

**EXPOSURE TO NOVEL ACOUSTIC STIMULI INDUCES PLASTICITY IN
AVIAN AUDITORY CORTEX**

by

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ABSTRACT OF THE DISSERTATION

Exposure to Novel Acoustic Stimuli Induces Plasticity in Avian Auditory Cortex

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While early auditory experience establishes the initial organization of the auditory system, cortical plasticity in adulthood enables the organism to adapt an ever-changing environment. The adult auditory system needs to maintain perceptual filters for efficient processing of native stimuli while, at the same time, being ready to respond to novel exemplars and sound categories if they are encountered. The importance of this juxtaposition of stability with plasticity is highlighted in adult learners of a second language who learn to process a novel category of sounds while still maintaining efficient coding for their native language. In the present study, we simulated the foreign immersion experience by exposing zebra finches, a socially gregarious songbird species, to passive playback of either conspecific aviary sounds or heterospecific aviary sounds. After different exposure durations, multiple microelectrodes were placed bilaterally in an area of the bird's auditory cortex, NCM, to record neural responses. The results of Experiment 1 revealed unexpectedly dynamic changes in the normal pattern of lateralization over time. In Experiment 2, birds were similarly exposed to conspecific and heterospecific environments for various times, and then were asked to perform a behavioral discrimination in an operant paradigm. Behavioral discrimination was

significantly enhanced in a subset of birds with previous exposure to heterospecific sounds. Experiment 3 tested spectro-temporal receptive fields (STRFs) in single NCM neurons before and after exposing naïve birds to novel conspecific and heterospecific sounds. Results indicated that brief exposure to song stimuli was sufficient to produce detectable changes in STRFs, and the magnitude of these changes depended on the category of stimuli presented. In sum, the adult auditory cortex is capable of passively detecting and adapting to changes in the statistics of the auditory environment, while maintaining pre-established filters for species-specific vocalizations. Division of labor between hemispheres and/or between neuronal subtypes could underlie this ability to respond to both old and new categories of stimuli, providing both stability and plasticity. These results advance the understanding of adult plasticity, with potential implications for second language learning.

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Chapter 1

General Introduction

The auditory system faces the challenging task of processing a variety of acoustic signals in a noisy environment and in a timely manner. In the large universe of possible sounds, conspecific vocalizations play an essential role in social communication, especially in species that learn, produce and recognize a large repertoire of complex sounds, e.g. humans and songbirds. Detection and discrimination of behaviorally relevant stimuli, i.e., conspecific vocalizations, or other relevant environmental sounds, may depend on a set of perceptual filters, which are selective for salient acoustic contrasts. Furthermore, these perceptual filters potentially improve processing efficiency by filtering out irrelevant stimuli. However, there is a trade-off between enhanced selectivity and discrimination for conspecific vocalizations and the ability to accommodate novel sounds and sound categories, such as new languages or heterospecific sounds. This trade-off is illustrated by the closure of the critical period in infants when the auditory system transitions from being able to discriminate contrasts from a wide variety of languages, to selective discrimination of contrasts in the native language (Kuhl 2004).

We hypothesize that in an auditory environment that is ever-changing, the most successful model for auditory processing is not one where the brain is eternally plastic and responsive to all stimuli, nor is it one where the preferred stimuli are fixed at the end of the critical period. Ideally, the adult auditory cortex should retain patterning established early in development by exposure to a set of behaviorally relevant sounds,

while also being ready to adapt to changes in the current sound environment as they occur. This dissertation will use the songbird, the best-developed model for studying the production and processing of learned vocal signals at the neural level, to 1) investigate both stability and plasticity of auditory cortex in the face of challenges by novel stimuli, 2) relate evidence of neural plasticity to behavioral performance, and 3) explore the dynamics of receptive field changes elicited by brief exposure to unfamiliar conspecific and heterospecific stimuli.

Location of perceptual filters

The auditory system represents spectral-temporal characteristics of the external auditory environment through feature maps (e.g. tonotopy) in primary auditory cortex (Schreiner 1995), with additional computations that give rise to sound localization (Miller and Knudsen, 2001), rhythm, and timbre (Bizley et al., 2009; Woolley et al., 2009) in higher auditory areas. As auditory input travels up the ascending auditory system, the ability to parse the incoming signal depends on the ability to detect the sound through genetically predetermined tonotopy in the cochlea, and also on categorization of the sound as a relevant or irrelevant signal by higher regions in auditory cortex that are selective for conspecific vocalizations or language. While cochlear tonotopy is relatively fixed and not subject to experiential effects, except for rapid adaptation that occurs on the order of milliseconds (Stauffer and Holt, 2007), cells in higher auditory areas encode complex sound categories and represent long-lasting memories of auditory objects as a result of auditory experience (Phan et al., 2006). The neural systems in higher auditory cortex that compute these features can be thought of as a set of perceptual filters that selectively filter/dampen neural responses to incoming stimuli, and adjust category

boundaries based on previous experience. From an ethological standpoint, these perceptual filters determine which sounds are ignored as background noise and which sounds are detected, processed, and translated into action.

Initial establishment of filters

Some perceptual filters are established early in life during a sensitive or critical period for sensory learning (Miller and Knudsen, 2001; Bao et al., 2013; Amin et al., 2013). Because the auditory system is most plastic during the early critical period, much research has been focused on developmental plasticity, its timecourse, and the underlying neural mechanisms (Froemke and Jones, 2010). The influence of early auditory experience on organization is seen in nuclei throughout the ascending auditory pathway, including the cochlear nucleus (Tierney et al., 1997; Mostafapour et al., 2002), inferior colliculus (Knudsen and Brainard, 2003; Gao and Suga, 2000), thalamus, (Speechley et al., 2007), and primary auditory cortex (Zhang et al., 2002; Woolley et al., 2010; Barkat et al., 2011; De Villers-Sidani et al., 2007). Organization of these filters in early development guides selectivity for a set of species-specific sounds (Suga et al., 1997; Maier and Scheich, 1987; Portfors et al., 2009; Ehret 1987; Belin et al., 2000; Amin et al., 2013).

Operant training effects on perceptual filters

Despite establishment and modification of perceptual filters by early experience, the auditory system remains labile in response to new auditory experience even in adulthood. For example, the tonotopic map, a topographically organized map of frequencies in A1, can be altered through operant training in adulthood. Operant training

paradigms that manipulate the behavioral salience of an auditory stimulus lead to map expansions and shrinkages in the area of representation of the target frequency (Bieszczad and Weinberger, 2010, Polley et al., 2006). Furthermore, adult rats trained to attend to a specific stimulus intensity increased cortical representation of the target intensity with no change in the frequency map (Polley et al, 2006). Increased area of representation for a target tone or intensity enhances the neural population response to stimuli at that frequency, leading to selectivity for tones of the target frequency over all other tones. Neural response was also increased after operant conditioning with a complex song stimulus. In a songbird study, adult starlings operantly conditioned to select a reinforced conspecific song in a two-choice paradigm showed increased firing rates in individual neurons, specifically to the reinforced song but not to other novel songs (Gentner and Margoliash 2003).

Possible mechanisms underlying operant training-induced plasticity were investigated by pairing electrical stimulation of nucleus basalis (a major source of excitatory neurotransmitter acetylcholine) with presentation of a 4 Khz tone. This pairing increased the excitation-inhibition (E:I) ratio of the paired frequency in auditory cortex (Polley et al., 2006). Stimulation of nucleus basalis most likely increased salience of the paired tone through release of acetylcholine, resulting in a shift of the E:I balance towards excitation. The change in E:I balance suggested a rapid modification of the tonotopic map and is a possible mechanism for plasticity. Similar modifications of cortical maps have been described in other sensory systems and may rely on the same underlying mechanisms (de Villers-Sidani and Merzenich, 2011).

Passive exposure effects on perceptual filters

Passive exposure to sounds in the environment can also produce plastic changes in auditory responses, although the underlying neural mechanism may be different from those driving plasticity after operant training. Plasticity after passive exposure suggests that implicit learning about altered distributions of sound, or stimulus statistics, is possible, even beyond closure of the critical period. When adult rats were passively exposed to white noise for 6 weeks, they showed changes in A1 tonotopy, tuning specificity and firing rate that reflected the lack of temporal structure in their new auditory environment. After noise-exposed rats were returned to their home environment, it took 8 weeks for A1 characteristics to return to baseline (Zhou et al., 2011), suggesting that plasticity can be induced through passive exposure, but may require an extended length of time.

Additional evidence of experience-induced plasticity was seen in caudomedial nidopallium (NCM), a region analogous to secondary auditory cortex or superficial layers of A1 in songbirds (Wang et al., 2010). Electrophysiological recordings of neurons in NCM of cross-housed zebra finches and canaries showed narrower tuning widths in both cross-housed groups relative to non-cross-housed controls (Terleph et al., 2008). On the contrary, housing in isolation with no acoustic stimulation led to a widening of tuning width (Terleph et al., 2008). Ongoing acoustic stimulation from conspecific sounds is required for maintenance of the *status quo ante*, while narrowing of tuning after exposure to foreign heterospecific song could reflect learning and fine tuning of perceptual filters to accommodate the new feature space. Auditory cortex adapts to hearing novel classes of

sounds, as well as to removal of acoustic stimulation as shown in the case of white noise presentation, or isolation (Zhou et al., 2011; Terleph et al., 2008).

Both these studies suggested that passive exposure or immersion in an auditory context was sufficient to bring about changes in adult auditory cortex and these changes may align auditory maps to better represent external distributions of sound (stimulus statistics). If auditory cortex is constantly adapting to accommodate the distribution of acoustic parameters in its environment, then how much of what we accept as normal or baseline patterning of auditory cortex is maintained by constant exposure to a stable, unchanging auditory environment and how much is genetically predetermined or established early in development? At one extreme, auditory cortex could be highly labile and organization is determined solely by recent experience, while at the other end, auditory cortex could have perceptual filters that are relatively fixed after closure of the critical period. The answer probably lies somewhere in between, with flexibility built on top of a relatively fixed mapping or filter organization.

In this view, plasticity in adult auditory cortex requires a balance between maintaining useful perceptual filters that were established during the early critical period, i.e., phonemic contrasts in the native language in humans, while also being able to adapt to challenges posed by novel stimuli, i.e., exposure to a second language. This juxtaposition of stability vs. plasticity, and old vs. new information, is a challenge for the auditory system. In addition to maintaining proficiency within the native category -- defined by sounds that have a high probability of existing together and are similar in some acoustic dimension -- adults acquiring a second language are tasked with forming a

new category of sounds and performing discriminations within the novel category. So, how might categories be formed? One might imagine that sounds from the native language form a probability distribution based on how those sounds are arranged in a multi-dimensional acoustic space, together with their frequencies of occurrence alone or in combination with other sounds. As auditory input is received, these prior distributions are updated with new exemplars whose frequency generates new probabilities. When one encounters sounds that fall far outside the distribution, they may be perceived at first as general non-native sounds. Thus, during early stages of exposure to a foreign language, with very few exemplars of foreign stimuli, there may exist only categories for native vs. non-native sounds. However, as exposure to foreign stimuli increase, these sounds begin to form their own distribution, and give rise to perceptual filters that underlie recognition of stimuli as belonging to a new category. Filters for both the native and the new category may now co-exist simultaneously in auditory cortex.

Where and how these category ‘memories’ could be stored has yet to be defined. The left hemisphere has been shown to respond preferentially to the contrasts that underlie the phonemes of a particular language, while the right hemisphere prefers stimuli with slower temporal and spectral modulation i.e. the prosody of speech, which is not category-specific (Telkemeyer et al., 2011). Bats also show a left hemisphere response bias for conspecific vocalizations, while the right hemisphere is used in echolocation (Kanwal 2012; Washington and Kanwal, 2012). The division of labor between the hemispheres could result in a stable category memory for the conspecific category in the left hemisphere, while remaining open to non-species-specific sounds in

the opposite hemisphere. Thus, functional hemispheric lateralization could be one way the adult brain solves the stability vs. plasticity conundrum.

In this dissertation, we will answer the following three questions regarding neural plasticity in avian auditory cortex after passive exposure to a set of novel heterospecific stimuli:

Experiment 1: Can exposure to a novel category of sounds in adult songbirds induce changes in response patterns reflecting neural plasticity?

Immersive passive exposure to stimuli with distinctly different acoustic features from conspecific vocalizations has been shown to change neural response properties in mature auditory cortex (Zhou et al., 2011; Terleph et al., 2008). However, if changes in neural response somehow represent acquisition of a new category, there may be fluctuations in neural response between the two hemispheres that occur over the course of passive exposure that are not captured by testing only at the conclusion of exposure. In Experiment 1, we aim to test changes in hemispheric lateralization at several time points over the course of passive novel experience. We hypothesize that some aspects of neural response will fluctuate over the course of passive exposure, ultimately returning to baseline if plasticity was driven by category novelty, while other aspects of the neural response will remain fixed, in order to maintain the ‘native’ category.

Experiment 2: Can exposure to a new sound category improve behavioral discrimination of novel sounds?

Some studies of adult plasticity show that operant conditioning can alter responses to the trained stimulus (Gentner and Margoliash 2003), or that there are

changes in the cortical representation of a target tone (Biezscad and Weinberger, 2010), but do not address whether these changes in auditory cortex are generalizable or how they could alter processing of future stimuli. In Experiment 2, we test whether changes in neural response after passive exposure to novel sounds generalize to other novel stimuli belonging to the same category. Subjects will be tested on discrimination of two novel exemplars from the same category of sounds that they were passively exposed to. We hypothesize that passive exposure to a novel category of sounds prior to operant training will improve performance relative to a control group with no previous experience.

Experiment 3: Does exposure to novel sounds and sound categories have an immediate effect on receptive field organization?

Changes in perception can occur on a short time scale, i.e., when heavily accented speech becomes more discernible over the course of 10-20 minutes (Bradlow and Bent 2008). In Experiment 3, we measure the plasticity of spectro-temporal receptive fields (STRFs) in aviary birds after adaptation to either a heterospecific or conspecific song set. We hypothesize that if adaptation to song modifies perceptual filters to more efficiently process incoming stimuli, heterospecific songs that are a poor fit for existing perceptual filters may cause more of a change in STRFs as auditory cortex stretches to accommodate them.

Background on the Songbird Auditory System

Songbirds are one of the few species capable of vocal learning from a live tutor, aside from humans, cetaceans and bats, which are harder to study in the laboratory. Other mammals, aside from those listed, have not been shown to acquire their vocalizations

from a tutor. Thus, the bird song system is an excellent animal model of vocal learning due to its accessibility and its many parallels with human language acquisition e.g. learned communication from a tutor, a critical period for vocal learning and selectivity for conspecific vocalizations. In addition, recent anatomical evidence suggests close analogies, if not homologies, between the thalamo-cortical auditory system of birds and mammals (Wang et al., 2010; Bolhuis et al., 2010; Haesler et al., 2004; Dugas-Ford et al., 2012; Karten, 2013; Jarvis et. al., 2013). Furthermore, the auditory system of both songbirds and mammals is lateralized for motor and sensory processing (Nottebohm et al., 1976; Floody and Arnold, 1997; Wild et al., 2000; Voss et al., 2007; Poirier et al., 2009; Phan and Vicario, 2010). Although zebra finches, a type of songbird, are primarily studied because they provide a model for vocal imitation early in life, forming auditory memories for song stimuli is not restricted to the critical period when the juvenile zebra finch first learns to produce its song. Because songs are learned by imitation, each juvenile songbird produces a slightly altered, imperfect copy of the tutor's song. Due to these slight differences, each song is a unique identifier of an individual male. These songs are not just used by females in the course of mate selection, but also serve as cues in a social context for individual recognition, and thus remain highly relevant in adulthood for both sexes. The auditory cortex in both songbirds and mammals remains labile to new experiences well after closure of the critical period (Terleph et al., 2008; Zhou et al., 2011; Polley et al., 2006; Bieszczad and Weinberger 2010), perhaps enabling the organism to encode, remember and respond appropriately to unpredictable or novel sounds in its auditory environment. For highly social songbirds, like the zebra finch studied here, this environment is likely to include the songs of unfamiliar birds.

Anatomy of NCM

Caudo-medial nidopallium (NCM) is an important auditory area involved in communication and auditory memory in songbirds (Chew et al., 1995, 1996; Phan et al., 2006). NCM is a region of avian auditory cortex analogous to superficial layers of mammalian A1 or secondary auditory cortex in humans (Wang et al., 2010; Theunissen et al., 2000). Auditory information travels from the ear, to the cochlear nucleus, then up the ascending auditory system through midbrain (MLD), auditory thalamus - nucleus ovoidalis (Ov) to primary auditory area Field L2. Field L2 projects to intermediate areas L1 and L3 which then project to higher auditory areas NCM and CMM (**Figure 1**).

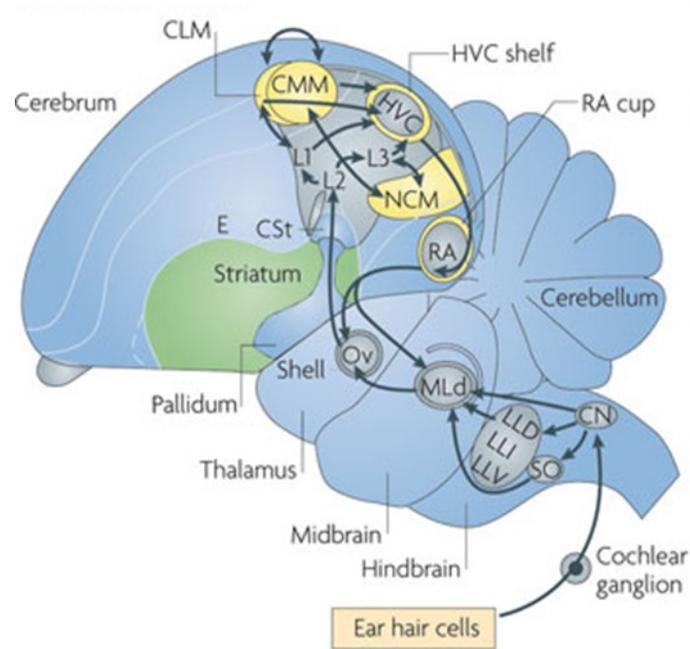


Figure 1. Avian auditory system. Ascending auditory inputs travel from hair cells to nucleus mesencephalicus lateralis pars dorsalis (MLD; avian homolog of mammalian central nucleus of the inferior colliculus), through auditory thalamus, nucleus Ovoidalis (Ov; avian homolog of mammalian medial geniculate nucleus). Ov then projects to forebrain region Field L2, thought to be homologous to thalamo-recipient layers III-IV of mammalian cortex (Wang et al., 2010; Dugas-Ford et al., 2012). Field L2 sends projections to adjacent areas, Field L1 and L3, which in turn project to the caudo-medial nidopallium (NCM) and caudal mesopallium (CLM and CMM).

