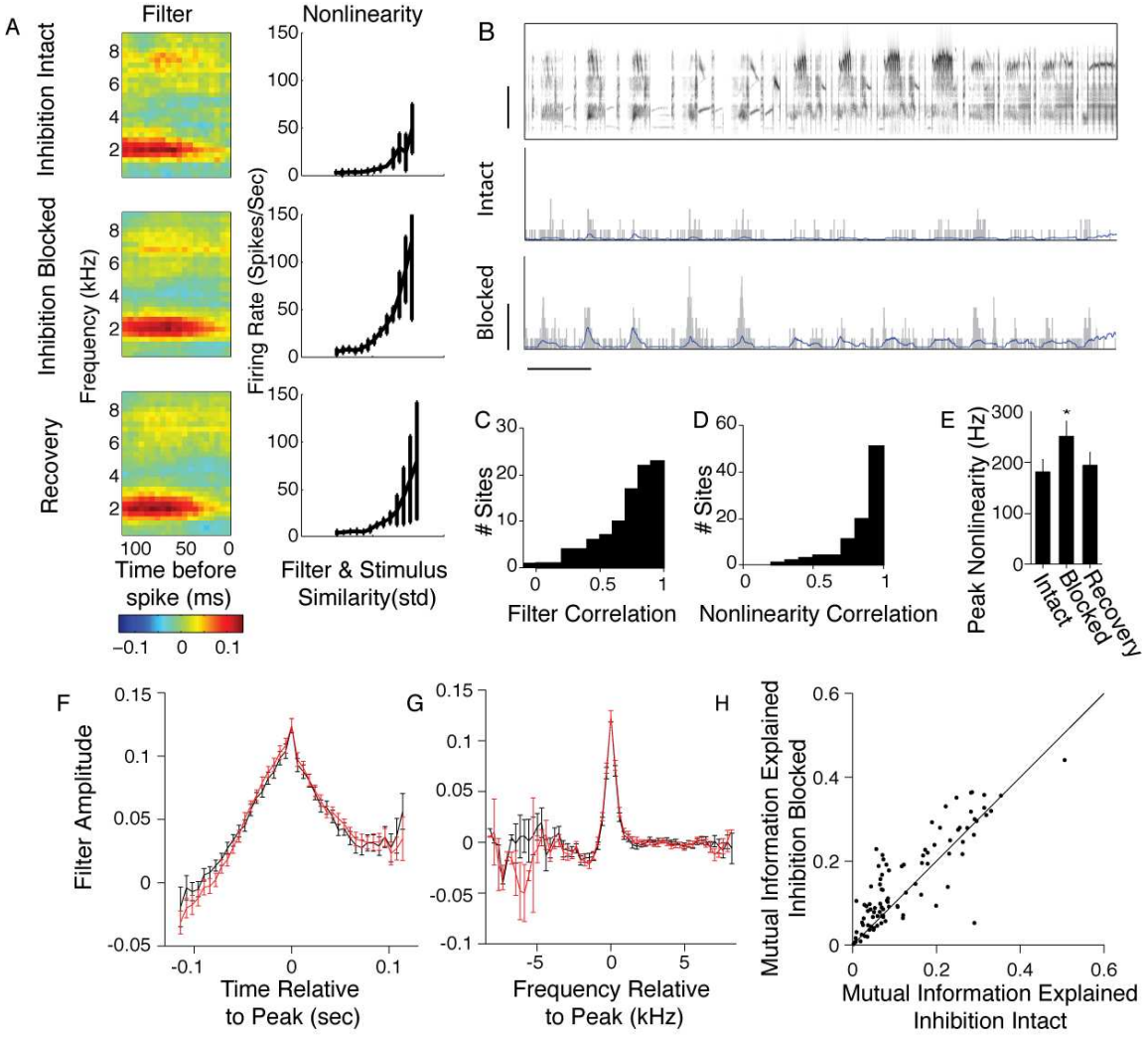

Figure 3.8. Effects of blocking inhibition on MID receptive fields. A) MID filter and nonlinearity for a sample NCM site with inhibition intact, blocked and during recovery. B) PSTH of the response (gray) and predicted based on the MID (blue) for the neuron in (A) with inhibition blocked and intact. The vertical scale bar marks 75 spikes/sec for the PSTH and the vertical scale bar for the spectrogram marks 4 kHz. Horizontal scale bar marks one second. C) R values from correlations between the MID filter of each site with inhibition intact and blocked. D) R-values for the correlation between the nonlinearity of each site with inhibition intact and blocked. E) Peak of the nonlinearity with inhibition intact, inhibition blocked and during recovery. F) Temporal Bandwidth of the MID filter with inhibition intact (black) and blocked (red). G) As in F) for spectral bandwidth. H) Mutual information explained by the MID with inhibition intact and blocked.

nonlinearity with inhibition blocked (Fig 3.8E). The peak of the nonlinearity was 181±24Hz with inhibition intact, increased to 250±29Hz with inhibition blocked and returned to 194±25Hz during recovery (repeated measures ANOVA, $p < 10^{-10}$). To further quantify any changes in the shape of the filters, we examined the bandwidth of the peak of the filter in time and frequency. Blocking inhibition had no major effects on the temporal (Fig 3.8F) or spectral bandwidth (Fig 3.8G). Overall we observed that blocking inhibition improved the predictive performance of the MIDs (Fig 3.8H). Blocking inhibition significantly increased the percentage of information explained by the MID models. The MID explained 12.5±1.1% of information with inhibition intact and 14.9±1.1% with inhibition blocked (paired t-test $p = 4.3\times10^{-5}$). Changes in the responses of NCM neurons when inhibition is blocked are at least in part due to increases in the gain of the spiking nonlinearity.