Tuning and Auditory Response in NCM

NCM is comprised of granule cells with subpopulations of inhibitory, narrow-spiking and excitatory, broad-spiking neurons (Calabrese and Woolley 2015, Pinaud et al., 2008). There is evidence of tonotopy along the dorsal-ventral axis, with higher frequencies coded at more ventral sites. This tonotopy aligns roughly with the clear tonotopy seen in Field L2 (Gehr et al., 1999; Terleph et al., 2006). Neurons in Field L2 have single peaked tuning curves with clear inhibitory sidebands (Lewicki, and Arthur, 1996; Nagel and Doupe, 2008; Terleph et al., 2006). In contrast, tuning curves of NCM

neurons are often multi-peaked with shallow or no inhibitory sidebands, suggesting convergent excitatory inputs from primary auditory cortex, Field L2. Using conspecific songs to probe for responses along the spectral and temporal dimensions revealed neurons with a variety of receptive field types in Field L2 (Woolley et al., 2009). NCM, located several synapses beyond Field L2 in the ascending auditory pathway, have even more complex receptive fields (Calabrese and Woolley, 2015). While click trains and simple tones are often used for mapping best responses from lower auditory structures in the brainstem and thalamus, these simple stimuli may fail to capture the complex non-linear receptive fields in higher associative cortices such as NCM (Calabrese and Woolley, 2015).

NCM is involved in conspecific song perception, identification, and memory. During the critical period, NCM forms a memory of the tutor's song that guides song development during the sensory-motor phase of vocal learning. Long-term memory of the tutor song has been detected in adult NCM (Phan et al., 2006) and adult birds with NCM lesions fail to show the normal behavioral preference for tutor song (Gobes and Bolhuis, 2007). Aside from the tutor song memory, NCM neurons in adult songbirds also respond selectively to conspecific vocalizations and undergo stimulus-specific adaptation to the unique songs of individual conspecifics (Chew et al, 1995; Stripling et al., 2001; Phan et al., 2006; Bolhuis et al., 2012). Electrophysiological responses in NCM differentiate between novel and familiar songs, indicating a neuronal memory for conspecific songs lasting for 20 hours or more (Phan et al., 2006; Tsoi et al., 2014).

Neural and Behavioral Conspecific Bias

Electrophysiological and genomic responses are higher to conspecific song than heterospecific song, white noise or tones in NCM (Chew et al 1995; 1996; Mello et al, 1992; Stripling et al., 2001). Presentation of conspecific song induces the highest amount of ZENK expression, an immediate early gene associated with learning and memory in the mammalian hippocampus. Heterospecific song induces less ZENK expression and pure tones elicit no ZENK expression at all (Mello et al, 1992). Not only are immediate responses to conspecific song more robust than to other stimuli, memory for conspecific songs lasts longer than memories for heterospecific songs. Responses to previously heard conspecific songs can be detected for more than 20 hours after song presentation while memory for heterospecific songs only lasts for up to 6 hours (Chew et al., 1995).

Innate or early developmental factors could selectively increase neural response to spectral-temporal structures typical of zebra finch song, giving rise to a behavioral and neural response bias for conspecific vocalizations. Several studies support a genetic or early developmental explanation for conspecific bias. Recordings of the cardiac response to conspecific and heterospecific songs in young swamp sparrows during the sensitive period for song learning revealed more deceleration of the cardiac response to conspecific song (Dooling and Searcy, 1980). Heightened attention to the relevant tutor song might increase accuracy of song copying. Juvenile white-crowned sparrows exposed to both conspecific and heterospecific song chose to copy only the conspecific song (Marler and Peters, 1977). However, heterospecific songs may present a greater physical challenge due to genetically predetermined attributes of the vocal chords, and thus lead to copying of the more easily reproducible conspecific song. Another study in fledgling white crown sparrows naïve to song quantified the number of call back responses to conspecific and

heterospecific songs and showed a behavioral response preference for conspecific vocalizations over heterospecific songs (Whaling et al, 1997).

In zebra finches, males and females individually reared in sound isolation chambers also showed a behavioral preference for conspecific song when given the choice to listen to either conspecific or heterospecific song (Braaten and Reynolds, 1999). However, an fMRI study in juvenile zebra finches showed sex differences in the origin of the conspecific bias. Normally reared juvenile male zebra finches had stimulus-specific responses to conspecific song while juvenile males raised in acoustic isolation did not – providing support for the early developmental exposure hypothesis. However, in the same study, juvenile female zebra finches raised in isolation did show a conspecific preference for song (Maul et al. 2010). One interpretation is that conspecific preference is innate and genetically coded in females but learned during early development in males. More support for an early experience based bias comes from a cross-fostering study where juvenile zebra finches were cross-fostered with bengalese finch parents, and hence exposed to bengalese finch calls and songs rather than their own-species vocalizations. Cross-fostered zebra finches showed no behavioral discrimination between conspecific and heterospecific (bengalese finch) vocalizations when tested in a dual playback paradigm where proximity and call responding to the playback speaker were measured (Campbell and Hauber, 2009). Thus what appears to be an innate bias in some studies may in fact be an early developmental priming of the auditory system, first by the parent's vocalizations, then by the bird's own vocalizations as it matures. A lack of behavioral preference for conspecific song in cross-housed birds suggests that either

conspecific bias is not genetically hard-coded, or that the young brain is plastic enough for early auditory experience to overcome an existing genetic bias.

Despite extensive research on the initial formation of native language/conspecific song biases in both humans (Werker and Tees, 1984; Rivera-Gaxiola et al., 2005; Kuhl et al., 2003; Kuhl, 2004; Belin et al., 2000; Best and McRoberts, 2003) and songbirds (Marler and Peters, 1977; Whaling et al., 1997; Braaten and Reynolds, 1999; Maul et al., 2010; Campbell and Hauber, 2009), respectively, plasticity in perceptual filters and responses biases in adulthood are less well understood. If perceptual filters are largely a representation of the auditory environment that has been recently experienced, then the bias for higher neural response to conspecific song seen in aviary housed zebra finches (Chew et al., 1995) could be a product of constant exposure to zebra finch songs. Based on this assumption, one would hypothesize that conspecific bias would be reduced if birds are removed from the zebra finch acoustic space and exposed only to canary vocalizations. On the other hand, if the conspecific bias is a result of innate or early developmental patterning of auditory cortex, then removal of conspecific sounds and exposure to heterospecific sounds for a prolonged period should not alter preferential response to conspecific songs.

Lateralization and plasticity in NCM

Hemispheric lateralization of auditory responses is ubiquitous across vertebrate species (Rogers and Andrew, 2002; Geschwind and Levitsky, 1968; Hauser and Andersson, 1994; Poremba et al., 2004; Ehret 1987; Miklósi et al., 1996; Siniscalchi et al., 2015; Kanwal 2012), and in multiple structures in the auditory system (Schönwiesner

et al., 2007); songbirds are no exception (George et al., 2002; Voss et al., 2007; Cynx et al., 1992). Lateralization of auditory processing in NCM has been demonstrated in both electrophysiological and IEG studies (Phan and Vicario 2010; Moorman et al., 2012). Neural responses to song are higher and adapt faster in right NCM. These patterns of lateralization appear to be established early on, and require normal exposure to conspecific song (Phan and Vicario, 2010). Similarly, lateralization in humans may be contingent upon normal auditory exposure during the critical period (Marcotte and Morere 1990; Leybaert and D'Hondt, 2003; Dehaene-Lambertz et al., 2002). Cellular processes such as neurogenesis are also lateralized in songbird NCM and accuracy of song copying was correlated with neurogenesis in the left hemisphere but not the right (Tsoi et al., 2014). Due to the many similarities in lateralization between songbirds and humans, songbirds are a good empirical model for studying how lateralization of function arises and later contributes to maintenance of perceptual filters for species-specific vocalizations.

Chapter 2

Experiment 1: Can exposure to a novel category of sounds in adult songbirds induce changes in response patterns reflecting neural plasticity?

INTRODUCTION

Both operant training and passive exposure to novel auditory stimuli have been shown to alter representations and response properties in auditory cortex in mature mammals (Polley et al., Bieszczad and Weinberger, 2010; Zhou et al., 2011) and songbirds (Terleph et al., 2008). Thus, adult auditory cortex retains a significant amount of plasticity even beyond the closure of the critical period. However, because many of these studies focused on primary auditory cortex, used pure tones as training and testing stimuli, and performed mapping of auditory cortex under anesthesia, the effects of full on immersive exposure to complex sounds in an awake subject is unknown. If the adult brain is capable of implicit learning of underlying stimulus statistics, as well as explicit learning through operant training, then we should see alterations in perceptual filters during the course of passive exposure to foreign stimuli.

One type of perceptual filter is the conspecific bias. In many vertebrates, early exposure to a set of species-specific sounds shapes perceptual filters to respond preferentially to these highly relevant communication signals (Suga et al., 1997; Maier and Scheich, 1987; Portfors et al., 2009; Ehret 1987; Belin et al., 2000; Amin et al., 2013). In some species that are capable of learning a complex set of vocal communication signals, both auditory perception and vocal production of these salient sounds have been shown to be lateralized, e.g. for native language in humans and song in songbirds

(Dronkers et al., 2007; Springer et al., 1999; Nottebohm et al., 1976; Floody and Arnold, 1997; Wild et al., 2000; Voss et al., 2007; Poirier et al., 2009; Phan and Vicario, 2010; Moorman et al., 2012; Ocklenburg et al., 2013). This raises the question of how adaptive changes in acoustic representations induced by experience with new sounds in adulthood interact with pre-existing patterns of lateralized processing.

Although it is unclear why lateralization is a common trait across multiple species and multiple sensory systems (Rogers and Andrew, 2002; Geschwind and Levitsky, 1968; Hauser and Andersson, 1994; Poremba et al., 2004; Ehret 1987; Miklósi et al., 1996; Siniscalchi et al., 2015; Kanwal 2012; Schönwiesner et al., 2007; George et al., 2002; Voss et al., 2007; Cynx et al., 1992), strongly lateralized responses are correlated with improved cognitive performance. In an fMRI study of baseline patterns of lateralization, Gotts et al., 2013 found that the degree of lateralization in auditory and visual cortices was positively correlated with verbal and visuospatial abilities. On the other hand, lack of lateralization or bilateral activation is correlated with speech deficits and other impairments in speech processing (de Guibert et al., 2011). A near-infrared spectroscopy (NIRS) study found that lateralization for phonemic contrasts was reduced in children and adults that stutter (Sato et al., 2011). Furthermore, children with autism or dysphasia showed a reversed pattern of hemispheric asymmetry when tested with a speech stimulus compared to normal children of the same age range (Dawson et al., 1989). In the autistic group only, the degree of reversed asymmetry was positively correlated with language impairments. Thus, both the existence of hemispheric lateralization and hemispheric dominance for language are predictive of language ability in certain populations. Instead of having two redundant hemispheres that respond equally

to all incoming stimuli, it seems to be a fundamental property that each hemisphere is specialized to process certain aspects of the environment. Division of labor between the two hemispheres may improve cognitive abilities by increasing speed of processing.

Studies that show a left hemispheric dominance for language are often done in right-handed monolinguals with phonemes of their native language. A review of the literature on functional lateralization in bilinguals or left-handed individuals presents a less definitive picture of the relationship between lateralization and cognitive performance. While atypical lateralization is seen in a higher percentage of language-impaired individuals, approximately 4-6% of normal right-handed people and 22-27% of normal left handed people are atypically lateralized for language with no deficits in language ability (Knecht et al., 2000; Szaflarski et al, 2002). On the other hand, in zebra finches, development of hemispheric lateralization is dependent on normal auditory experience during the critical period and deprived early auditory experience does lead to deficits in song quality (Phan et al., 2010). Thus, functional lateralization for language or conspecific vocalizations, may be indicative of early auditory exposure, rather than a genetically hard-wired trait.

If the above narrative is true, functional lateralization for native stimuli would reflect underlying perceptual filters formed during the critical period and maintained in adulthood. While many studies treat the pattern of left hemispheric dominance for language as a fixed, or even desirable characteristic of auditory cortex in normal adults, recent studies have shown that patterns of lateralization are labile and can be modified by experience in songbirds (Yang and Vicario, 2015). Changes in functional lateralization

for a newly acquired grammar are seen in human adults, and are correlated with proficiency (Newman-Norlund et al., 2006).

In the following experiment, we passively exposed adult zebra finches to either conspecific or heterospecific auditory immersion environments and tested for changes in the pattern of lateralization at various time points. These results have been partially published in Yang and Vicario (2015).

METHODS

Subjects and Housing

Adult male zebra finches (>120 days of age) were obtained from the zebra finch breeding aviary at Rutgers University, New Brunswick, NJ or purchased from a local supplier. At the beginning of the experiment, subjects were removed from the general aviary and individually housed in sound-proof isolation boxes (33" x 16" x 18") containing a speaker on a 12/12 light/dark cycle with *ad libitum* access to food and water.

Acoustic Environment Manipulation

During lights-on, subjects heard continuous 12h passive playback of recordings from either a control conspecific zebra finch aviary (zebra finch; CONENV) or an experimental heterospecific aviary (canary; HETENV). Playback lasted for either 4, 9, 14 or 30 consecutive days. Subjects were randomly assigned to one of 8 groups: 4d, 9d, 14d or 30d HETENV or 4d, 9d, 14d or 30d CONENV.

Playback environments were recorded over 12h periods from zebra finch and canary aviaries at Rockefeller University, Millbrook, NY to ensure that all acoustic

environments were novel at the start of the experiment. Exposure to canary songs and calls in the HETENV group simulated a ‘foreign’ acoustic environment, to replicate acoustic experience of zebra finches in the “cross housed” condition described in Terleph et al, 2008. Exposure of the CONENV group to zebra finch songs and calls represented the ‘native’ acoustic environment. HETENV stimuli contain relatively more energy at higher frequencies and longer durations than CONENV stimuli (**Figure 2A**). The HETENV sounds presented contained acoustic features typical of canary vocalizations such as rapid trills and high pitched whistles with long durations and many repetitions. In contrast, CONENV sounds contained acoustic features commonly found in zebra finch vocalizations such as broadband harmonic stacks, fast frequency modulations, and shorter syllables (**Figure 2B**). Vocalizations in the two environments occupied distinct, but partially overlapping, sectors of acoustic space, such that CONENV and HETENV present two separate combinations of sounds, both of which lie within the perceptual range of the zebra finch (Okanoya and Dooling, 1987). Sound intensities for the two playback environments were matched to each other and to sound intensity in the general zebra finch aviary and were well below the threshold for causing peripheral damage (Rubel and Ryals, 1982). Playback amplitude at the end of the cage closest to the speaker averaged 67dB SPL (A-scale) with occasional brief peaks not exceeding 74dB SPL (A-scale, fast) in both CONENV and HETENV. All procedures conformed to a protocol approved by the Animal Care and Use Committee at Rutgers University.

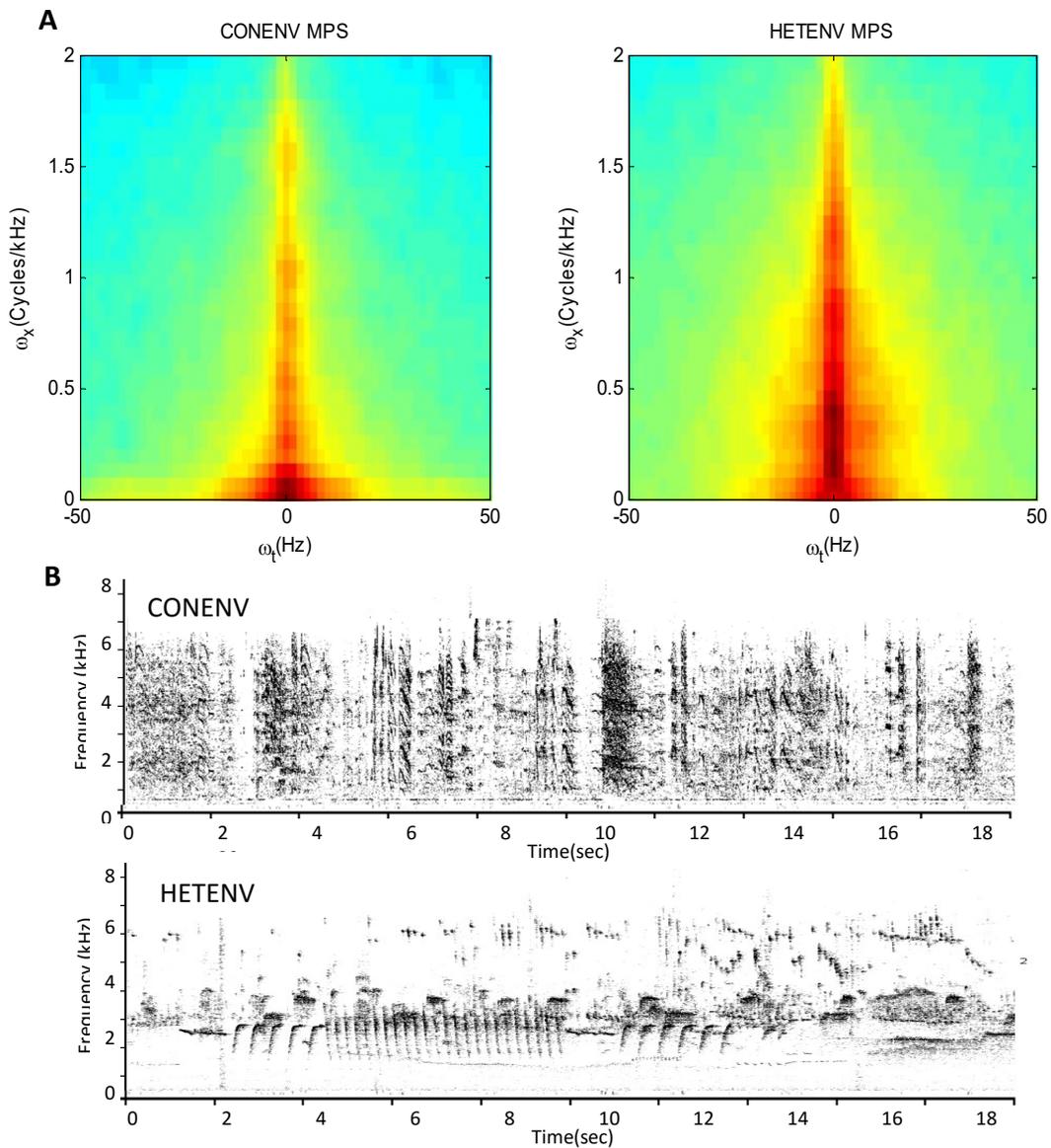


Figure 2. A) Modulation Power Spectra (MPS) generated from zebra finch (CONENV) and canary (HETENV) playback tapes show the temporal and spectral modulations of vocalizations recorded in the two species environments. The X axis represents the temporal modulation as oscillations of power over time. The Y axis represents the spectral modulation as oscillations of power over a short-interval frequency spectrum. HETENV stimuli contain relatively more energy at higher frequencies and longer durations than CONENV stimuli. B) Spectrograms of CONENV and HETENV from a representative 20s sample of each environment.