Discussion

Learning shapes the response properties of neurons in sensory regions of the brain (Buonomano and Merzenich, 1998; Weinberger, 1995). Here, we demonstrate that local inhibition can inactivate changes in sensory representations induced by associative learning. Inhibition weakens the preference for unfamiliar songs in ventral NCM and inactivates an underlying preference for learned songs in dorsal NCM. Previous studies have suggested a role for inhibition in learning-related plasticity of sensory representations. Following fear conditioning, levels of GABA and GABA receptors are increased in primary sensory cortices (Gierdalski et al.,

2001; Lech et al., 2001; Liguz-Lecznar et al., 2009). Furthermore, fear conditioning with the whisker system increased the density of inhibitory synapses and strengthened GABAergic transmission in the primary somatosensory cortex (Jasinska et al., 2010; Tokarski et al., 2007). Similarly, habituation of whisker inputs increased the density of inhibitory synapses in the primary somatosensory cortex (Knott et al., 2002). Our results demonstrate a functional role for inhibition in the plasticity of sensory representations caused by learning. Inhibition is also involved in the plasticity related to altered sensory exposure during development and adolescence. In the somatosensory cortex of rats, GABA-A mediated inhibition is involved in receptive field changes following whisker plucking, such that blocking inhibition shifts receptive fields back towards their normal shape (Foeller et al., 2005). In contrast, blocking inhibition in ventral and dorsal NCM enhances, not reduces, the plasticity in responses that accompany learning. Inhibition serves a similar function in the early stages of learning in barn owls that have been equipped with visual prisms, where inhibition opposes the adaptive changes in the auditory space map of the inferior colliculus (Zheng and Knudsen, 2001). As learning progresses, inhibition switches to inactivate the normal map and enhance the representation of the new learned map (Zheng and Knudsen, 1999). Inhibition must perform a different function in the dorsal NCM of songbirds, because the starlings in this experiment were trained extensively and were well beyond the initial stage of learning. In dorsal NCM, the preference for learned songs may be expressed in the early stages of song recognition learning and then inactivated by inhibition in the

late stages. It is unclear why inhibition inactivates the representation of learned songs in dorsal NCM. Inhibition may counteract excitatory inputs that respond strongly to learned songs in order to maintain a similar representation of learned and unfamiliar songs. CMM is a possible source of this input because neurons in CMM respond preferentially to learned songs and project to NCM (Gentner and Margoliash, 2003; Vates et al., 1996).

In ventral NCM, neurons respond more strongly to unfamiliar songs than to songs that songbirds have learned to recognize (Thompson and Gentner, 2010). We hypothesized that blocking inhibition in ventral NCM would inactivate the preference for unfamiliar songs and make the responses to learned and unfamiliar songs similar. Instead, blocking inhibition in ventral NCM increased the preference for unfamiliar songs. This shows that the preference for unfamiliar songs in ventral NCM is not caused by increased inhibition during learned songs and suggests that it is instead caused by weakened excitatory drive to learned songs.

Inhibition shapes selectivity in the responses of neurons in several regions of the brain (Ingham and McAlpine, 2005; Muller and Scheich, 1987; Sillito, 1975; Wang et al., 2002b; Xie et al., 2005). Blocking inhibition in these areas reduces the stimulus selectivity of neurons. In the auditory forebrain regions field L and CM of chicks, inhibition controls the selectivity for natural sounds (Muller and Scheich, 1987). Blocking local inhibition in NCM reduced the selectivity for song motifs. This demonstrates that selectivity for complex features of conspecific vocalizations in NCM is in part caused by local inhibition. However, blocking inhibition did not

completely abolish the tuning for song motifs. A degree of selectivity may be inherited from other parts of NCM or CMM, where neurons are also highly selective for song motifs (Gentner and Margoliash, 2003; Meliza et al., 2010). A similar role for inhibition has been found in the inferotemporal cortex of primates, where blocking inhibition reduces selectivity for complex visual objects (Wang et al., 2000). Typically studies that report a decrease in stimulus selectivity when inhibition is blocked observe an expansion of receptive fields (Andoni et al., 2007; Chen and Jen, 2000; Muller and Scheich, 1987; Sillito, 1975; Thiele et al., 2004; Wang et al., 2002a; Wang et al., 2002b). Most of the studies examining the effect of inhibition on neuronal responses use artificial stimuli such as tones (Chen and Jen, 2000; Wang et al., 2002a). In this experiment we used natural starling songs, which are spectrotemporally complex and vary greatly over time. We found that blocking inhibition had no consistent effects on the shapes of NCM receptive fields, but increased the gain in spiking nonlinearities. Similarly, blocking inhibition in NCM has little effect on tonal receptive fields (Pinaud et al., 2008). Blocking inhibition significantly increased the percentage of information explained by the receptive fields. Inhibition may be responsible for the nonlinearity in the responses of neurons that has limited the performance of receptive field models in other sensory regions (Carandini et al., 2005).

While we did not detect changes in the shape of receptive fields of NCM sites, we still observed large changes in the responses to songs with inhibition blocked. The magnitude of the release from inhibition varied across songs and blocking

inhibition reduced stimulus selectivity. We also found that blocking inhibition unmasks responses to portions of song that elicited no response with inhibition intact. Interestingly, decomposing song motifs into notes unmasked responses in CMM neurons to specific notes that are silenced when motifs are played as a whole (Meliza et al., 2010). Similar inhibitory interactions operating on a longer timescale may be responsible for the masked responses in NCM. In several earlier studies, when blocking inhibition widened receptive fields, neurons began to respond to stimuli that elicited no response when inhibition was intact (Chen and Jen, 2000; Muller and Scheich, 1987; Sillito, 1975; Thiele et al., 2004; Wang et al., 2002a; Wang et al., 2002b). The changes observed here extend these results to show how blocking inhibition can uncover responses to select regions of complex stimuli and dramatically change the spike pattern in responses. It is unclear why inhibition silences robust and specific responses to portions of song. One possibility is that inhibition selectively silences excitatory inputs to change the representation of songs as experience changes their behavioral relevance. Consistent with the notion that learning causes inhibition to cover and uncover responses in order to change representations; we find that in dorsal NCM there is a higher frequency of uncovered responses to learned songs than to unfamiliar songs. This may be a general mechanism that allows the representations in sensory regions of the brain to change by modulating levels of inhibition.