Electrophysiological recording

Two days prior to testing, subjects were deeply anesthetized with 1.5-2.0% isoflurane in oxygen and placed in a stereotaxic apparatus. A metal pin was attached to the skull with dental cement and a craniotomy was performed on the skull above NCM in preparation for awake, head-fixed electrophysiological recording. Post-surgery, subjects were observed for recovery from the short acting anesthetic (Roberson et al, 2000). After full recovery (30-60mins), subjects were returned to their respective acoustic environments for continued exposure to sound playback. At the end of their respective exposure periods and 48h post-implantation surgery, subjects were removed from isolation boxes and placed in a soundproof booth (IAC Inc., Bronx, NY) for electrophysiological recording. Subjects were restrained in a custom made plastic tube and head-fixed to the stereotaxic apparatus using the previously implanted head pin. A multi-electrode microdrive (Thomas Recording, Giessen, Germany) was used to place 16 tungsten micro-electrodes (Type ESI2ec, impedance: 2–4 M ohm, Thomas Recording) in NCM bilaterally (8 in each hemisphere, **Figure 3A**). Coordinates used for placement of micro electrodes within the boundaries of NCM were 0.5-1.5mm rostral and 0-1 mm lateral to the bifurcation of the mid-sagittal sinus (the zero point for songbird stereotaxis). Each electrode was lowered into the brain while white noise stimuli with the amplitude envelope of canary song were played to identify the first responsive site along the track. Once responsive sites were located on all electrodes, playback of the testing stimuli commenced. Multi-unit recordings of neural spike activity were taken simultaneously

from all electrodes using Spike 2 software (CED, Cambridge, England). Recorded activity was amplified (x19,000) and band-pass filtered from 0.5-5kHz.

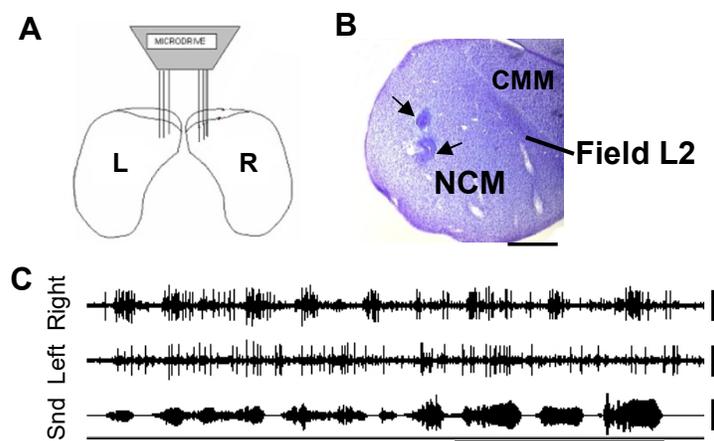


Figure 3. NCM anatomy and electrophysiology. A) Schematic in coronal view of bilateral electrode placement in NCM. B) Sagittal section of NCM stained with cresyl violet. Black arrows indicate location of 2 electrolytic lesions made in NCM at the conclusion of recording. Scale bar: 1mm. Labels show locations of NCM, CMM and the primary auditory area, Field L2. C) Raw electrophysiological recording from electrodes placed in left and right NCM showing bilateral responses to conspecific song. X-axis time bar: 0.5s. Y-axis scale bar: 25 μ V

Auditory testing stimuli

Auditory stimulus sets consisted of 5 novel zebra finch (zfstim) and 5 novel canary songs (canstim) that were not part of either playback environment. A separate set of novel stimuli was used at each group of simultaneously recorded sites. In each set, the 25 repetitions of each of the 10 songs (duration: 800-1500ms; sampling rate: 40 KHz) were presented in shuffled order at 8s ISI. All song stimuli were played back at 70dB through a speaker centered 30cm in front of the subject. Each set of song stimuli was followed by presentation a set of pure tone stimuli to obtain tuning curves and best

frequencies for each recording site. Tone stimuli ranged from 500Hz to 5000Hz at 250Hz increments (20 stimuli; duration: 260ms; sampling rate: 40 KHz; 3 repetitions each in shuffled order; 6s ISI). After presentation of both song and tone stimuli, all electrodes were lowered 300 μ m along the dorsal-ventral axis and a new set of song stimuli was played, followed by the tone set. This was repeated until all 4 sets of novel songs had been played at their respective depths in NCM. Upon completion of testing, 3 small electrolytic lesions (10 μ A for 10s) were made at the final recording depth in each hemisphere for histological verification that sites were within the boundaries of NCM.

Histology

Three days post-test, subjects were deeply anesthetized with Nembutal and transcardially perfused with saline and 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde. Sagittal sections (50 μ m) were taken through left and right NCM on a Vibratome, stained with cresyl violet and visualized under light microscopy to identify lesioned recording sites (**Figure 3B**). Sites determined to be outside of the histological boundaries of NCM were excluded. In addition, only those sites in NCM that showed adaptation profiles (see Data Analysis, below) characteristic of NCM neurons were included in the analyses. The vast majority of neurons and sites in NCM are known to exhibit stimulus specific adaptation, while those in the adjacent primary auditory area, Field L, do not exhibit this complex form of adaptation (Chew et al, 1995), although they do show simple adaptation akin to deviance detection in mismatch negativity (Nelken and Ulanovsky, 2007). Thus, we eliminated recording sites that did not meet the adaptation criterion (rate < -0.05) established in earlier work (Phan

et al, 2006). Across the different groups, 10-12% of sites were excluded based on this criterion.

Data Analysis

Neural recordings were quantified in two ways: by measuring response magnitude and rate of adaptation across repeated presentation of the same stimulus. Both these measures have been shown to differ in a lateralized manner in NCM (Phan and Vicario, 2010).

Absolute Response Magnitude (ARM) measures the strength of neural responses to auditory stimuli, and is calculated as follows: the root mean square (rms) of neural activity during the control period (500ms prior to stimulus onset) was subtracted from the rms of the stimulus-on period (stimulus duration plus 100ms), following established methods in the laboratory (Phan et al., 2006; Phan and Vicario, 2010, **Figure 4**). ARMs were averaged for trials 2-6 of each song stimulus and each stimulus category (zfstim or canstim) to yield a response strength for conspecific songs and a response strength for heterospecific songs for each recording site to be used in further analysis.

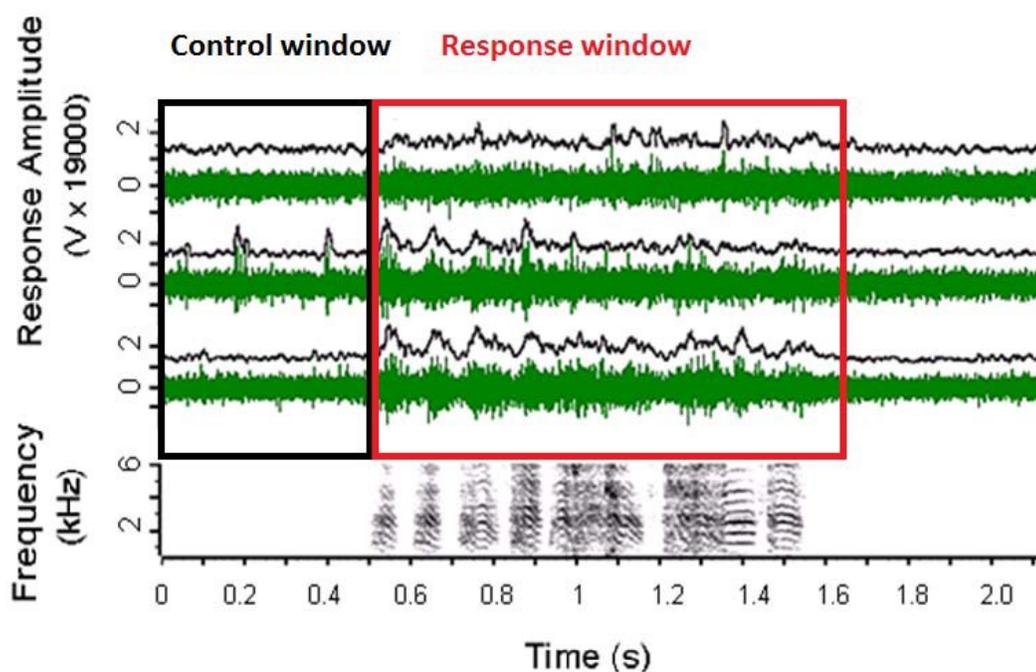


Figure 4. Calculation of RMS – Multi-unit activity is recorded from 16 electrodes, amplified (x19,000) and band-pass filtered from 0.5-5kHz. The absolute response magnitude at each recording site is calculated as the root mean square of (RMS) of the response window during stimulus onset (red box) minus the RMS of the control window 500 ms prior to stimulus onset (black box). The bottom panel contains the spectrogram of a zebra finch song presented during testing.

Adaptation Rate (Adpt Rate) measures the normalized rate of decrease in response size to a repeated stimulus (Chew et al, 1995; Phan et al., 2006). Adaptation rate is calculated as the slope of the regression line over repeated presentations for trials 6-25 with a given stimulus divided by the average ARM of those trials (**Figure 5**). Dividing by the average ARM normalizes for differences in absolute response strength between recording sites. Thus, adaptation rate is the percentage drop in response amplitude per stimulus repetition at each recording site. In NCM, repeated novel stimuli elicit robust responses which rapidly adapt, while responses to familiar stimuli are already adapted and only undergo

minimal additional adaptation. High adaptation rates signify novelty, while low rates indicate familiarity for a stimulus - a kind of long-lasting neuronal memory (Chew et al, 1996).

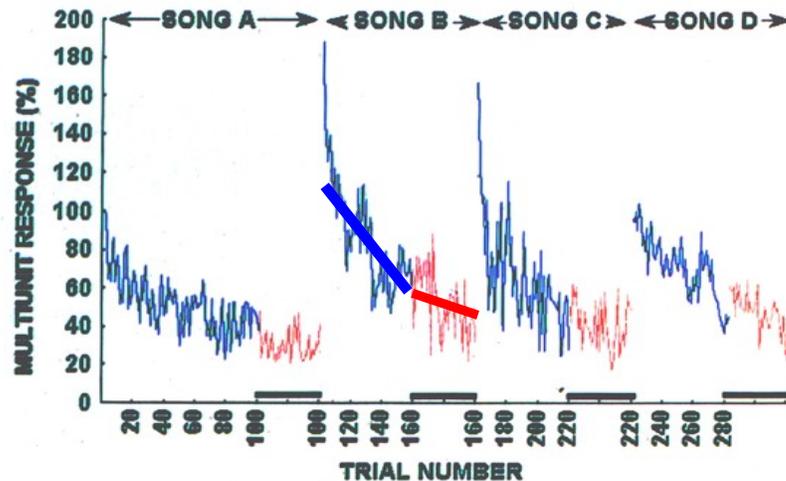


Figure 5. Calculation of Adpt Rate –Adaptation rate over trials 6-25 was calculated for each song stimulus presented as follows: the slope of the regression line over trials 6-25 was with a divided by the average ARM of trials 6-25 to normalize against variation in response magnitude. Adaptation rates are usually taken as a measure of novelty or familiarity. Initial presentations of a novel song generate a robust response that adapts quickly (blue line). Once that song is familiar, adaptation rates have a shallower slope (red line).

Statistical Tests

Effects of auditory environment on ARM and adaptation rate were quantified using repeated measures ANCOVA for main effects and interactions of auditory environment (CONENV, HETENV) and hemisphere (left, right) with stimulus category (zfstim, canstim) as the repeated measure. ARMs tend to decrease with depth along the dorsal-ventral axis; therefore, our analyses included depth as a covariate.

RESULTS

Changes in Hemispheric Lateralization of Absolute Response Magnitude -4d

When data for 4d CONENV (n=5) and HETENV birds (n=6) were combined in an overall model ANCOVA where ARMs for trials 2-6 were analyzed for main effects of environment and hemisphere, with stimulus type as the repeated measure and depth as the covariate, there were no main effects of environment or hemisphere, but a significant interaction of environment by hemisphere came through ($F(1,258) = 9.443$, $p < 0.002$, **Figure 6A**). The interaction is due to ARMs being slightly higher in the right hemisphere in 4d CONENV birds and significantly higher in the left hemisphere in 4d HETENV birds. Within 4d CONENV birds, a repeated measures ANOVA revealed a trend towards higher ARMs in the right hemisphere ($F(1,135) = 3.542$, $p = 0.062$, n.s., **Figure 6A**). A repeated measures ANOVA in 4d HETENV birds revealed the opposite pattern, with the left hemisphere showing significantly higher ARMs than the right ($F(1,125) = 8.949$, $p < 0.003$, **Figure 6A**). When average ARMs for sites in the left and right hemispheres were compared within each bird, 5 out of 6 birds in HETENV showed higher ARMs in the left hemisphere, while 5 out of 5 birds in CONENV showed higher ARMs in the right hemisphere.

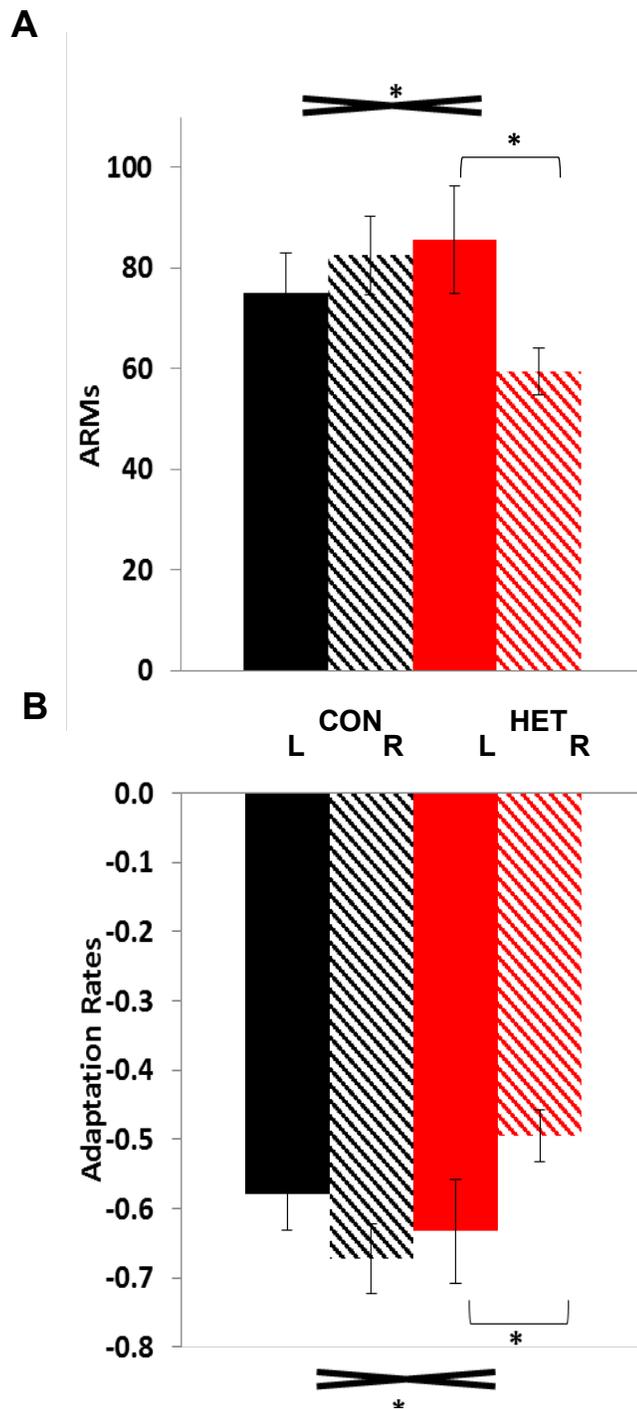


Figure 6. Effects of 4d auditory exposure on neural responses. A) Absolute Response Magnitudes for trials 2-6 in the left (solid bar) and right (striped bar) hemispheres for birds that spent 4d in either CONENV (black) or HETENV (red). CONENV birds had slightly higher ARMs in the right hemisphere while HETENV birds had higher ARMs in the left hemisphere, yielding a significant environment by hemisphere interaction ($F(1,258)=8.231, P<0.005$). B) Adaptation Rates for trials 6-25. CONENV birds had faster adaptation rates in the right hemisphere while HETENV birds had faster adaptation rates in the left hemisphere, leading to a significant environment by hemisphere interaction ($F(1, 258)=8.044, P<0.005$). Error bars represent SEM. Asterisks denote significant main effects and interactions.

Changes in Hemispheric Lateralization of Absolute Response Magnitude -9d

Similar to results in 4d birds, when data for 9d CONENV (n=12) and HETENV (n=13) birds were combined in an overall model ANCOVA where ARMs for trials 2-6 were analyzed for main effects of environment and hemisphere, with stimulus type as the repeated measure and depth as the covariate, there were no main effects of environment or hemisphere, but a significant interaction of environment by hemisphere came through (ANCOVA: $F(1, 369)=8.506, P<0.004$). The interaction is a result of CONENV birds having the expected pattern of lateralization, with the right hemisphere responding more robustly than the left (Phan and Vicario, 2010), but HETENV birds having stronger responses in the left hemisphere than the right (**Figure 7A**). Interestingly, this change in lateralization was seen for both canary and zebra finch testing stimuli.

In zebra finches with 9d auditory exposure to CONENV (conspecific zebra finch aviary sounds), mean ARMs during trials 2-6 were higher in the right hemisphere than the left to both zfstim and canstim (ANOVA for left vs. right hemisphere with stimulus type at each site as a repeated measure; Hemisphere $F(1, 179)= 5.58, P < 0.019$; **Figure 7A**). These results are consistent with previous findings (Phan and Vicario, 2010). In contrast, zebra finches with 9d auditory exposure to HETENV (heterospecific canary aviary sounds), had higher mean ARMs during in the left hemisphere than the right (ANOVA $F(1, 191)=4.5665, P < 0.034$; **Figure 7A**). When average ARMs for sites in the left and right hemispheres were compared within each bird, 10 out of 13 birds in HETENV showed higher ARMs in the left hemisphere, while 11 out of 12 birds in CONENV showed higher ARMs in the right hemisphere.

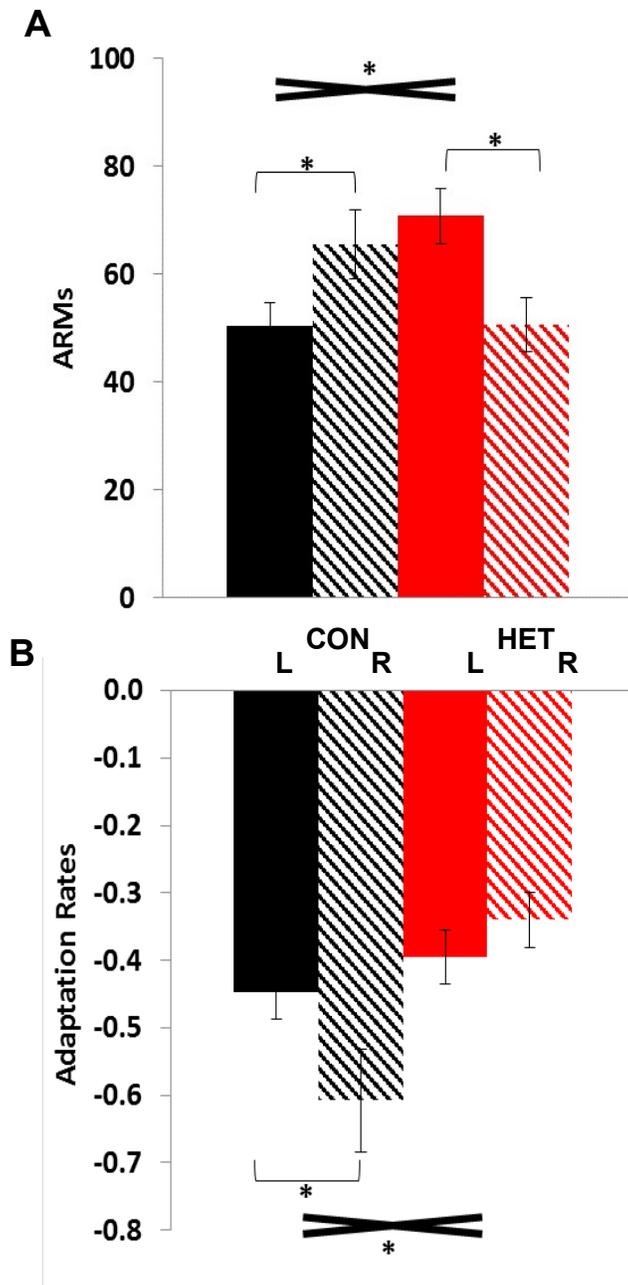


Figure 7. Effects of 9d auditory exposure on neural responses. A) Absolute Response Magnitudes (ARMs) for trials 2-6 in the left (solid bar) and right (striped bar) hemispheres for birds that spent 9d in either CONENV (black) or HETENV (red). CONENV birds had higher ARMs in the right hemisphere (CONL vs CONR) while HETENV birds showed higher ARMs in the left hemisphere (HETL vs HETR) yielding a significant environment by hemisphere interaction ($F(1,369)=8.506$, $P<0.004$). B) Adaptation Rates for trials 6-25. CONENV birds had faster adaptation in the right hemisphere while HETENV birds did not have a hemispheric difference in adaptation rates, once again showing a significant environment by hemisphere interaction ($F(1,369)=5.508$, $P<0.020$). Error bars represent standard error of the mean (SEM). Asterisks denote significant main effects and interactions.

Conspecific Bias in ARMs is maintained after exposure to HETENV

In control 9d CONENV birds, there was a clear response bias for conspecific song over heterospecific song (zfstim vs. canstim, $F(1, 179)=29.422$, $P < 0.00001$), as previously shown (Chew et al., 1995). Furthermore, there was a strong trend towards an interaction between hemisphere and stimulus type ($F(1, 179)=3.8615$, $P = 0.051$, n.s.), suggesting a greater degree of lateralization when tested with zfstim than with canstim.

There was no change in the conspecific bias in birds who were exposed to HETENV for 9d: zebra finch songs still evoked a significantly higher response than canary songs ($F(1, 191)=27.253$, $p < 0.00001$). Unlike the 9d CONENV birds, there was no trend towards an interaction between hemisphere and stimulus type ($F(1, 191)= 2.479$, $p=0.117$, n.s.). In 9d HETENV birds, the degree of lateralization did not differ between zfstim and canstim. Conspecific bias appears to be established early on and remains fairly robust in adulthood despite our manipulations. However, other forms of plasticity cannot be ruled out.

One interesting observation is that responses to zfstim are more lateralized than responses to canstim in the 9d CONENV birds, but responses are equally lateralized to both zf stim and canstim in 9d HETENV birds. This is reminiscent of human studies that show greater lateralization for native speech vs. non-native speech in monolinguals. In bilinguals, unfamiliar language constructs initially evoke a bilateral response (i.e., less lateralized canstim responses in the 9d CONENV birds) but as proficiency increases, responses become lateralized like responses to the native language (i.e., lateralized canstim responses in 9d HETENV birds).

Changes in Hemispheric Lateralization of Adaptation Rate – 4d

Adaptation rates during trials 6-25 are used to measure novelty or familiarity with a stimulus following established procedures (Phan et al., 2006). When adaptation rates for birds exposed for to 4d CONENV and 4d HETENV were combined in an ANCOVA and evaluated for main effects and interactions, there was a significant interaction of environment with hemisphere ($F(1,258)=8.044$, $p<0.005$, **Figure 6B**). When 4d CONENV birds were tested with a repeated measures ANOVA, the right hemisphere showed a trend towards faster adaptation ($F(1,135)=3.623$, $p<0.059$) while the reverse was true in 4d HETENV birds where the left hemisphere adapts significantly faster than the right ($F(1,125)=4.756$, $p<0.031$).

Changes in Hemispheric Lateralization of Adaptation Rate – 9d

Similarly, analysis of 9d CONENV and 9d HETENV data in an ANCOVA also showed a significant interaction of environment with hemisphere ($F(1, 369)=5.508$, $p<0.020$; **Figure 7B**). Bonferroni post-hoc revealed that most of the interaction effect was due to the right hemisphere in CONENV birds having steeper adaptation than all of the other groups (left hemisphere in CONENV birds and both hemispheres in HETENV birds) ($P<0.05$ for each Bonferroni post-hoc comparison). ANOVA of data from 9d CONENV birds revealed the typical pattern (Phan and Vicario, 2010) of faster adaptation rates in the right hemisphere ($F(1, 179)=5.392$, $p<0.021$; **Figure 7B**). In contrast, ANOVA of adaptation rates in 9d HETENV birds showed no significant difference between the left and right hemispheres ($F(1, 191)=1.178$, $p=0.279$, n.s.; **Figure 7B**).

A main effect of environment was seen in the 9d birds but was not observed in the 4d birds. Adaptation rates to both zfstim and canstim was slower in HETENV birds than in CONENV birds ($F(1, 369)=12.233, p<0.001$). If we take adaptation rates as a measure of novelty, perhaps testing stimuli are less surprising overall to 9d HETENV birds because they have previous experience with canary-like sounds while birds in 9d CONENV are hearing canary songs for the first time during testing.

Effects of Recent Auditory Experience on Responses to Tones

In order to assess whether the changes in hemispheric lateralization were specific to complex stimuli, or showed a general reversal of functional lateralization to all stimuli, we measured ARMs for pure tones in 4d and 9d HETENV and CONENV birds. ARMs for tones were defined as the average response across all trials at the best frequency of each site. There was no hemispheric difference in ARMs to pure tones in birds housed in either environment (ANOVA: CONENV: $F(1,156)=0.002, p=0.969$, HETENV: $F(1,154)=0.799, p=0.373$) and there was no interaction of environment with hemisphere (ANCOVA: $F(1,321)=0.127, p=0.722$). Responses to simple stimuli like pure tones do not appear to be lateralized, as previously observed (Phan & Vicario, 2010), regardless of the environment to which the birds were exposed.

In a previous study in the laboratory that tested tone stimuli in birds exposed to novel sound environments for 9d, the width of tuning functions showed evidence of plasticity (Terleph et al., 2008). That study measured tuning width for pure tones as the contiguous frequency range over which absolute responses were at least two standard deviations above baseline. Using the same method, we found that tuning width was found

to be significantly narrower in 4d HETENV than 4d CONENV birds ($F(1, 267)=11.212$, $p<0.001$). Tuning width was similarly narrower in 9d HETENV than 9d CONENV birds ($F(1, 304)=5.645$, $p<0.018$, **Figure 8**). Our findings are consistent with results of a cross-housing experiment (Terleph et al., 2008). Since our study controlled for confounds of social interaction by presenting CONENV and HETENV sounds to isolated birds, we conclude that narrowing of tuning width in NCM neurons occurs primarily as a result of experience with foreign acoustic stimuli and is not due to other variables such as multi-sensory cues or social interactions.

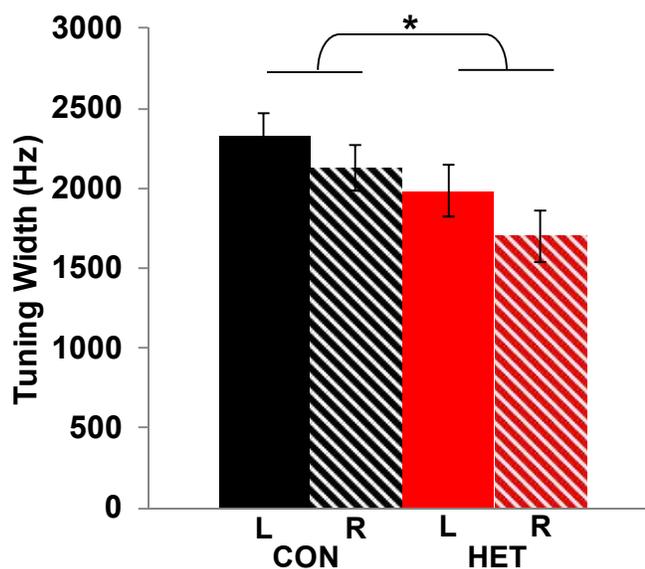


Figure 8. Effects of exposure on tuning. Tuning widths in the left (solid bars) and right (striped bars) hemispheres for birds exposed to either CONENV (black) or HETENV (red) for 9 consecutive days are shown. Tuning width is significantly narrower in birds exposed to HETENV compared to CONENV ($F(1, 304)=5.645$, $P<0.018$) but there was no effect of hemisphere. Error bars represent SEM. Asterisks denote significant main effects.

Experiment 1b: Effects of Prolonged HETENV exposure on patterns of lateralization

We observed changes in the normal pattern of lateralization in subjects that had 4d of exposure in HETENV and these changes persisted in the 9d HETENV group. However, it was unclear whether these changes would persist or reverse after prolonged exposure to HETENV. One hypothesis is that the acoustic characteristics of canary song syllables are better processed in the left hemisphere and perhaps lead to a use-dependent increase in left hemispheric activity that manifests as higher ARMs to all stimuli. If the change in lateralization we observe is purely due to the stimulus presented, we should continue to see enhanced responses in the left hemisphere ARMs when exposure to HETENV is continued to 14 and 30d time points. On the other hand, if changes in lateralization reflect a reorganizational process triggered by exposure to novel categories of sound, we may expect these changes to revert to baseline after a period of prolonged exposure causes canary stimuli to become familiar as a sound category.

Lateralization in ARMs returns to normal after 14d exposure to HETENV

After prolonged 14d exposure, ARMs in HETENV (n=6) were not different from ARMs in CONENV (n=4). There was no main effect of housing environment when 14d HETENV ARMs were compared to 14d CONENV ARMs in a repeated measures ANCOVA with housing environment and hemisphere as factors, depth as a covariate and stimulus type as a repeated measure. There was a significant main effect of hemisphere ($F(1, 199)=10.036, p=0.002$, **Figure 9A**): the right hemisphere had higher ARMs than the left in both 14d CONENV and 14d HETENV groups and there was a significant effect of stimulus type ($F(1, 199)=13.569, p=0.0003$), indicating preservation of the conspecific response bias for zebra finch stimuli in both groups. There was an interaction between hemisphere and stimulus type ($F(1, 199)=6.392, p=0.012$) due to a stronger response bias

for zebra finch stimulus in the right hemisphere, similar to that observed in the 4d CONENV group. ARMs to zebra finch and canary stimuli were approximately equal in the left hemisphere. There is a trend towards a three-way interaction between environment, hemisphere and stimulus type ($F(1, 199)=2.999$, $p=0.085$, n.s.) due to a high conspecific bias in both hemispheres in the 14d CONENV group but a detectable conspecific bias only in the right hemisphere in the 14d HETENV group.

When 14d HETENV subjects were directly compared to 4d and 9d HETENV birds in a repeated measures ANCOVA (as above), there was a significant interaction of environment with hemisphere ($F(2, 430)=8.055$, $p=0.0004$, **Figure 10A**) with both the 4d and 9d HETENV birds having higher ARMs in the left hemisphere contrary to the 14d HETENV birds that had higher ARMs in the right hemisphere.

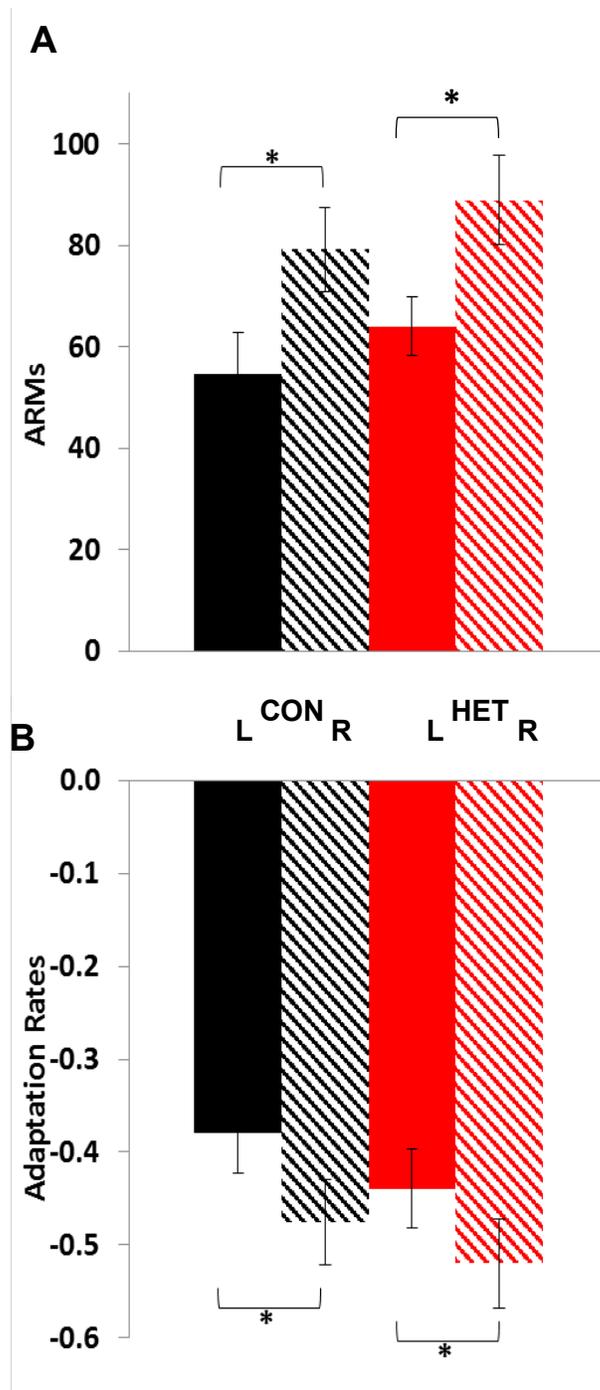


Figure 9. Effects of 14d auditory exposure on neural responses. A) Absolute Response Magnitudes for trials 2-6 in the left (solid bar) and right (striped bar) hemispheres for birds that spent 14d in either CONENV (black) or HETENV (red). Both CONENV birds and HETENV birds had higher ARMs in the right hemisphere so there was no significant environment by hemisphere interaction. B) Adaptation Rates for trials 6-25. Both CONENV birds and HETENV birds had faster adaptation rates in the right hemisphere and again there was no significant environment by hemisphere interaction. Error bars represent SEM. Asterisks denote significant main effects and interactions.