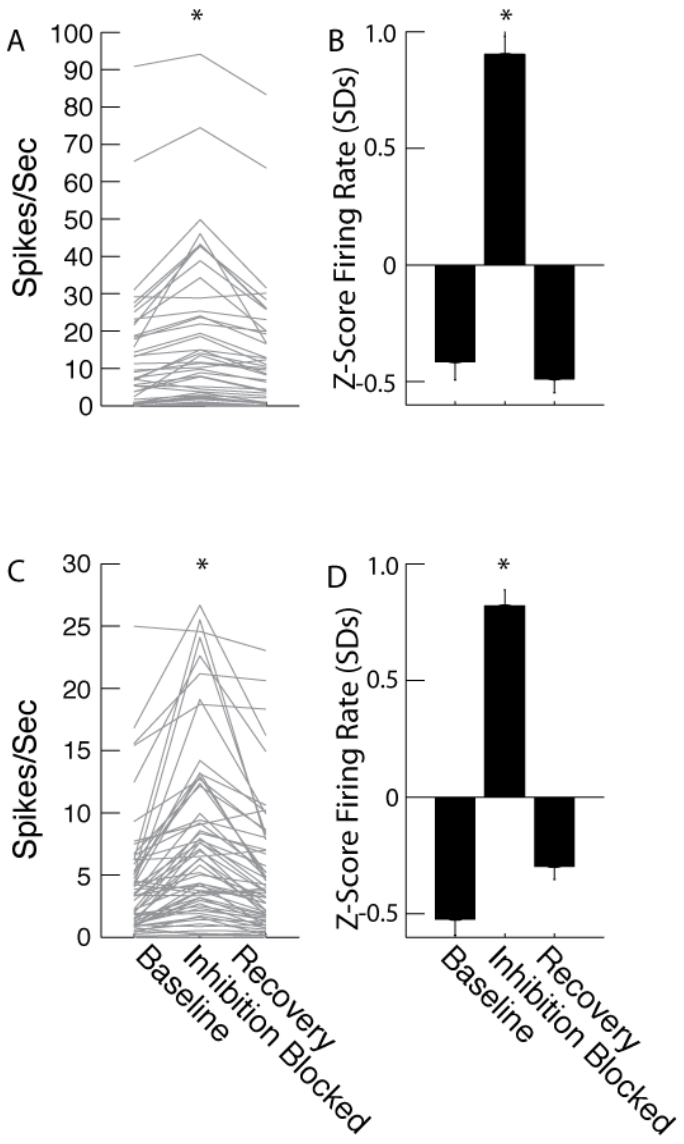

Figure 3.S1. Blocking local inhibition increases spontaneous firing rates. (A) Change in spontaneous firing rate with inhibition blocked for ventral sites. Each line shows the change in firing for an individual site. (B) Z-score of spontaneous firing rates to song for ventral sites in baseline, inhibition blocked and recovery conditions. (C) Same as in A) for dorsal sites. (D Same as in B) for Dorsal Sites.

References

Andoni, S., Li, N., and Pollak, G.D. (2007). Spectrotemporal receptive fields in the inferior colliculus revealing selectivity for spectral motion in conspecific vocalizations. J Neurosci *27*, 4882-4893.

Atencio, C.A., Sharpee, T.O., and Schreiner, C.E. (2008). Cooperative nonlinearities in auditory cortical neurons. Neuron *58*, 956-966.

Bakin, J.S., and Weinberger, N.M. (1990). Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. Brain Res *536*, 271-286.

Bao, S., Chan, V.T., Zhang, L.I., and Merzenich, M.M. (2003). Suppression of cortical representation through backward conditioning. Proc Natl Acad Sci U S A *100*, 1405-1408.

Bieszczad, K.M., and Weinberger, N.M. (2009). Learning strategy trumps motivational level in determining learning-induced auditory cortical plasticity. Neurobiol Learn Mem.

Buonomano, D.V., and Merzenich, M.M. (1998). Cortical plasticity: from synapses to maps. Annu Rev Neurosci *21*, 149-186.

Carandini, M., Demb, J.B., Mante, V., Tolhurst, D.J., Dan, Y., Olshausen, B.A., Gallant, J.L., and Rust, N.C. (2005). Do we know what the early visual system does? The Journal of neuroscience : the official journal of the Society for Neuroscience *25*, 10577-10597.

Chen, Q.C., and Jen, P.H. (2000). Bicuculline application affects discharge patterns, rate-intensity functions, and frequency tuning characteristics of bat auditory cortical neurons. Hear Res *150*, 161-174.

Chew, S.J., Vicario, D.S., and Nottebohm, F. (1996). A large-capacity memory system that recognizes the calls and songs of individual birds. Proc Natl Acad Sci U S A *93*, 1950-1955.

Diamond, D.M., and Weinberger, N.M. (1984). Physiological plasticity of single neurons in auditory cortex of the cat during acquisition of the pupillary conditioned response: II. Secondary field (AII). Behav Neurosci *98*, 189-210.

Feldman, D.E. (2009). Synaptic mechanisms for plasticity in neocortex. Annu Rev Neurosci *32*, 33-55.

Foeller, E., Celikel, T., and Feldman, D.E. (2005). Inhibitory sharpening of receptive fields contributes to whisker map plasticity in rat somatosensory cortex. Journal of neurophysiology *94*, 4387-4400.

Fritz, J., Shamma, S., Elhilali, M., and Klein, D. (2003). Rapid task-related plasticity of spectrotemporal receptive fields in primary auditory cortex. Nat Neurosci *6*, 1216-1223.

Galindo-Leon, E.E., Lin, F.G., and Liu, R.C. (2009). Inhibitory Plasticity in a Lateral Band Improves Cortical Detection of Natural Vocalizations. Neuron *62*, 705-716.

Gentner, T.Q., and Margoliash, D. (2003). Neuronal populations and single cells representing learned auditory objects. Nature *424*, 669-674.

George, I., Cousillas, H., Richard, J.-P., and Hausberger, M. (2008). A potential neural substrate for processing functional classes of complex acoustic signals. PLoS One *3*, e2203.

Gierdalski, M., Jablonska, B., Siucinska, E., Lech, M., Skibinska, A., and Kossut, M. (2001). Rapid regulation of GAD67 mRNA and protein level in cortical neurons after sensory learning. Cereb Cortex *11*, 806-815.

Hensch, T.K. (2005). Critical period plasticity in local cortical circuits. Nat Rev Neurosci *6*, 877-888.

Hensch, T.K., Fagiolini, M., Mataga, N., Stryker, M.P., Baekkeskov, S., and Kash, S.F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science *282*, 1504-1508.

Ingham, N.J., and McAlpine, D. (2005). GABAergic inhibition controls neural gain in inferior colliculus neurons sensitive to interaural time differences. The Journal of neuroscience : the official journal of the Society for Neuroscience *25*, 6187-6198.

Jasinska, M., Siucinska, E., Cybulska-Klosowicz, A., Pyza, E., Furness, D.N., Kossut, M., and Glazewski, S. (2010). Rapid, learning-induced inhibitory synaptogenesis in murine barrel field. J Neurosci *30*, 1176-1184.

Jenkins, W.M., Merzenich, M.M., Ochs, M.T., Allard, T., and Guic-Robles, E. (1990). Functional reorganization of primary somatosensory cortex in adult owl monkeys after behaviorally controlled tactile stimulation. Journal of neurophysiology *63*, 82-104.

Knott, G.W., Quairiaux, C., Genoud, C., and Welker, E. (2002). Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. Neuron *34*, 265-273.

Lech, M., Skibinska, A., and Kossut, M. (2001). Delayed upregulation of GABA(A) alpha1 receptor subunit mRNA in somatosensory cortex of mice following learning-dependent plasticity of cortical representations. Brain Res Mol Brain Res *96*, 82-86.

Liguz-Lecznar, M., Waleszczyk, W.J., Zakrzewska, R., Skangiel-Kramska, J., and Kossut, M. (2009). Associative pairing involving monocular stimulation selectively mobilizes a subclass of GABAergic interneurons in the mouse visual cortex. J Comp Neurol *516*, 482-492.

Maffei, A., Nataraj, K., Nelson, S.B., and Turrigiano, G.G. (2006). Potentiation of cortical inhibition by visual deprivation. Nature *443*, 81-84.

Meliza, C.D., Chi, Z., and Margoliash, D. (2010). Representations of conspecific song by starling secondary forebrain auditory neurons: toward a hierarchical framework. Journal of neurophysiology *103*, 1195-1208.

Müller, C.M., and Leppelsack, H.J. (1985). Feature extraction and tonotopic organization in the avian forebrain. Experimenatl Brain Research *59*, 587-599.

Muller, C.M., and Scheich, H. (1987). GABAergic inhibition increases the neuronal selectivity to natural sounds in the avian auditory forebrain. Brain research *414*, 376-380.

Ohl, F.W., and Scheich, H. (1996). Differential frequency conditioning enhances spectral contrast sensitivity of units in auditory cortex (field Al) of the alert Mongolian gerbil. Eur J Neurosci *8*, 1001-1017.

Pinaud, R., Terleph, T.A., Tremere, L.A., Phan, M.L., Dagostin, A.A., Leao, R.M., Mello, C.V., and Vicario, D.S. (2008). Inhibitory network interactions shape the auditory processing of natural communication signals in the songbird auditory forebrain. J Neurophysiol *100*, 441-455.

Pinaud, R., Velho, T.A., Jeong, J.K., Tremere, L.A., Leao, R.M., von Gersdorff, H., and Mello, C.V. (2004). GABAergic neurons participate in the brain's response to birdsong auditory stimulation. Eur J Neurosci *20*, 1318-1330.

Recanzone, G.H., Schreiner, C.E., and Merzenich, M.M. (1993). Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. J Neurosci *13*, 87-103.

Ribeiro, S., Cecchi, G.A., Magnasco, M.O., and Mello, C.V. (1998). Toward a song code: evidence for a syllabic representation in the canary brain. Neuron *21*, 359-371.

Rolls, E.T., and Tovee, M.J. (1995). Sparseness of the neuronal representation of stimuli in the primate temporal visual cortex. Journal of neurophysiology *73*, 713-726.

Sharpee, T., Rust, N.C., and Bialek, W. (2004). Analyzing neural responses to natural signals: maximally informative dimensions. Neural Comput *16*, 223-250.

Sharpee, T.O., Sugihara, H., Kurgansky, A.V., Rebrik, S.P., Stryker, M.P., and Miller, K.D. (2006). Adaptive filtering enhances information transmission in visual cortex. Nature *439*, 936-942.

Sillito, A.M. (1975). The contribution of inhibitory mechanisms to the receptive field properties of neurones in the striate cortex of the cat. J Physiol *250*, 305-329.

Stripling, R., Volman, S.F., and Clayton, D.F. (1997). Response modulation in the zebra finch neostriatum: relationship to nuclear gene regulation. J Neurosci *17*, 3883-3893.

Thiele, A., Distler, C., Korbmacher, H., and Hoffmann, K.P. (2004). Contribution of inhibitory mechanisms to direction selectivity and response normalization in macaque middle temporal area. Proceedings of the National Academy of Sciences of the United States of America *101*, 9810-9815.

Thompson, J.V., and Gentner, T.Q. (2010). Song recognition learning and stimulus-specific weakening of neural responses in the avian auditory forebrain. J Neurophysiol.

Tokarski, K., Urban-Ciecko, J., Kossut, M., and Hess, G. (2007). Sensory learning-induced enhancement of inhibitory synaptic transmission in the barrel cortex of the mouse. Eur J Neurosci *26*, 134-141.

Tolhurst, D.J., Smyth, D., and Thompson, I.D. (2009). The sparseness of neuronal responses in ferret primary visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience *29*, 2355-2370.

Vates, G.E., Broome, B.M., Mello, C.V., and Nottebohm, F. (1996). Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. J Comp Neurol *366*, 613-642.

Vinje, W.E., and Gallant, J.L. (2000). Sparse coding and decorrelation in primary visual cortex during natural vision. Science *287*, 1273-1276.

Vinje, W.E., and Gallant, J.L. (2002). Natural stimulation of the nonclassical receptive field increases information transmission efficiency in V1. The Journal of neuroscience : the official journal of the Society for Neuroscience *22*, 2904-2915.

Wang, J., McFadden, S.L., Caspary, D., and Salvi, R. (2002a). Gamma-aminobutyric acid circuits shape response properties of auditory cortex neurons. Brain research *944*, 219-231.

Wang, Y., Fujita, I., and Murayama, Y. (2000). Neuronal mechanisms of selectivity for object features revealed by blocking inhibition in inferotemporal cortex. Nature neuroscience *3*, 807-813.

Wang, Y., Fujita, I., Tamura, H., and Murayama, Y. (2002b). Contribution of GABAergic inhibition to receptive field structures of monkey inferior temporal neurons. Cerebral cortex (New York, N Y : 1991) *12*, 62-74.

Weinberger, N.M. (1995). Dynamic regulation of receptive fields and maps in the adult sensory cortex. Annu Rev Neurosci *18*, 129-158.

Wiesel, T.N., and Hubel, D.H. (1965). Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J Neurophysiol *28*, 1029-1040.

Xie, R., Meitzen, J., and Pollak, G.D. (2005). Differing roles of inhibition in hierarchical processing of species-specific calls in auditory brainstem nuclei. Journal of neurophysiology *94*, 4019-4037.

Zhang, L.I., Bao, S., and Merzenich, M.M. (2001). Persistent and specific influences of early acoustic environments on primary auditory cortex. Nat Neurosci *4*, 1123-1130.

Zheng, W., and Knudsen, E.I. (1999). Functional selection of adaptive auditory space map by GABAA-mediated inhibition. Science *284*, 962-965.

Zheng, W., and Knudsen, E.I. (2001). Gabaergic inhibition antagonizes adaptive adjustment of the owl's auditory space map during the initial phase of plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience *21*, 4356-4365.