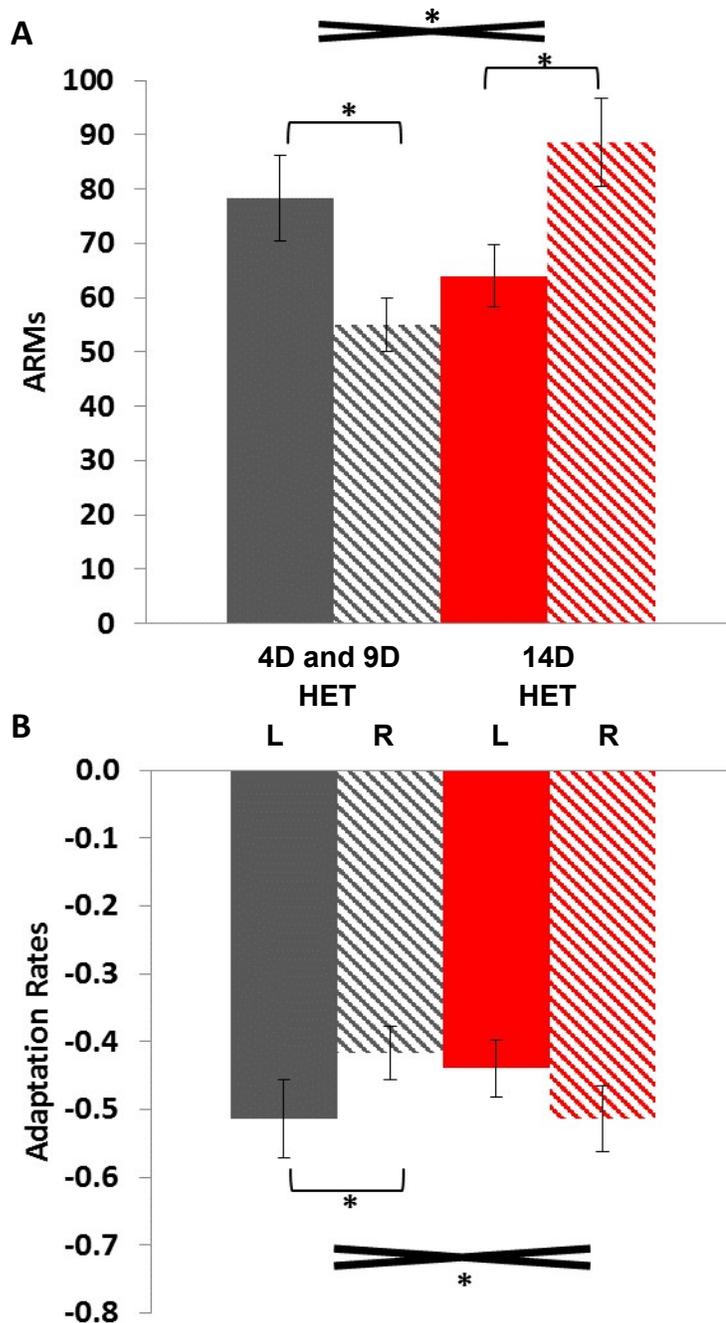


Figure 10. 14D HETENV compared to pooled 4D and 9D data A) 4D and 9D HETENV ARMs are significantly higher in the left hemisphere (gray) compared to the right (gray stripes). 14D HETENV ARMs are higher in the right hemisphere (red stripes) compared to the left (red). Environment by Side interaction is significant. Significant interactions are denoted by asterisks. Error bars represent standard error of the mean. B) 4D and 9D HETENV Adpt Rates are significantly faster in the left hemisphere (gray) compared to the right (gray stripes). 14D HETENV Adpt Rates are not significantly different between the left (red) and right (red stripes) hemispheres.

Lateralization in ARMs remains stable after 30d exposure to HETENV sounds

Lateralization patterns in ARMs remain right lateralized when tested after month long exposure to passive CON or HET environment playbacks. When 30d HETENV (n=5) birds were compared in a repeated measures ANCOVA to the 30d CONENV (n=4) group, there was no interaction of housing environment by hemisphere, indicating that patterns of lateralization were the same in both groups. Both groups had higher ARMs in the right hemisphere as indicated by a significant effect of hemisphere ($F(1, 367)=16.861, p=0.00005$, **Figure 11A**). Once again, there was a strong response bias for zebra finch stimuli ($F(1, 367)=27.711, p=0.000001$) in both groups, consistent with previous findings. Similar to results in the 14d cohorts, there was an interaction of hemisphere by stimulus type ($F(1, 367)=4.998, p=0.026$) where the right hemisphere contributed most of the conspecific bias while responses to zebra finch and canary stimuli were roughly equal in the left hemisphere. When 4d and 9d HETENV cohorts are included in the ANCOVA and compared against the 30d HETENV group, there was a significant environment by hemisphere interaction ($F(2, 492)=11.783, p=0.00001$, **Figure 12A**) due to left hemisphere higher ARMs in the 4d and 9d HETENV groups vs. right hemisphere higher ARMs in the 30 HETENV group.

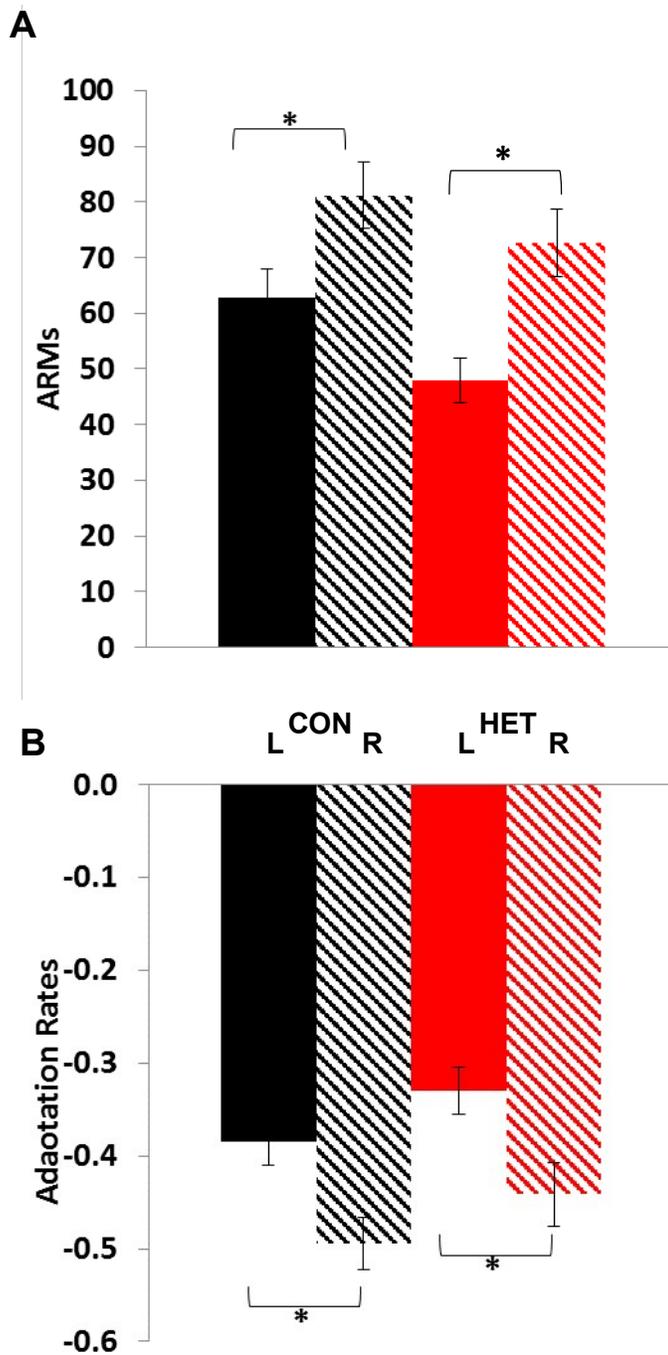


Figure 11. Effects of 30d auditory exposure on neural responses. A) Absolute Response Magnitudes for trials 2-6 in the left (solid bar) and right (striped bar) hemispheres for birds that spent 30d in either CONENV (black) or HETENV (red). Both CONENV birds and HETENV birds had higher ARMs in the right hemisphere so there was no significant environment by hemisphere interaction. B) Adaptation Rates for trials 6-25. Both CONENV birds and HETENV birds had faster adaptation rates in the right hemisphere and again there was no significant environment by hemisphere interaction. Error bars represent SEM. Asterisks denote significant main effects and interactions.

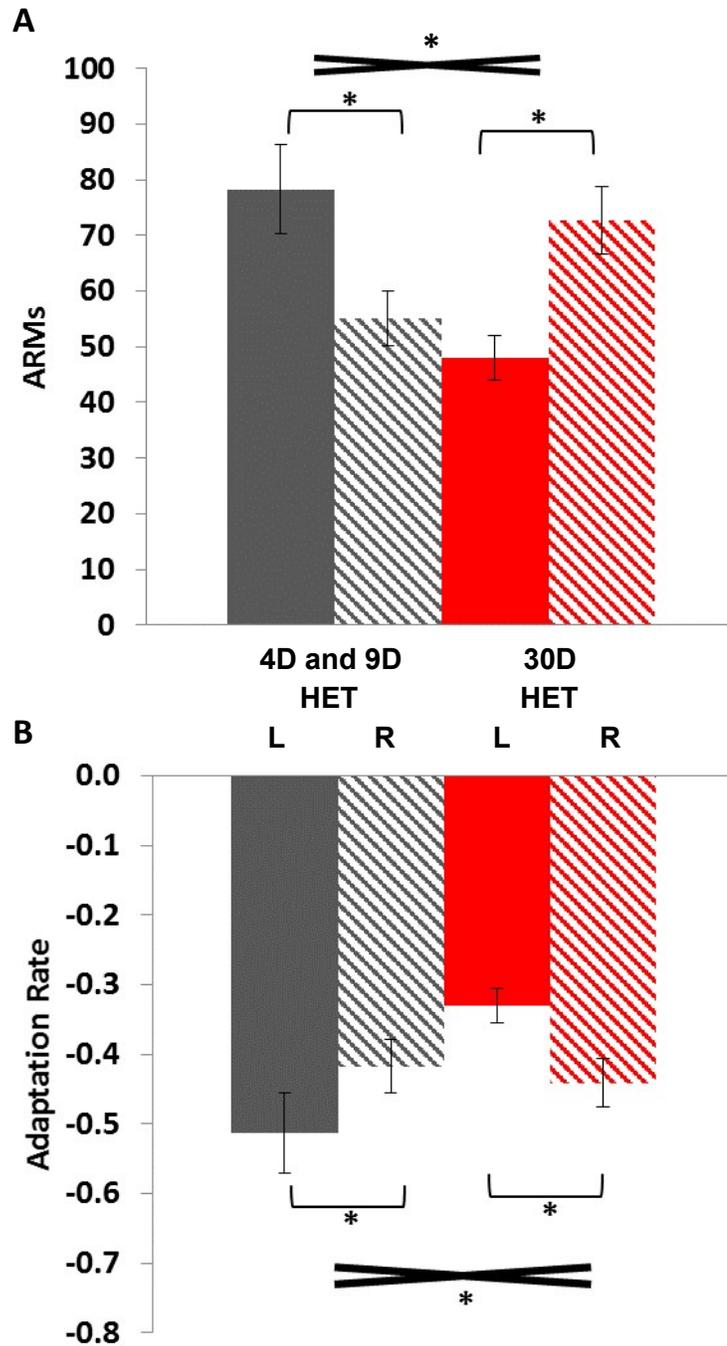


Figure 12. 30D HETENV compared to pooled 4D and 9D data A) 4D and 9D HETENV ARMs are significantly higher in the left hemisphere (gray) compared to the right (gray stripes). 30D HETENV ARMs are higher in the right hemisphere (red stripes) compared to the left (red). Environment by Side interaction is significant. Significant interactions are denoted by asterisks. Error bars represent standard error of the mean. B) 4D and 9D HETENV Adpt Rates are significantly faster in the left hemisphere (gray) compared to the right (gray stripes). 30D HETENV Adpt Rates are faster in the right hemisphere (red stripes) compared to the left (red). Environment by Side interaction is significant.

Lateralization of adaptation rates is in transition after 14d of HETENV exposure

Adaptation rates were compared in 14d HETENV and 14d CONENV birds using a repeated measures ANCOVA. There was no environment by hemisphere interaction but we observed a strong trend towards faster adaptation in the right hemisphere ($F(1, 199)=3.821$, $p=0.052$, n.s., **Figure 9B**) that was mostly driven by the 14d CONENV group. When adaptation rates in the 14d HETENV group were compared with the 4d and 9d HETENV groups in a repeated measures ANCOVA, there was a trend towards an environment by hemisphere interaction ($F(2, 430)=2.779$, $p=0.063$, n.s., **Figure 10B**) that was due to faster adaptation in the left hemisphere in the 4d HETENV group but equal rates of adaptation across hemispheres in the 9d and 14d HETENV groups. Patterns of lateralization in adaptation rates of 9d and 14d HETENV birds represent an intermediate stage between left lateralized 4d HETENV and right lateralized 30d HETENV birds discussed below.

Lateralization of adaptation rates returns to baseline patterns after 30d of HETENV exposure

Comparison of adaptation rates between 30d HETENV and 30d CONENV cohorts using a repeated measures ANCOVA once again revealed no interaction of housing environment with hemisphere, but yielded a main effect of hemisphere with significantly faster adaptation rates in the right hemisphere than the left in both groups ($F(1,367)=16.508$, $p=0.0001$, **Figure 11B**). When 30d HETENV adaptation rates were compared to 4d and 9d HETENV adaptation rates, there was a significant environment by hemisphere interaction driven by faster adaptation in the right hemisphere in the 30d

HETENV group in contrast to faster adaptation in the left hemisphere of the 4d HETENV group and equal lateralization in the 9d HETENV group ($F(2, 492)=5.3620$, $p=0.005$, **Figure 12B**). The pattern right side faster adaptation in 30d HETENV was consistent with controls, indicating that lateralization of adaptation rates eventually returned to baseline but may take longer to do so than ARMs.

DISCUSSION

Exposure to HETENV Reverses the Normal Pattern of Lateralization

Novel sounds presented in the HETENV condition effectively change the statistics of the bird's acoustic environment by expanding the universe of probable sounds. The changes in lateralization that we observe can be interpreted as a reorganization of auditory processing in response to this challenge, as follows. In order to efficiently represent the new stimulus statistics encountered in HETENV, the auditory system modifies existing neural connectivity to represent the new sounds. The data show that the majority of the overall environment by hemisphere interaction in adaptation rates is contributed by the loss of faster right side adaptation in 4d and 9d HETENV birds. In contrast, the reversal of lateralization measured in ARMs is a result of both increased responding in the left and decreased responding in the right hemisphere in 4d and 9d HETENV birds when compared to CONENV birds. The right hemisphere responds to HETENV exposure with a slowing of adaptation rates, while the left side shows increased ARMs to song stimuli. Both hemispheres are affected by changes in the auditory environment but each responds differently when exposed to novel categories of

sounds, perhaps because the left and right hemispheres have different roles in parsing auditory input.

Roles of Left and Right Auditory Cortex

Our data show that the challenge of novel auditory experience induces plasticity in different response parameters in the left and right hemispheres, suggesting an underlying difference in function. We speculate that, in the normal conspecific environment, the right hemisphere performs the fine discriminations necessary for recognition of particular conspecific songs as shown by rapid adaptation to each stimulus. In contrast, the left processes sounds according to their acoustic category (in this case, combinations of features that characterize conspecific vs. heterospecific song) and responds to the foreign environment with heightened responses (seen as higher ARMs) to category violations. Repetition of these violations would ultimately lead to forming new categories. This division of labor may be established early in life through an interaction between latent hemispheric predispositions and exposure to native sounds, i.e. conspecific songs or contrasts in the native language (Phan et al., 2010; Dehaene-Lambertz et al., 2002; Rogers 2008). In humans, the left hemisphere responds preferentially to the contrasts that underlie the phonemes of a particular language, while the right hemisphere integrates inputs over a longer temporal window, allowing it to process stimuli with slower temporal and spectral modulation i.e. the prosody of speech (Telkemeyer et al., 2011). In the bat auditory cortex, the hemispheres differ in their selectivity for different classes of vocalizations; the left hemisphere seems tuned to social vocalizations, while the right processes echolocation calls (Kanwal 2012; Washington and Kanwal, 2012). Division of labor between the two hemispheres may lead to more

efficient processing of sensory stimuli (Ocklenburg and Güntürkün, 2012; Hirnstein et al., 2008; Güntürkün et al., 2000). In songbirds, hemispheric lateralization may enable the rapid discrimination of specific sounds while concurrently allowing for plasticity in the formation of new category clusters and boundaries. Given the many parallels between humans and songbirds, the songbird model could serve as a window into the mechanisms underlying cortical plasticity for speech processing in humans.

Changes in behavioral state during passive exposure could unlock plasticity

Changes in behavioral state induced by the novelty of the HETENV exposure could trigger gene cascades involved in reorganization. From an ethological standpoint, immersive experience with novel foreign stimuli may place the bird in a different state than exposure to novel conspecific stimuli, although effects of social isolation are controlled for. In a previous study, tuning width narrowed in both zebra finches cross housed in a canary aviary and canaries cross housed in a zebra finch aviary (Terleph et al., 2008). However, it was unclear whether changes observed were due to purely auditory cues or to the unfamiliar and possibly stressful social interaction caused by housing in an aviary filled with strange birds. In order to control for effects of social interaction in the case of birds housed in a conspecific aviary and the stress for birds housed in a heterospecific aviary, all subjects in the present experiment were housed in soundproof isolation boxes and only the auditory variable was manipulated.