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IV. Conclusion

The preceding chapters describe results that make several important contributions to the understanding of vocal recognition. In chapter one, we demonstrated that song recognition learning modified responses in NCM, which suggests that NCM is involved in the storage of vocalizations. In a broader context, our results add to a growing literature showing that learning can lead to diverse types of changes in the auditory system. In chapter two, we demonstrated a functional role for local inhibition in learning-related plasticity and surprisingly we showed that this role was to inactivate plastic representations. Finally, we showed that local inhibition contributes to the selectivity for vocalizations in the songbird.

Our results demonstrate that learning to recognize conspecific songs changes the representation of auditory information in NCM of songbirds. Overall, we found that in ventral NCM, learning decreased the responses of ventral NCM neurons to the songs that were learned in recognition training, effectively enhancing the representation of unfamiliar songs. In the dorsal region of NCM, learning had no effect on responses and neurons responded similarly to the learned and unfamiliar songs. We determined that associative learning caused the changes in ventral NCM, rather than stimulus exposure, because neurons continued to respond strongly to songs that were heard passively, while responding weakly to the learned songs. These results suggest that ventral NCM is involved in song recognition learning, however the function of ventral NCM is unclear. The enhanced representation of unfamiliar (and passively heard) songs may indicate that ventral NCM is specialized

for processing songs that songbirds have not yet learned to recognize. In their social environment, songbirds interact with conspecifics and process song stimuli that they have not learned to recognize (Eens, 1997). In addition, starlings continue to add songs motifs to their repertoires throughout adulthood and must process unfamiliar songs before they can be memorized (Chaiken et al., 1994). The representation of unfamiliar songs in ventral NCM may be necessary for processing the songs of individuals before they are learned for either recognition or for song production. Ventral NCM could also be involved in the initial stages of learning to recognize new songs. In our studies, we recorded the activity of NCM neurons long after the songbirds had initially learned to recognize the songs. When songs are being acquired, they may be represented differently in ventral NCM than after learning is complete. As songs are learned, the representation in ventral NCM could gradually weaken and once learning has stabilized, ventral NCM would no longer be involved in the processing of the learned song. Other studies support a role for NCM in the acquisition of new song associations. IEG expression is enhanced in NCM during the acquisition stage of song recognition learning, but not during the recognition of songs after the association is learned or while learning new associations for already learned songs (Gentner et al., 2004). Several studies have suggested that NCM is most involved in processing song stimuli when they are initially heard. Neural responses in NCM adapt rapidly, such that responses decrease as song stimuli are repeated (Chew et al., 1995; Mello et al., 1995; Stripling et al., 1997). Our results also showed that NCM neurons are tuned to respond to

novel songs and showed that the responses of NCM neurons are strongly shaped by recognition learning. Our findings in NCM might extend to other animals that use vocal recognition. A specialized region for representing vocalizations that have not been learned might be important for efficient recognition.

When local inhibition was blocked in NCM, we uncovered a strong representation of learned songs in dorsal NCM that is normally inactivated. This suggests that dorsal NCM has the potential to be involved in the storage of learned songs. The demonstration of plasticity in dorsal NCM from song learning is consistent with earlier work showing that IEG expression is elevated in both ventral and dorsal NCM during the acquisition stage of song recognition learning (Gentner et al., 2004). The plasticity in dorsal NCM may only be inactivated by inhibition in certain stages of learning. All of the songbirds in this study were trained well beyond acquisition of song recognition and it is not known whether the representation of learned songs in dorsal NCM is inactivated during earlier stages of learning. In the inferior colliculus of the barn owl, inhibition inactivates the learned representation of auditory space during the initial stages of learning (Zheng and Knudsen, 2001). There is currently no evidence to support a similar mechanism in dorsal NCM. One possibility is that the representation of learned songs in dorsal NCM is important in the early stages of learning and does not become inactivated by inhibition until later stages of learning. Once the acquisition of song associations is complete, the representation of learned songs may become inactivated in order to restore an equal representation of both learned and unfamiliar songs.

NCM has also been implicated in tutor song learning and memory (Bolhuis et al., 2000; Gobes and Bolhuis, 2007; Phan et al., 2006; Terpstra et al., 2004). The level of IEG expression after hearing tutor song and the adaptation rate to tutor song is correlated to how well a songbird's song matches the tutor song (Bolhuis et al., 2000; Phan et al., 2006; Terpstra et al., 2004). However, using the similarity between a bird's song and the tutor song as a measure of the strength of memory has several assumptions that may be problematic. This assumes that only the strength of the tutor song memory is responsible for how well the bird copies the tutor song. This has not been established and it is not clear that all birds are trying to match the song of the tutor as closely as possible. Other factors may be involved, such as physical limitations in singing. A bird may have a perfect memory of the tutor song, but may not be able to sing it due to imperfect motor control. Further studies are required to clearly demonstrate a role for NCM in tutor song memory. How the roles of NCM in general conspecific song learning and tutor song learning are related has not been investigated. It is unknown whether tutor song is represented differently in NCM than other conspecific songs. One study reported increased IEG expression following tutor song exposure (Bolhuis et al., 2000), but it was later shown that this increase was similar to the increase following exposure to conspecific songs (Terpstra et al., 2004). No study has reported either increased or decreased electrophysiological responses to tutor song versus other conspecific songs. Electrophysiological responses to a bird's own song, which is similar to the tutor song, have been shown to be similar to responses to other conspecific songs

(Chew et al., 1996; Stripling et al., 1997). The plasticity we observed following song recognition learning may also be involved in tutor song memory. The storage of songs for recognition and song production could be stored with the same neurons and plasticity mechanisms. It may be that ventral NCM responses to tutor song are weakened similarly to songs that songbirds learn to recognize. Information about songs learned for recognition and production could be processed and stored similarly in the auditory system and then transferred to other parts of the brain for more specialized processing. Projections from the auditory system to the songs system could provide a path for song memories to be accessed by the song production system. The mechanisms that are thought to be involved in the storage of tutor song memory in NCM could also be involved in the memory of songs that birds have learned to recognize. The adaptation of NCM responses to learned conspecific songs has not been examined. We did not observe any effects of adaptation in our experiments, though we did not actively try to induce adaptation. Functional studies, such as the targeted inactivation of NCM during different stages of song recognition learning and tutor song learning are required to determine the roles of NCM in song learning.

NCM is likely part of a functional circuit for song processing and memory with the other secondary auditory nuclei CMM and CLM. Recognition learning also changes the representation of songs in CMM. Following recognition training, CMM neurons respond more strongly to learned songs than to unfamiliar songs (Gentner and Margoliash, 2003). CMM neurons are active during several stages of song

recognition learning. IEG expression was increased during the acquisition of new song associations, while recognizing learned songs and while learning new associations for songs that have been already learned (Gentner et al., 2004). While it is tempting to suggest that the strong representation of learned songs in CMM improves the perceptual representation and contributes to the memory for learned songs, a functional role has not yet been demonstrated. No study has recorded the activity of CMM and NCM neurons simultaneously, but combined with our results, the findings on CMM suggest that as learning progresses the representation of songs decreases in ventral NCM and increases in CMM. Having segregated representations of sensory stimuli that are behaviorally relevant and that are not behaviorally relevant may have evolved as part of the mechanisms underlying song recognition learning. The representation of learned songs in ventral NCM and CMM may be functionally connected. NCM and CMM are connected through reciprocal projections (Vates et al., 1996) and both NCM and CMM contain large portions of inhibitory neurons (Pinaud and Mello, 2007). If ventral NCM neurons, that represent all songs strongly except learned songs, projected onto inhibitory neurons in CMM, this could inhibit the responses to all unfamiliar songs and enhance the representation of learned songs. Responses in CLM also change with learning. Following song recognition learning, the magnitude of responses to learned and unfamiliar songs is similar, but the learned songs are represented with higher information (J. Jeanne et al. submitted). Inputs from CLM and NCM could combine to form the representation of learned songs in CMM. Regardless of the mechanism, it is clear that CMM, NCM

and CLM all change the representation of songs with learning and are likely performing different functions that contribute to the processing and learning of songs. The network of secondary auditory nuclei may be involved in not only the memory of conspecific songs, but also in the memory of tutor songs. There is currently no evidence showing that CLM or CMM are important for tutor song learning, however, determining how these regions are involved is an interesting avenue for future study.

Our finding of weakened responses to learned songs after recognition learning are surprising given the large body of work on plasticity in the auditory system of mammals. Numerous studies over the last few decades have shown that learning most often leads to increases in the responses of neurons in the auditory system (Weinberger, 2004). Training animals with classical conditioning increases the firing rates of single neurons in the auditory cortex to training sounds and shifts the receptive fields towards the training sounds (Bakin and Weinberger, 1990; Diamond and Weinberger, 1984, 1986). Training animals on auditory discrimination tasks shifts the receptive fields of single neurons towards training sounds and increases the region of the cortex responsive to the training sound (Edeline and Weinberger, 1993; Recanzone et al., 1993). A few studies have shown that learning does not always lead to an increase in the responses to learned sounds. Training animals on discrimination tasks with multiple target or reference tones leads to a decrease in the firing rate to training tones and increases the firing rate to surrounding frequencies (Ohl and Scheich, 1996, 1997; Witte and Kipke, 2005).

However, the unusual design of these tasks and the lack of behavioral measures to explain what the animals learn, make these studies difficult to interpret (Weinberger 04). Using a simple song recognition task where songbirds clearly learn to recognize the songs, we showed that in ventral NCM, learning leads to a decrease in responses to learned songs. The plasticity that blocking inhibition uncovered in dorsal NCM is similar to most of the findings in auditory cortex, however, this representation of learned song is inactivated by inhibition. There are several differences between our study and most studies of auditory plasticity. Most studies are performed on mammals, whereas we focused on songbirds due to their rich communication system that relies on auditory learning (Stoddard, 1996). Our results likely are not due to a species difference because recognition learning causes neurons to increase their response in CMM, consistent with most studies in the auditory cortex in mammals (Gentner and Margoliash, 2003). In addition, most studies examine plasticity in the primary auditory cortex, whereas we examined plasticity in a secondary auditory region that receives input from the analogue to the primary auditory cortex in birds. Investigating the changes in the belt and parabelt regions of the auditory cortex, where neurons have more complex receptive fields, is necessary to understand auditory learning in mammals (Rauschecker et al., 1995). Most previous studies also examine plasticity caused by training animals with simple sounds such as tones or frequency sweeps, whereas we trained starlings with segments of natural song. Learning with natural sounds may engage a wider variety of plasticity mechanisms than learning with simple sounds

such as tones. This is supported by a study showing that a natural form of learning increases the suppression in responses to ultrasonic pup calls in mice (Galindo-Leon et al., 2009). Examining changes in the auditory cortex of mammals following training with natural stimuli may reveal more results similar to ours. With earlier work (Gentner and Margoliash, 2003), our results show that vocal recognition learning involves a variety of plasticity mechanisms including both increases and decreases in the responses to learned vocalizations. These results may extend to other species that practice vocal recognition such as humans.

Local inhibition is important for plasticity in sensory systems that results from altered sensory exposure during development and adolescence (Feldman, 2009). Local inhibition in the visual cortex controls the onset of the critical period for monocular deprivation plasticity (Hensch et al., 1998). After eye closure, the responses to the deprived eye in the visual cortex are weakened through a strengthening of inhibitory synapses (Maffei et al., 2006). Similarly, plucking whiskers causes local inhibition to weaken the responses to the deprived whisker in the somatosensory cortex (Foeller et al., 2005). Inhibition is also involved in the plasticity of the auditory space map of owls with altered visual input from wearing visual prisms. Inhibition inactivates the normal map of space to enhance the learned map of space (Zheng and Knudsen, 1999). We hypothesized that local inhibition might also be important for plasticity underlying vocal recognition learning. To investigate the mechanisms of vocal recognition learning in songbirds, we manipulated local inhibition in NCM of starlings following song recognition learning.

In ventral NCM, local inhibition weakened the preference for learned songs that results from song recognition learning. In dorsal NCM, local inhibition inactivated a strong preference for learned songs. These results show that local inhibition does not cause the plasticity in the representation of songs in NCM, but that inhibition has a modulatory effect. This is the first demonstration of a functional role for local inhibition in plasticity that results from learning. Several studies have pointed to a role for inhibition in learning-related plasticity. Fear conditioning with visual or somatosensory stimuli increases the level of GABA and GABA receptors in the primary visual or somatosensory cortex respectively (Gierdalski et al., 2001; Lech et al., 2001; Liguz-Lecznar et al., 2009). In addition, fear conditioning increased the density of inhibitory synapses and strengthened GABAergic transmission in the primary somatosensory cortex (Jasinska et al., 2010; Tokarski et al., 2007). It is not clear what the functional role of inhibition is in these systems. The inactivation of plastic representations may be important for other forms of learning. This may be an effective mechanism for modifying representations that must change on a short timescale. Nevertheless, our results show one way in which inhibition influences learning-related plasticity. Further, our results demonstrate a role for local inhibition in vocal recognition learning.