Although we interpret our results in terms of adaptive changes in NCM elicited by novel stimulus statistics, we cannot fully rule out a role for non-specific effects. Social isolation itself may be stressful for normally gregarious birds; however, that effect should

be the same in both HETENV and CONENV. Another factor is that stimulus novelty in the HETENV condition is much greater than in the CONENV. In HETENV, subjects are exposed to a completely novel class of stimuli while in CONENV, stimuli are class-familiar conspecific songs, although the songs are individually novel. The extreme novelty of HETENV experience may place the bird in an altered state (due to a stressor beyond isolation itself) that makes the brain more plastic and receptive to external stimuli, leading to changes in lateralization not seen in the CONENV group.

In conclusion, we have demonstrated that patterns of lateralization are labile and vary with challenges presented by the current auditory environment even in the mature brain. The left and right hemispheres perform distinct roles in processing auditory input, and thus also respond to changes in the acoustics of the environment by shifting their neural responses in opposite directions. Although we observe experience-induced plasticity in neural response properties, we do not yet know if these changes represent a fine-tuning of perceptual filters that contributes to processing for future stimuli. In the following experiment, we aim to demonstrate that shifts in patterns of lateralization seen in HETENV birds correspond to improvements in behavioral discrimination of sounds from the novel category.

Chapter 3

Experiment 2: Can exposure to a new sound category improve behavioral discrimination of novel sounds?

INTRODUCTION

Discrimination of heterospecific vocalizations or non-native phonemes is a difficult and a somewhat unnatural task from an ethological standpoint. Finely tuned filters for conspecific vocalizations will enable a zebra finch to identify its mate among other zebra finches but finely tuned filters for heterospecific stimuli are rarely useful, especially in a socially gregarious flock species such as the zebra finch. When zebra finches, canaries and budgerigars were tested on conspecific and heterospecific call discriminations, all three species performed significantly better on the conspecific discrimination (Dooling et al., 1992). General aviary housed zebra finches are better at discriminating zebra finch vocalizations than at discriminating heterospecific vocalizations (Dooling et al., 1992). This enhanced ability to discriminate conspecific stimuli could be due to an alignment of perceptual filters in auditory cortex with the informative dimensions that differentiate one conspecific song from the next. However, if perceptual filters are labile and can be modulated through recent experience, then perhaps an organism is constantly updating its filters based on incoming distributions of sound. When the native distribution of sound is drastically altered, i.e., when traveling to a foreign country, one might need to adapt to a new set of stimulus statistics and discover the relevant dimensions that differentiate stimuli in the new acoustic space. After being given some time to update its perceptual filters, the subject may be able to perform an

auditory discrimination in its new environment more quickly than a subject who had had no previous experience with the environment.

Statistical learning is often credited with enabling implicit learning of L1 in children (Saffran et al., 2008). However, whether adults access the same mechanisms for acquisition of L2 is debated. Bley-Vroman's Fundamental Difference Hypothesis (1990) suggests children's acquisition of language is guided by access to the universal grammar and that the difficulty of learning second language in adulthood is due to no longer having access to the universal grammar. Indeed, when a group of Korean adults were tested, mastery of grammatical structures in English was directly correlated with age of arrival in the US (Johnson and Newport 1989). On the other hand, testing adults on a word segmentation task in an artificial language revealed that adults, like children, are capable of using transitional probabilities in a speech stream to distinguish words from non-words (Saffran et al., 1996). Furthermore, the ability to learn statistical probabilities was not domain-specific to linguistic stimuli, but could be seen in segmentation of a tone stream as well (Saffran et al., 1999). Given the likelihood that adults, as well as children, implicitly detect regularities in the auditory environment, immersive experience in a foreign language environment should improve discrimination of words in that language.

Unfortunately, much of second language instruction places emphasis on explicit teaching of the grammatical and syntactical rules of the language, leaving the benefits of immersive experience and the mechanisms that support implicit learning to be less well understood. While some studies show that adults learn most effectively through a direct teaching of the rules of the language (Norris and Ortega, 2000), other studies have shown the benefit of immersion programs or have shown that the same level of proficiency can

be achieved through either means (VanPatten and Oikkenon 1996; Batterink and Neville, 2013; Morgan Short et al., 2011). Subjects taught a subject-verb agreement rule in a foreign language, either passively or explicitly, achieved similar levels of proficiency and demonstrated similar patterns of neural activation (Batterink and Neville, 2013). An ERP study also showed that explicit and implicit learning of grammatical structure in an artificial language resulted in the same level of proficiency but only implicit immersion style learning lead to native-like patterns of activation for the new language (Morgan-Short et al., 2011). Thus, statistical and implicit learning, previously believed to be the domain of children during the critical period, is also a plausible means of learning in adulthood. The extent to which passive immersion in a set of novel sounds can improve behavioral discrimination has yet to be demonstrated in an animal model.

The results of Experiment 1 confirmed that immersion in a novel heterospecific auditory environment induces changes in neural responses that reflect neural plasticity in NCM. Observable effects include a narrowing of tuning width, and reversal of the pattern of lateralization with a complex timecourse. These changes may be evidence of reorganization of the auditory cortex to better represent a new range of stimulus statistics. We predict that, if shifts in lateralization lead to finer perception of heterospecific canary sounds, then birds with previous passive experience in HETENV will do better at discriminating between two canary songs than birds that have had no previous exposure to canary stimuli. Experiment 2 tests this prediction.

METHODS

Subjects and Housing

Separate groups of adult male zebra finches ($n=5$ in each condition) were exposed to the auditory environments as described for subjects, housing, and acoustic environments in Experiment 1 above. However, instead of undergoing electrophysiological testing, subjects were trained in a Go/No-Go operant discrimination paradigm after short (9d), or long (14 or 30d) passive exposure in CONENV or HETENV.

Operant Go/NoGo Procedure

After the termination of the period of passive exposure, subjects were removed from their isolation boxes and placed in an operant training chamber (**Figure 13A**). All shaping and conditioning contingencies were controlled using ARTSy (Gess et al., 2011). Subjects were acclimated to their new boxes and shaped to peck a sensor. Once subjects were pecking reliably (1-2days), training on the Go/NoGo paradigm commenced. Discrimination stimuli were two novel canary songs (duration =1.2s, 70dB) that were 70% similar in their acoustic characteristics (%similarity measure in Sound Analysis Pro, Tchernichovsky et al., 2000). One song was arbitrarily assigned as the Go stimulus, and another song was assigned as the NoGo stimulus (counterbalanced across subjects).

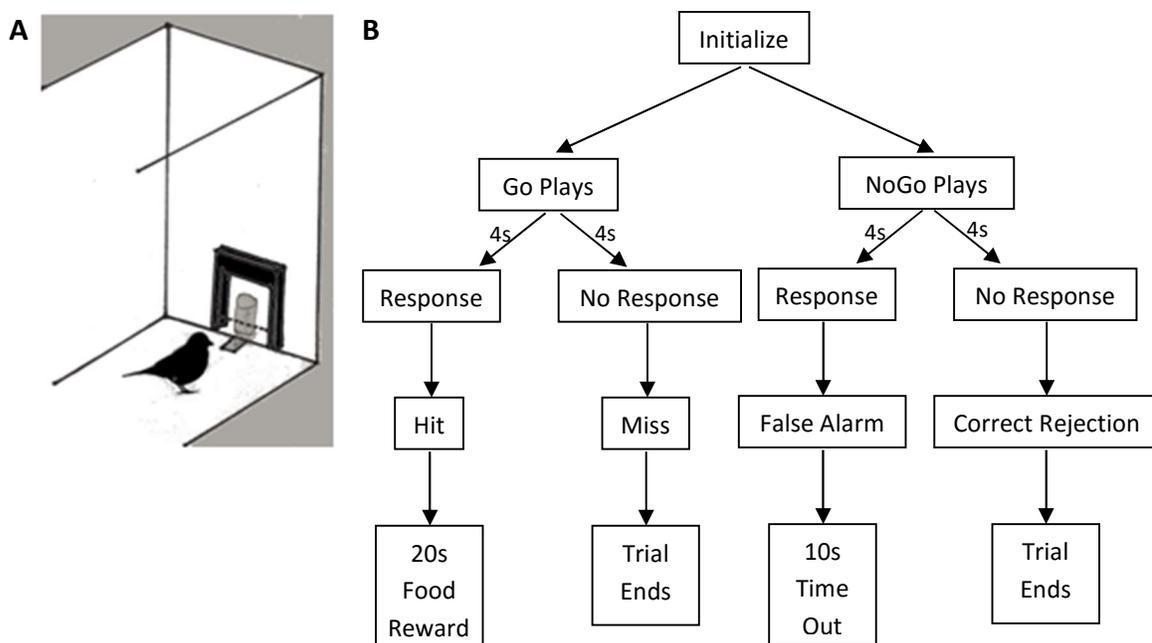


Figure 13. Operant Go/NoGo Training – A) Operant training apparatus consisted of a cage with a sensor to detect pecking response and a cutout on the floor of the cage for access to a retractable feeder. B) Flow chart of contingencies for operant training. Subjects were shaped to peck for trial initiation. Once a trial was initiated, a Go or NoGo song (50/50 probability) would be played through the speakers. If a Go song was presented, subjects had 4s to respond with a second peck in order to obtain access to a food reward for 20s. If a Nogo song was played, subjects had to withhold pecking to avoid receiving a 10s lights-out timeout punishment.

The apparatus contained an infrared beam, a response key, and a retractable food hopper. Subjects had to peck the key once to initiate a trial; approach to the key broke the infrared beam, and a response was recorded. Once a trial was initiated, a Go or NoGo song (50/50 probability) would be played through the speakers (**Figure 13B**). If a Go song was presented, subjects had 4s to respond with a second peck in order to obtain access to a food reward from the extended food hopper for 20s. If a Nogo song was played, subjects had to withhold pecking to avoid receiving a 10s lights-out timeout punishment. The trial terminated after 6s if there was no response.

The operant training chambers had a 12:12 light cycle. Subjects had access to the training apparatus for 8h a day and received 4h of free access to food during a non-training lights-on period, in accordance with protocols approved by the Rutgers University Institutional Animal Care and Use Committee. Subjects were housed in the operant training chamber for 5 days, then they were returned for 2 days to their passive environment box where they received refresher playback of their respective auditory environments (CONENV or HETENV). Training continued until subjects reached 80% criterion on 2 consecutive blocks of 50 trials each or until 21 days of operant training had elapsed, whichever came first. Final performance on the operant discrimination test was defined as the percentage correct the day subjects reached or exceeded 80% criterion and the number of days to reach criterion was recorded. Training was terminated for subjects that failed to reach criterion after 21d.

RESULTS

Canary discrimination improves after 14d and 30d exposure to HETENV but not 9d

Behavioral testing was carried out in birds who spent 9d (short exposure), 14d or 30d (long exposure) in either the CONENV or HETENV environment (N=5 in each group: short CONENV, short HETENV, long CONENV, and long HETENV). There were no main effects or interactions in final performance scores between 14d and 30d long exposure CONENV (n=2 and n=3) or between 14d and 30d long exposure HETENV (n=2 and n=3), so data from 14d and 30d groups were combined into a long exposure CONENV and a long exposure HETENV group. Subjects with 9d short exposure to HETENV did not perform any better than those housed in 9d CONENV prior

to discrimination training ($F(1, 8)=1.681, p=0.231, n.s., \mathbf{Figure\ 14A}$). However, subjects in the long HETENV exposure groups (14d and 30d HETENV) had significantly higher final performance scores than subjects with 14d and 30d long exposure to CONENV ($F(1, 8)=7.074, p<0.029, \mathbf{Figure\ 14B}$), a consequence of all subjects in long HETENV reaching 80% criterion. To further illustrate this difference, 5 out of 5 subjects tested on the operant training task after long HETENV exposure reached criterion of 80% correct, while only 1 out of 5 reached criterion in each of the three other groups (short HETENV, short CONENV, long CONENV). Furthermore, of the subjects that reached 80% criterion, those with long exposure to HETENV reached criterion at 11 days on average while it took subjects in the other conditions 19, 16 and 21 days to hit criterion, respectively.

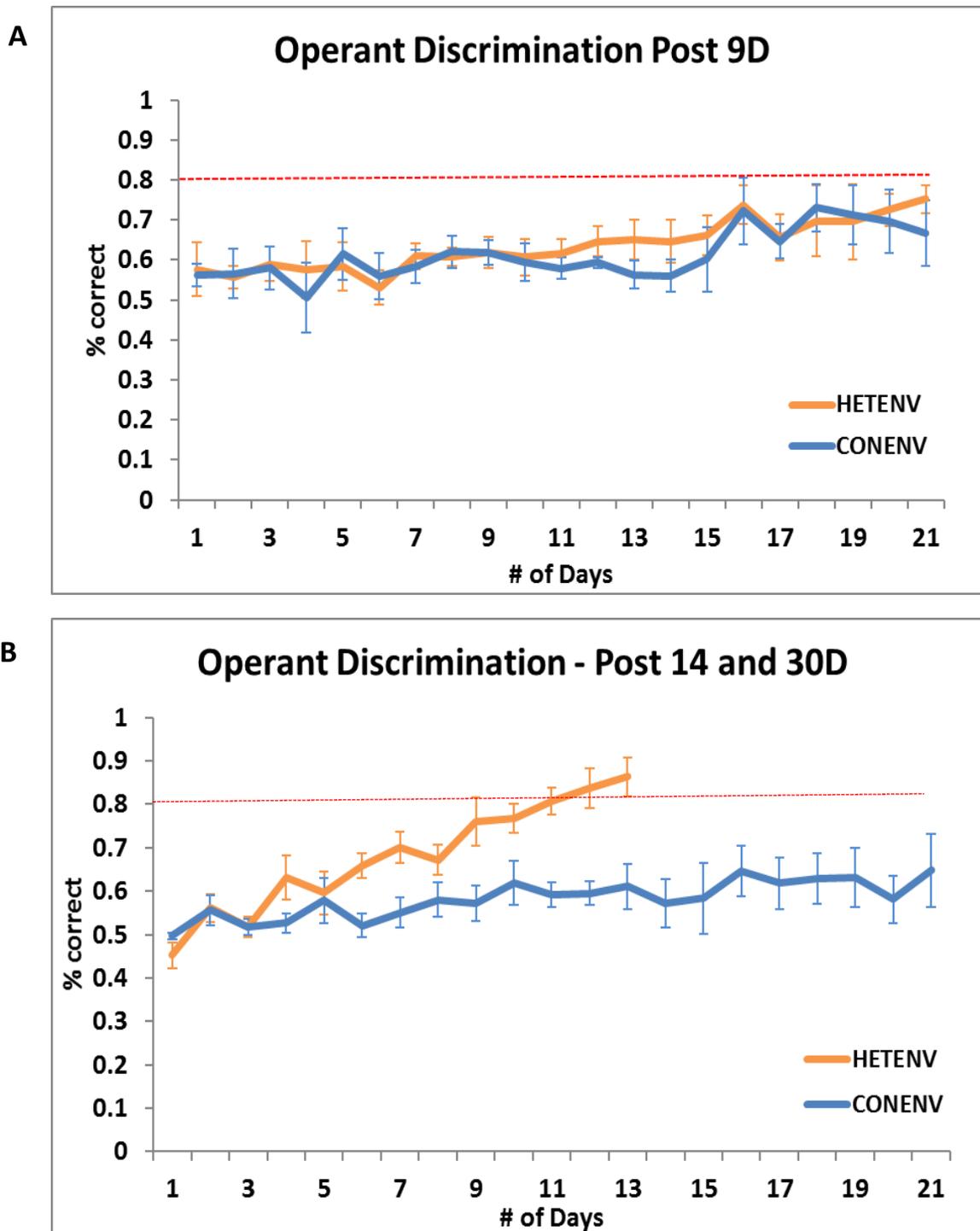


Figure 14. Operant Discrimination Performance A) % correct after 9D passive exposure in CONENV (blue) and HETENV (orange). The X axis represents number of days in the operant training paradigm excluding days of refresher training in CONENV and HETENV. The Y axis represents the average % correct on go and no-go trials. B) % correct after 14D and 30D (combined) of passive exposure to CONENV (blue) and HETENV (orange). Dotted red line denotes 80% correct criterion. Error bars represent standard error of the mean.

DISCUSSION

Prolonged Passive Exposure to a Novel Category Improves Discrimination of Category Exemplars

Naïve zebra finches have a response bias for conspecific vocalizations and are naturally better at discriminating two conspecific vocalizations than two heterospecific vocalizations (Dooling et al., 1992). However, our data show that this behavioral deficit can be overcome by passively exposing subjects to the novel category prior to operant discrimination training. Subjects that were passively exposed to 14 and 30d HETENV performed significantly better than subjects with only 9d of HETENV (and all CONENV subjects) in a difficult behavioral discrimination task where they had to distinguish between two foreign stimuli that shared 70% of their acoustic characteristics. The improvement in performance is delayed relative to the initial shift in lateralization. Subjects with 9d of passive exposure to HETENV had a reversed pattern of lateralization compared to subjects with 9d CONENV exposure. However, the reversed lateralization at 9d does not predict improved performance on the operant discrimination task. Performance on the discrimination task was the same between 9d HETENV and 9d CONENV controls. Surprisingly, performance on the discrimination task was better after 14 and 30d of HETENV exposure (vs. 14d and 30d of CONENV), when patterns of lateralization had reverted to baseline. Thus, there is an interesting dissociation between the pattern of lateralization at any point in time vs. behavioral discrimination performance.

One caveat is that we performed electrophysiological testing to determine patterns of lateralization after passive exposure on one group of birds and did the operant training after passive exposure experiment on a separate group of birds because the electrophysiological experiment was terminal. We were not confident that we could interpret electrophysiological results collected in birds after operant training. Although we do not have direct electrophysiological confirmation of the lateralization pattern after passive exposure, but prior to operant training, in each of the operantly trained birds, we assume that the asymmetries measured electrophysiologically after various periods of HETENV exposure occurred in both groups of birds at the same time points. Therefore, we interpret the combined results from both groups as evidence that reversion of lateralization after prolonged exposure to HETENV (but not the initial change in lateralization at 9d) predicted improved behavioral discrimination of heterospecific stimuli.

Transient shifts in lateralization might be evidence for an underlying process of memory consolidation for novel sound categories. Behavioral improvements in discrimination were only seen after 14d in HETENV, when the left hemisphere had stopped responding with higher ARMs and faster adaptation rates seen in the 4d and 9d HETENV birds. Traditionally lower ARMs and slower adaptation rates have been used to demonstrate familiarity with a stimulus, while higher ARMs and faster adaptation rates signify novelty (Chew et al., 1995). Perhaps the return to lower ARMs and slower adaptation on the left side after 14d reflects the left hemisphere's participation in a recognition memory for the category of canary sounds. Thus, a reversion to control patterns of lateralization could represent learning about the new category, and/or a

stabilization of newly formed connections which are now available for use in heterospecific stimulus discriminations.

Relationship to Shifts in Lateralization as a Function of Proficiency in Humans

These results have a possible parallel in human studies, where shifts in the pattern of lateralization occur during language learning and may reflect levels of proficiency with the language. In bilinguals, the second language is lateralized in the same hemisphere as the native language if acquired early in life, but bilaterally activated if acquired later in life (Hull and Vaid, 2007). Although lateralization patterns between the two groups may differ due to age of acquisition, differences may also be a result of language proficiency, where later learners are less proficient than earlier learners. Since the human subjects were sampled for lateralization in adulthood, changes in lateralization over the course of learning are unknown. In a separate human study where an artificial grammar was taught to adults, lateralization for the new grammar changed from bilateral to left lateralized over the course of training as proficiency increased (Newman-Norlund et al., 2006). Another fMRI study also showed increasing left hemisphere lateralization over the course of one experimental session for novel Norwegian words presented in a randomized auditory stream to English speakers. Once again, shifts in lateralization coincided with improved ability to identify previously heard Norwegian words from pseudo-words (Plante et al., 2015).

Our data also show shifts in lateralization related to an increased ability to discriminate two novel exemplars of foreign song after passive experience with the foreign category. However, our data do not mirror results in the above human studies

exactly. For one, foreign stimuli were processed bilaterally in humans and then move towards native patterns of left hemisphere activation (Newman-Norlund et al., 2006). Heterospecific songs in our birds were initially right lateralized like conspecific songs, then show a reversal of lateralization after short term 4d and 9d exposure, followed by a reversion to the normal pattern of lateralization after 14d and 30d in CONENV. Similar to results in human studies, a reversion to native-like patterns of lateralization in our birds is also correlated with proficiency as demonstrated by better performance in the operant discrimination paradigm. Thus, our data suggest a relationship between lateralization and familiarity with the stimulus class.

However, one distinction from the human comparison is that ARMs in all of our birds were lateralized for both conspecific zebra finch stimuli as well as heterospecific canary stimuli, unlike in humans where responses to native language are lateralized but responses to non-native languages are bilaterally activated. In our data, any changes in the pattern of lateralization occurred equally for both categories of sound. In 4d and 9d HETENV birds, ARMs increased for both zf and canstim in the left hemisphere. Likewise, in 14d and 30d HETENV birds, ARMs reverted to right side higher responses for both zf and can stim. In all cases, zf stim continued to evoke a higher response than can stim, although there are some group effects indicating less lateralization of responses to canstim especially at deeper depths. Thus, it is not apparent that foreign sounds (canstim) are more bilaterally activated or that native sounds (zfstim) are more lateralized.

There are several reasons why our results may not agree completely with findings in human language studies. For one, the hemispheric lateralization observed using fMRI

relies on taking the difference of the brain-oxygen-dependent-level (BOLD) signal between regions of interest (ROIs) in the two hemispheres. The BOLD signal is taken as a measure of total neural activity, based on the premise that blood releases oxygen to active neurons at a greater rate than inactive neurons. Thus, the hemodynamic response measures total neural activity, including both inhibitory and excitatory neurons. This same caveat applies studies using near-infrared spectroscopy (NIRS) which measures changes in hemoglobin concentrations. Thus, greater left hemisphere signal in human fMRI or NIRS could actually reflect higher inhibitory activity.

Another reason our results differ in pattern of lateralization from human studies could be due to anatomical differences. Birds lack a corpus callosum and may have less cross-talk between hemispheres compared to mammals, although interhemispheric transfer of information can occur through the anterior commissure. To the extent that there are large interhemispheric modulations in human processing of language through the corpus callosum, we may not see the same interactions between the hemispheres in birds. Currently, there are two conflicting theories on how the corpus callosum may increase laterality (van der Knapp et al., 2011). One theory suggests that the corpus callosum exerts its effects through excitatory control, since most callosal fibers are glutamatergic (Conti and Manzoni, 1994). In simple tasks, i.e., processing of the native language, the left hemisphere is sufficient and does not recruit the right hemisphere. However, if the task is difficult, i.e., processing a foreign language, the left hemisphere recruits the right hemisphere via excitatory stimulation through the corpus callosum, resulting in the observed bilateral activation for foreign phonemes (Minagawa-Kawai et al., 2005; Moser et al., 2009).

Perhaps we do not see the same pattern of bilateral activation for heterospecific sounds at baseline because there is no excitatory signaling through the callosum. Instead we see a lower but still lateralized response to heterospecific stimuli. The other model suggests that the corpus callosum generates lateralization through inhibition of the opposite hemisphere. This theory is supported by evidence from aging populations where corpus callosum thinning is correlated with decreased lateralization (Langan et al., 2010). The theory suggests that in language processing, the dominant left hemisphere sends inhibitory signals to the right in order to reduce noise and increase efficiency in processing the native language but does not send inhibitory signals when presented with foreign language.

On the surface, our data do not support this model since our subjects show functional lateralization even without an intact corpus callosum, as do split brained patients. However, the possibility of inhibitory or excitatory cross-talk between hemispheres cannot be completely ruled out since there are inter-hemispheric connections through the anterior-commissure in the avian brain. In fact, one study comparing visual responses in chicks that were lateralized (normally reared) vs. un lateralized (dark reared), showed that integration of visual information from both hemispheres is required to solve a transitive reasoning task where only half the information was presented to each hemisphere (Manns and Römling, 2012). Interestingly, chicks that were dark-reared and as a result un lateralized, could not solve the task. The extent of interhemispheric interactions through the anterior commissure in birds compared to information transfer through the corpus callosum in mammals needs further study, and the difference amount

of cross-talk could explain some of the lateralization differences we observe between birds and mammals.

Another reason we may see activation for zebra finch and canary vocalizations lateralized to the same hemisphere is because zebra finch and canary songs share similar acoustics and occupy overlapping regions of acoustic space as seen by their modulation power spectrums (**Figure 2A**). Thus, if the right hemisphere responds more robustly to zebra finch stimuli, it may also be the stronger responding hemisphere to canary stimuli because the two categories of stimuli are acoustically similar. Along the same vein, studies in bilingual humans have revealed different patterns of activation for the second language (L2) relative to the first (L1) depending on the linguistic distance between L2 and L1 (Jeong et al., 2007). The right ear advantage (REA) for English words is greater in native German speakers than in native Italian speakers presumably because German and English are more linguistically similar (D'Anselmo et al., 2013). In fact, it is debated whether initial learning of L2 piggybacks off of L1 neural structures. Limited research shows that it depends on how similar L1 and L2 are (Kotz 2009). The more similar they are, the more likely L2 is likely to scaffold off L1 but as a result also experiences more interference from L1. One interesting future direction may be to try different types of heterospecific vocalizations of varying spectro-temporal distance from zebra finch vocalizations to decouple similarity with L1 from pattern of lateralization.

Lastly, because the flip and reversion of lateralization is not specific to the category of the stimulus (zf or can stim), but rather, a general increase in activity in the left hemisphere of 4d and 9d HETENV birds to all stimuli, the shift could be due to a lateralized neuromodulatory mechanism. To date, little is known about lateral differences

in the expression of receptors for neuromodulators such as noradrenaline. There are lateral differences in oxytocin receptor expression in auditory cortex of mice (Marlin et al., 2015) but lateralization of other neuromodulatory systems in auditory cortex has not been documented.

Conspecific vs. Heterospecific Go/NoGo Discriminations

Our subjects were operantly trained to discriminate two heterospecific songs belonging to a stimulus category, or feature space, that was unfamiliar to a subset of our birds (CONENV), and recently familiar to the rest (HETENV). The challenges involved in performing such a task may involve first moving from a within vs. with-out category discrimination (i.e., zf vs. not zf), to formation of a novel category (i.e., zf, canary, other), and finally to a within category discrimination (i.e., canary 1 vs. canary 2). The unique challenges of discriminating two heterospecific songs may require additional neural resources not required in a conspecific song discrimination. There is an interesting juxtaposition of lateralization and performance between our study and another go/nogo operant discrimination study in zebra finches, using conspecific songs (Bell et al., 2015). In that study, subjects were median split into fast and slow learners based on performance, fast learners taking between 9-17 days to reach criterion and slow learners taking between 18-41 days. Interestingly, the study found that fast learners were left lateralized in ARMs at the end of training, while slow learners were either not lateralized or slightly right lateralized. However, the causal relationship between lateralization and performance could not be determined. It was unclear whether birds who started off left lateralized tended to be faster learners, or if better performance caused a shift in lateralization such that all birds started off right lateralized but only the faster learners

were left lateralized at the end. Since previous studies have found that the majority of birds are right lateralized for song (Phan et al., 2010), it is more likely that the experience of the operant training caused some birds to shift their pattern of lateralization.

At first glance, our results seem to contradict Bell et al, 2015 since our fast learners (using the criterion of learning in less than 17 days) were the 14D HETENV group who were presumably right lateralized at the beginning of operant training, while our 9D HETENV birds who were left lateralized at the start of training had trouble reaching criterion. One major difference between the two studies is Bell et al., 2015 trained subjects on a conspecific song discrimination, something songbirds already do naturally, whereas we trained subjects on a much more challenging heterospecific song discrimination which has proven difficult for birds in the control condition (**Figure 14A**). Therefore, any shifts in lateralization in Bell et al. are likely due to the novelty of the operant training context, rather than to novelty of the auditory category, as in our experiment. Also, in Bell et al., both fast and slow learners were ultimately successful at the task, despite opposite patterns of lateralization so lateralization at any given point cannot accurately predict performance. Perhaps it is not the final pattern of lateralization that determines performance, but rather, whether exposure to a novel environment was sufficient to trigger reorganization of auditory cortex, seen here as a reversal in the pattern of lateralization. In this light, the fast learners from Bell et al., 2015 may have a more plastic auditory cortex where reorganization is easily triggered by novelty, thus allowing these individuals to learn the new task in less time than their less labile counterparts who do not show a shift in lateralization. Once again, we highlight the

importance of using the dynamic shifts in lateralization as a metric for learning, rather than taking a snapshot of lateralization at a fixed point in time.

Immersive Experience Breaches a Threshold for Neural Reorganization

In groups with prolonged exposure to HETENV, the amount of novel stimulus exposure may exceed a minimum needed for improvement in operant discrimination. One reason to believe there is a minimum quantity is that repeated exposure to the two canary songs repeatedly heard in the operant paradigm was not sufficient to improve performance for either the 9d HETENV group or any of the control CONENV groups. Although the 9d HETENV birds continue to be exposed to canary vocalizations in the operant training beyond the passive exposure, 9d of constant 12h playback with many different canary songs, followed by 21 days of hearing 2 canary songs during training was not enough to help them discriminate songs better than controls. Perhaps brief periods of novel stimulus exposure are sufficient to initiate hemispheric reorganization but continued exposure until stimuli become familiar as a class is necessary to stabilize modifications to perceptual filters in a way that can serve behavior.

It may also be that exposure to many exemplars of the new class is required to encourage category formation. In this view, the 14d HETENV birds are able to establish a new category that serves discrimination of two novel canary songs because they hear many exemplars of canary song. In contrast, 14d and 30d CONENV controls are exposed to only 2 canary songs during operant training, and perhaps receive too small of a sample to form a category, and show no behavioral improvement. These findings have implications for how second languages are taught and may be one reason why language

immersion programs abroad help build up proficiency faster than programs teaching a foreign language using a smaller stimulus set in the participant's native country.

Enriched Environment Effects on Behavioral Performance

Although we have attributed faster rates of learning in our HETENV group to modification of perceptual filters to better process canary-like vocalizations, we cannot rule out the effects of HETENV exposure on neurogenesis and neurochemistry in other brain regions such as prefrontal cortex (nidopallium caudolaterale (NCL) in avians; Güntürkün 2005) and hippocampus. These regions have been shown to modulate selective attention and contextual memory – both necessary in the go/no-go task. Coincidentally, both abilities are improved by exposure to an enriched environment in rodents. The beneficial effects of enriched environment exposure are largely due to increased novelty of stimuli (Li et al., 2013). Although enriched environments usually involve novel objects that challenge the visual and tactile senses, our HETENV exposure presents a novel category of sounds that may mimic an enriched auditory environment. Housing in an enriched environment has been shown to improve cognitive function in other species. Rats housed in enriched environments had increased levels of brain-derived neurotrophic factor (BDNF), a neurotransmitter involved with long term memory (Bekinschtein et al., 2008), and better performance on spatial memory tasks such as the morris water maze (Yuan et al., 2012). Odor discrimination and object exploration were also improved in enriched environment housed rats relative to normally housed rats (Gobbo and O'Mara, 2004). We see similar enhancements in auditory discrimination in HETENV birds relative to CONENV controls. Better performance on the discrimination task could be due to a better contextual memory for the go-no-go songs for birds

challenged with novel HETENV. Enhancements in cognitive function are seen in children with immersion experience in a foreign language (Bamford and Mizokawa, 1991) and bilingual children demonstrate greater inhibitory control when solving cognitive tasks that require selective attention (Bialystok 2009). In our study, one of the primary reasons for poor performance on the go/no-go task was the inability to withhold pecking responses to the no-go song. In general, performance on go trials was better than performance on no go trials and learned at a faster rate across all groups. In fact, the distinction between the prolonged HETENV group that learned and the other groups that did not was performance on the no-go trials (**Figure 15**). The prolonged HETENV group learned to inhibit responses to the no-go song faster than the other groups, and therefore reached criterion performance faster.

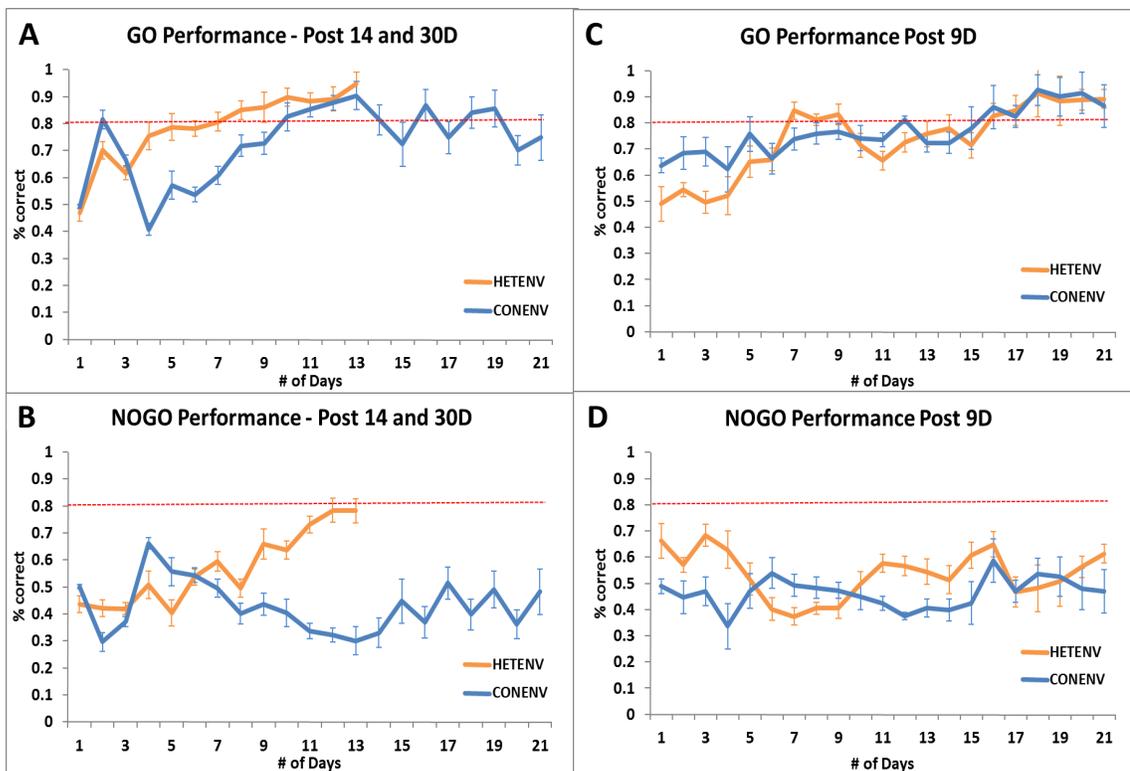


Figure 15. Go/Nogo Performance A) % correct for GO trials after 14 and 30D passive exposure in CONENV (blue) and HETENV (orange). The X axis represents number of days in the operant training paradigm excluding days of refresher training in CONENV and HETENV. The Y axis represents the average % correct on go trials. Dotted red line denotes 80% criterion. Error bars represent standard error of the mean. B) % correct for NOGO trials after 14 and 30D passive exposure in CONENV (blue) and HETENV (orange). C) % correct for GO trials after 9D passive exposure in CONENV (blue) and HETENV (orange). D) % correct for NOGO trials after 9D passive exposure in CONENV (blue) and HETENV (orange).