Neurons in many higher-level sensory areas are selective for complex stimuli such as vocalizations. In the inferotemporal cortex (IT) of the visual system, neurons respond selectively to complex objects (Gross et al., 1972) and subpopulations of neurons have been identified that are highly selective for human faces (Bruce et al.,

1981). Neurons that are highly selective for vocalizations have been found in several regions of the auditory system. In songbirds, neurons in CMM respond selectively to segments of conspecific songs (Gentner and Margoliash, 2003; Meliza et al., 2010). We showed that many neurons in NCM are also selective for specific motifs of conspecific songs. In primates, neurons in the belt of the auditory cortex respond selectively to vocalizations (Rauschecker et al., 1995). Recently a region in the insular cortex of primates was found to respond selectively to monkey vocalizations (Remedios et al., 2009). Local inhibition contributes to stimulus selectivity in several regions of the brain. Inhibition controls part of the selectivity for complex visual objects in IT cortex (Wang et al., 2000; Wang et al., 2002b). Blocking inhibition in IT causes neurons to respond to larger numbers of complex objects (Wang et al., 2000; Wang et al., 2002b). Inhibition has been shown to contribute to the selectivity of neurons throughout the auditory system. Blocking inhibition in IC reduces the selectivity for sounds (Klug et al., 2002; LeBeau et al., 2001; Xie et al., 2005). Similarly, in AI, inhibition increases the selectivity for frequency (Chang et al., 2005; Chen and Jen, 2000; Wang et al., 2002a). Inhibition has also been shown to contribute to the selectivity for complex sounds in field L and CM of chicks (Muller and Scheich, 1987). Despite the well-established role for inhibition in stimulus selectivity, it was unclear whether this extends to the selectivity for vocalizations. We showed that local inhibition contributes to the selectivity for components of conspecific song in NCM. Blocking local inhibition reduced the selectivity for song motifs and caused sites to respond to larger numbers of motifs. The highly selective

responses to song stimuli are thought to be important for the hierarchical processing of songs for vocal recognition. This suggests that while inhibition does not cause the plasticity that results from song learning, the selectivity it causes may be important for the accurate recognition of vocalizations. This may be a general mechanism that contributes to the recognition of vocalizations in other species.

The results described here raise several new questions for future research. Why does ventral NCM respond weaker to learned songs than to unfamiliar songs? Is this plasticity specific to song stimuli? What is the role of ventral NCM in song recognition learning? What is the role of dorsal NCM in song recognition learning? How do NCM and CMM interact during song processing and song learning? Why does local inhibition weaken plasticity in ventral NCM and inactivate plasticity in dorsal NCM? Does inhibition play a similar role in other forms of learning-related plasticity? Answering these questions will continue to improve our understanding of the neural mechanisms of vocal recognition.

References

Bakin, J.S., and Weinberger, N.M. (1990). Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. Brain Res *536*, 271-286.

Bolhuis, J.J., Zijlstra, G.G., den Boer-Visser, A.M., and Van Der Zee, E.A. (2000). Localized neuronal activation in the zebra finch brain is related to the strength of song learning. Proc Natl Acad Sci U S A *97*, 2282-2285.

Bruce, C., Desimone, R., and Gross, C.G. (1981). Visual properties of neurons in a polysensory area in superior temporal sulcus of the macaque. J Neurophysiol *46*, 369-384.

Chaiken, M., Bohner, J., and Marler, P. (1994). REPERTOIRE TURNOVER AND THE TIMING OF SONG ACQUISITION IN EUROPEAN STARLINGS. Behaviour *128*, 25-39.

Chang, E.F., Bao, S., Imaizumi, K., Schreiner, C.E., and Merzenich, M.M. (2005). Development of spectral and temporal response selectivity in the auditory cortex. Proc Natl Acad Sci U S A *102*, 16460-16465.

Chen, Q.C., and Jen, P.H. (2000). Bicuculline application affects discharge patterns, rate-intensity functions, and frequency tuning characteristics of bat auditory cortical neurons. Hear Res *150*, 161-174.

Chew, S.J., Mello, C., Nottebohm, F., Jarvis, E., and Vicario, D.S. (1995). Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. Proc Natl Acad Sci U S A *92*, 3406-3410.

Chew, S.J., Vicario, D.S., and Nottebohm, F. (1996). A large-capacity memory system that recognizes the calls and songs of individual birds. Proc Natl Acad Sci U S A *93*, 1950-1955.

Diamond, D.M., and Weinberger, N.M. (1984). Physiological plasticity of single neurons in auditory cortex of the cat during acquisition of the pupillary conditioned response: II. Secondary field (AII). Behav Neurosci *98*, 189-210.

Diamond, D.M., and Weinberger, N.M. (1986). Classical conditioning rapidly induces specific changes in frequency receptive fields of single neurons in secondary and ventral ectosylvian auditory cortical fields. Brain Res *372*, 357-360.

Edeline, J.M., and Weinberger, N.M. (1993). Receptive field plasticity in the auditory cortex during frequency discrimination training: selective retuning independent of task difficulty. Behav Neurosci *107*, 82-103.

Eens, M. (1997). Understanding the complex song of the European starling: An integrated ethological approach. In Advances in the Study of Behavior, Vol 26 (San Diego, Elsevier Academic Press Inc), pp. 355-434.

Feldman, D.E. (2009). Synaptic mechanisms for plasticity in neocortex. Annu Rev Neurosci *32*, 33-55.

Foeller, E., Celikel, T., and Feldman, D.E. (2005). Inhibitory sharpening of receptive fields contributes to whisker map plasticity in rat somatosensory cortex. Journal of neurophysiology *94*, 4387-4400.

Galindo-Leon, E.E., Lin, F.G., and Liu, R.C. (2009). Inhibitory Plasticity in a Lateral Band Improves Cortical Detection of Natural Vocalizations. Neuron *62*, 705-716.

Gentner, T.Q., Hulse, S.H., and Ball, G.F. (2004). Functional differences in forebrain auditory regions during learned vocal recognition in songbirds. J Comp Physiol A Neuroethol Sens Neural Behav Physiol *190*, 1001-1010.

Gentner, T.Q., and Margoliash, D. (2003). Neuronal populations and single cells representing learned auditory objects. Nature *424*, 669-674.

Gierdalski, M., Jablonska, B., Siucinska, E., Lech, M., Skibinska, A., and Kossut, M. (2001). Rapid regulation of GAD67 mRNA and protein level in cortical neurons after sensory learning. Cereb Cortex *11*, 806-815.

Gobes, S.M., and Bolhuis, J.J. (2007). Birdsong memory: a neural dissociation between song recognition and production. Curr Biol *17*, 789-793.

Gross, C.G., Rocha-Miranda, C.E., and Bender, D.B. (1972). Visual propoerties of neurons in the inferotemporal cortex of the macaque. Journal of Neurophysiology *35*, 96-111.

Hensch, T.K., Fagiolini, M., Mataga, N., Stryker, M.P., Baekkeskov, S., and Kash, S.F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science *282*, 1504-1508.

Jasinska, M., Siucinska, E., Cybulska-Klosowicz, A., Pyza, E., Furness, D.N., Kossut, M., and Glazewski, S. (2010). Rapid, learning-induced inhibitory synaptogenesis in murine barrel field. J Neurosci *30*, 1176-1184.

Klug, A., Bauer, E.E., Hanson, J.T., Hurley, L., Meitzen, J., and Pollak, G.D. (2002). Response selectivity for species-specific calls in the inferior colliculus of Mexican free-tailed bats is generated by inhibition. Journal of neurophysiology *88*, 1941-1954.

LeBeau, F.E., Malmierca, M.S., and Rees, A. (2001). Iontophoresis in vivo demonstrates a key role for GABA(A) and glycinergic inhibition in shaping frequency response areas in the inferior colliculus of guinea pig. J Neurosci *21*, 7303-7312.

Lech, M., Skibinska, A., and Kossut, M. (2001). Delayed upregulation of GABA(A) alpha1 receptor subunit mRNA in somatosensory cortex of mice following learning-dependent plasticity of cortical representations. Brain Res Mol Brain Res *96*, 82-86.

Liguz-Lecznar, M., Waleszczyk, W.J., Zakrzewska, R., Skangiel-Kramska, J., and Kossut, M. (2009). Associative pairing involving monocular stimulation selectively mobilizes a subclass of GABAergic interneurons in the mouse visual cortex. J Comp Neurol *516*, 482-492.

Maffei, A., Nataraj, K., Nelson, S.B., and Turrigiano, G.G. (2006). Potentiation of cortical inhibition by visual deprivation. Nature *443*, 81-84.

Meliza, C.D., Chi, Z., and Margoliash, D. (2010). Representations of conspecific song by starling secondary forebrain auditory neurons: toward a hierarchical framework. Journal of neurophysiology *103*, 1195-1208.

Mello, C., Nottebohm, F., and Clayton, D. (1995). Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene's response to that song in zebra finch telencephalon. J Neurosci *15*, 6919-6925.

Muller, C.M., and Scheich, H. (1987). GABAergic inhibition increases the neuronal selectivity to natural sounds in the avian auditory forebrain. Brain Res *414*, 376-380.

Ohl, F.W., and Scheich, H. (1996). Differential frequency conditioning enhances spectral contrast sensitivity of units in auditory cortex (field Al) of the alert Mongolian gerbil. Eur J Neurosci *8*, 1001-1017.

Ohl, F.W., and Scheich, H. (1997). Learning-induced dynamic receptive field changes in primary auditory cortex of the unanaesthetized Mongolian gerbil. J Comp Physiol [A] *181*, 685-696.

Phan, M.L., Pytte, C.L., and Vicario, D.S. (2006). Early auditory experience generates long-lasting memories that may subserve vocal learning in songbirds. Proc Natl Acad Sci U S A *103*, 1088-1093.

Pinaud, R., and Mello, C.V. (2007). GABA immunoreactivity in auditory and song control brain areas of zebra finches. J Chem Neuroanat *34*, 1-21.

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

Recanzone, G.H., Schreiner, C.E., and Merzenich, M.M. (1993). Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. J Neurosci *13*, 87-103.

Remedios, R., Logothetis, N.K., and Kayser, C. (2009). An auditory region in the primate insular cortex responding preferentially to vocal communication sounds. J Neurosci *29*, 1034-1045.

Stoddard, P.K. (1996). Vocal recognition of neighbors by territorial passerines. In Ecology and evolution of acoustic communication in birds, D.E. Kroodsma, and E.H. Miller, eds. (Ithaca, Comstock/Cornell), pp. 356-374.

Stripling, R., Volman, S.F., and Clayton, D.F. (1997). Response modulation in the zebra finch neostriatum: relationship to nuclear gene regulation. J Neurosci *17*, 3883-3893.

Terpstra, N.J., Bolhuis, J.J., and den Boer-Visser, A.M. (2004). An analysis of the neural representation of birdsong memory. J Neurosci *24*, 4971-4977.

Tokarski, K., Urban-Ciecko, J., Kossut, M., and Hess, G. (2007). Sensory learning-induced enhancement of inhibitory synaptic transmission in the barrel cortex of the mouse. Eur J Neurosci *26*, 134-141.

Vates, G.E., Broome, B.M., Mello, C.V., and Nottebohm, F. (1996). Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. J Comp Neurol *366*, 613-642.

Wang, J., McFadden, S.L., Caspary, D., and Salvi, R. (2002a). Gamma-aminobutyric acid circuits shape response properties of auditory cortex neurons. Brain Res *944*, 219-231.

Wang, Y., Fujita, I., and Murayama, Y. (2000). Neuronal mechanisms of selectivity for object features revealed by blocking inhibition in inferotemporal cortex. Nat Neurosci *3*, 807-813.

Wang, Y., Fujita, I., Tamura, H., and Murayama, Y. (2002b). Contribution of GABAergic inhibition to receptive field structures of monkey inferior temporal neurons. Cereb Cortex *12*, 62-74.

Weinberger, N.M. (2004). Specific long-term memory traces in primary auditory cortex. Nat Rev Neurosci *5*, 279-290.

Witte, R.S., and Kipke, D.R. (2005). Enhanced contrast sensitivity in auditory cortex as cats learn to discriminate sound frequencies. Brain Res Cogn Brain Res *23*, 171-184.

Xie, R., Meitzen, J., and Pollak, G.D. (2005). Differing roles of inhibition in hierarchical processing of species-specific calls in auditory brainstem nuclei. Journal of neurophysiology *94*, 4019-4037.

Zheng, W., and Knudsen, E.I. (1999). Functional selection of adaptive auditory space map by GABAA-mediated inhibition. Science *284*, 962-965.

Zheng, W., and Knudsen, E.I. (2001). Gabaergic inhibition antagonizes adaptive adjustment of the owl's auditory space map during the initial phase of plasticity. J Neurosci *21*, 4356-4365.