Possible Underlying Neural Mechanisms Based on Timecourse

Exposure to the stimulus statistics of canary vocalizations in HETENV for 14 and 30d is sufficient to improve operant discrimination of two novel canary songs but 9d of exposure is not. The timecourse of changes in lateralization as well as the shift in behavioral performance between 9d and 14d provides clues about the underlying neural mechanisms. Neural processes that occur on the time scale of 14d could include migration and incorporation of new neurons into functional circuits. Greater rates of neurogenesis in the left hemisphere are correlated with better song copying (Tsoi, et al., 2014). However, it is unclear whether greater rates of neurogenesis in the left hemisphere are required for better song copying, or if producing a better song copy stimulated neurogenesis in the left hemisphere due to a better match with the tutor song memory. Although our birds are adults and have achieved stable song, neurogenesis could still play a part in the formation and incorporation of new auditory memories. Perhaps increased activity in the left hemisphere in our 4d and 9d HETENV birds led to rescue of more new neurons, which were then incorporated as mature neurons into NCM circuitry at 14d and 30d time points. We can speculate that more new neurons in the left hemisphere combined with increased excitability of immature neurons could explain the initial left side higher ARMs seen in 4d and 9d HETENV groups.

Exposure to an enriched environment in rats has been shown to increase survival of immature neurons (Mongiati et al., 2009). If more new neurons are rescued as a result of novel auditory environment exposure, they will likely integrate into existing circuits and could contribute to plasticity that underlies overall discrimination of heterospecific canary stimuli. In this view, as these rescued neurons mature over the course of 14 -30

days, their excitability decreases, bringing ARMs in the left hemisphere down to the normal levels seen in our 14d and 30d HETENV groups. These mature neurons that received exposure to HETENV when they were at their most plastic, are now integrated into the network as mature neurons, aiding in discrimination of a new class of sounds.

Chapter 4

Experiment 3: Does exposure to novel sounds and sound categories have an immediate effect on receptive field organization?

INTRODUCTION

The long term changes we observe in NCM after extended exposure to a novel category of sounds could be an accumulation of smaller modifications to perceptual filters in NCM on a short time scale, stimulated by ongoing category violations (as discussed above). The auditory cortex may adapt to the current distribution of sounds by tweaking perceptual filters to extract relevant information. Rapid changes in perception have been demonstrated in humans listening to foreign-accented speech. Over the course of 10-20 minutes of listening to a speaker with a foreign accent, the accent becomes less noticeable and perception of speech is improved (Bradlow and Bent 2008).

A change in neural response that occurs on a similar time-scale in songbirds is stimulus-specific adaptation (SSA). SSA can be interpreted as a form of neuronal memory for individual songs that is seen in higher auditory areas NCM and CMM but not in earlier structures along the auditory pathway such as Field L, although other forms of simpler SSA similar to deviance detection in mismatch negativity have been observed as early as inferior colliculus (Nelken and Ulanovsky, 2007) and Field L in songbirds (Dong, unpublished data). SSA as it is referred to here is an effect of stimulus repetition (even with interstimulus intervals of 50s or more) that produces a long lasting adaptation for individual conspecific songs that can be detected >20 hours after initial presentation of the song (Chew et al, 1995, 1996; Tsoi et al., 2014). Repeated presentations of a song

lead to decreased neuronal response for that stimulus while responses to other novel song stimuli remain robust and can adapt independently (Chew et al, 1996; Stripling et al., 2001). SSA to an individual song could be driven by an overall decrease in response amplitude to all elements and syllables of the song, or a selective sparsening of response such that responses to some syllables decrease but responses to other syllables are retained. Both scenarios would manifest as a decrease in overall response strength. However, the latter scenario would suggest that perceptual filters were being modulated in a specific way as a result of recent auditory experience. These changes in the perceptual filters would be a sort of “memory” for recently heard stimuli, but the changes in sensitivity to component sounds could potentially influence processing of all future incoming stimuli.

If repeated exposure to a novel stimulus leads to increased selectivity in the neuronal response for specific spectral-temporal features, then we should be able to detect sharpening of temporal and spectral tuning, or shifts along the spectral-temporal dimensions of the spectral temporal receptive field (STRF) for a particular neuron. Furthermore, the amount of adaptation might correlate with the degree of change in the STRF such that units with steeper adaptation will have a greater change in their STRF. In order to detect real-time changes in the STRF of an individual neuron in response to auditory experience with either category familiar conspecific songs, or category novel heterospecific songs, we want to compare the STRF of single neurons before and after auditory experience. However, the first step in this experiment is to establish a valid method of measuring STRFs in NCM.

There are two issues that make obtaining STRFs in NCM challenging. First, in order to measure the receptive field of a neuron, one must present a large collection of sounds that cover the entire acoustic space that the neuron could be responsive to. Receptive fields in NCM may be multi-dimensional and non-linear so simple tones and bandpass noise are not sufficient. A large enough collection of conspecific songs could be used to generate a STRF but the reverse correlation method of measuring STRFs requires obtaining enough spikes to generate an average, so song stimuli would have to be presented multiple times. However, NCM neurons adapt to song stimuli (Chew et al., 1995; Stripling et al., 2001) and we hypothesize that adaptation to song could change the underlying STRF. Thus, if we use song stimuli to probe for STRFs, as has been done for STRFs in Field L (Woolley et al., 2009), we could be causing changes in the STRFs as we are attempting to measure them. A better method for sampling all of acoustic space, while not confounding the results with song adaptation, is to use ripple noise stimuli to generate the STRFs. Ripple noise stimuli provide a basis set, developed for use in auditory neurophysiology (Escabi and Schreiner, 2002), that can be adjusted to produce a stream that spans the acoustic space of zebra finch song. Ripple noise is produced by summing multiple ripple; as a result, it contains no explicitly repeated structure. In a series of pilot series tests, we confirmed that repeated presentations of a stream of ripple noise do not produce significant adaptation in NCM neurons (unpublished data). Thus, in this experiment, we were able to use repeated tests with ripple noise to measure STRF changes after exposure to novel stimuli.

METHODS

Subjects and Housing

Adult male zebra finches (>120 days of age; n=14) were obtained from the zebra finch breeding aviary at Rutgers University, New Brunswick, NJ or purchased from a local supplier. At the beginning of the experiment, subjects were individually housed in cages in the main aviary. Subjects remained in the general aviary on a 12/12 light/dark cycle with *ad libitum* access to food and water until removal for electrophysiological testing.

Electrophysiological recording

Two days prior to testing, subjects were deeply anesthetized with 1.5-2.0% isoflurane in oxygen and placed in a stereotaxic apparatus. A metal pin was attached to the skull with dental cement and a craniotomy was performed on the skull above NCM in preparation for awake, head-fixed electrophysiological recording. Post-surgery, subjects were observed for recovery and returned to their individual cages in the general aviary. On test day, subjects were removed from the general aviary and placed in a soundproof booth (IAC Inc., Bronx, NY) for electrophysiological recording. Electrophysiological recording protocol and electrode placements were the same as in Experiments 1 and 2.

Testing Stimuli

All song stimuli were presented at 70dB through a speaker centered 30cm in front of the subject. Testing stimuli consisted of Ripple stimuli and Song stimuli. Ripple stimuli were generated in MATLAB (courtesy of M. Escabi; Escabi and Schreiner, 2002) from 16 dynamic ripples that mimic the modulation spectrum of zebra finch song. The 16 dynamic ripples were layered to create a ripple noise stimulus with characteristics of natural zebra finch songs, but similar to white noise, so that there was no discernable

spectro-temporal structure. Unlike unique conspecific songs, ripple noise stimuli do not elicit adaptation. Testing began with playback of a 10 minute stream of ripple noise to establish the baseline pre-adaptation STRF at a given recording site. Then, an adaptation set was presented, consisting of either silence (control condition- 17 mins), 5 novel canary songs or 5 novel zebra finch songs (shuffled order, 25 repetitions each, 8s ISI; 17 mins total duration). This was followed by a second presentation of the same ripple noise used in pre-adaptation STRF to obtain the post-adaptation STRF.

Data Analysis

Single units were isolated from multi-unit activity using clustering algorithms in Spike2 software (CED, Cambridge, UK). Units were excluded from the analysis if greater than 2% of inter spike intervals were less than 2ms, suggesting contamination. Refractory periods of neurons in NCM are unlikely to be less than 2ms; therefore, all inter spike intervals under 2ms likely to contain contaminated spikes from another unit. After isolation of single units, the spike trains of each individual unit were reverse correlated to the ripple stimulus that had been heard in order to generate STRF matrices (Matlab, code courtesy of Monti Escabi) both pre- and post-adaptation. Pre and post STRF matrices were normalized to their respective means so that positive or negative values (red or blue respectively in the heat map, **Figure 16**) represent a percentage above or below the mean. Normalized STRFs were thresholded using criteria that selected for only regions that were significantly different from the mean ($p < 0.05$) in order to eliminate background noise and define major features in spectro-temporal space. STRF changes were analyzed in several ways.

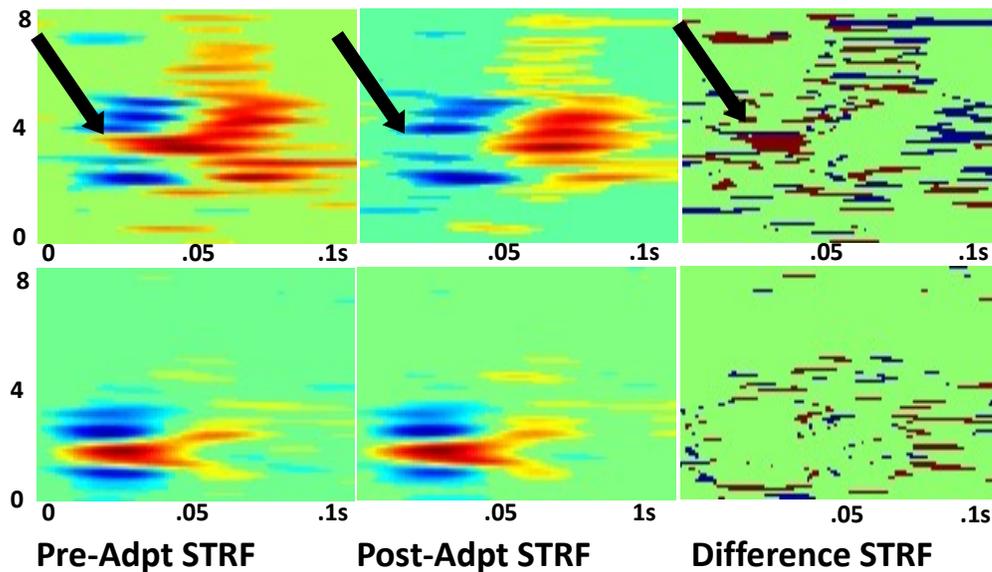


Figure 16. Example of STRFs A) STRF that changed in temporal and spectral space (black arrows) after song presentation X-axis represents time (s) and y-axis represents frequency (kHz). B) STRF that remained stable after song presentation. Pre and Post adaptation STRFs were binarized so all responsive regions were assigned a value of 1. The Post STRF was subtracted from the pre STRF to obtain a binarized matrix of all pixels that changed. The difference matrix was absolute valued then summed to yield the total pixel change between Pre and Post STRFs.

Total STRF change was calculated by first converting thresholded pre- and post- STRFs into binary value matrices, 0 for cells with no response and 1 for cells with a response.

The post binary STRF was subtracted from the pre binary STRF to obtain a binary

Difference STRF containing the pixels that shifted in either the frequency or time

dimension. Pixels in the Difference STRF were summed to obtain the total STRF change post adaptation.

Pos Sum was calculated as follows: The thresholded pre STRF was subtracted from the post STRF, yielding a Difference STRF that contained pixels that increased in value from Pre to Post (positive values) and pixels that decreased in excitation (negative values), relative to the mean of all pixels. To obtain the amount of total excitatory changes in the STRF, we summed only the positive values in the Difference STRF.

Neg Sum was calculated by summation of the negative values in the Difference STRF, obtained as above. Thus, the Neg Sum represents regions of the STRF that either decreased in excitation or increased in inhibition relative to the mean.

Cumulative frequency distributions of total STRF change, Pos Sum and Neg Sum were compared between silence, canary and zf intervening stimuli conditions in a two sample KS test. Further, an ANOVA hemisphere (left, right) and stimulus type (silence, canary, zf) as factors was performed to uncover possible interactions between the stimulus played and STRF change in each hemisphere that might be analogous to shifts in lateralization after long term exposure to CONENV or HETENV (Experiment 1).

Spike Waveforms

Templates of spike waveforms were extracted for each single unit and subjected to a cluster analysis to establish neuronal subtypes (narrow vs. wide), possibly corresponding to inhibitory (narrow) vs. excitatory (wide) neurons. About half of cells in NCM are known to be inhibitory (Pinaud et al., 2008; Jeong et al., 2009). To cluster the units, the spike shapes of all extracted single units were characterized in terms of their spike width, rising slope, and peak amplitude (**Figure 17**). Spikes were typically triphasic (initially down-going); spike width was taken as the time between the two minima of the

waveform. Rising slope was measured from the first minimum to the first maximum. Peak amplitude was measured as the height of the first maximum. All single units extracted were classified as narrow or wide spikes based on k-means clustering. An ANOVA was used to further analyze differences between units with narrow and wide spikes, i.e., total STRF change, Pos Sum, Neg Sum, Rising Slope and Peak Amplitude. The amount of total STRF change and the adaptation rate were also subjected to a regression analysis. All data collected (STRFs, adaptation rate, waveforms) were analyzed for differences in response patterns, adaptation and STRF changes between the two hemispheres.

Two sample Kolmogorov-Smirnov tests were performed to test for differences between distributions of STRF change scores after subjects heard either novel canary songs, novel zebra finch songs, or silence.

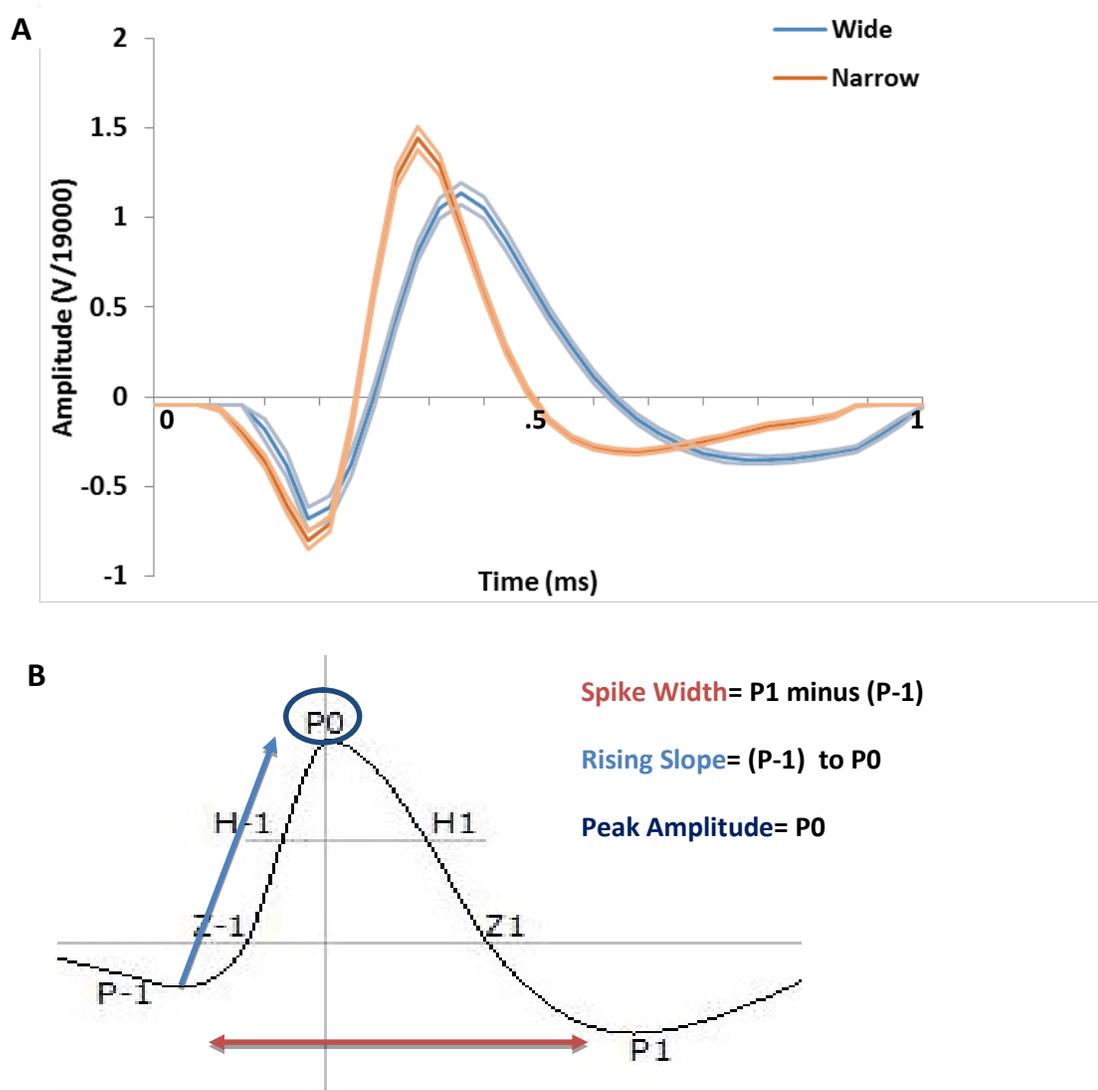


Figure 17 Spike shape templates A) Average spike waveforms of Wide (blue) and Narrow (red) spikes. Y-axis represents amplitude (Volts/19,000 amplified gain) and x-axis represents time (ms). B) Schematic of spike waveform measurements (adapted from Spike2 manual). Spike Width (red) is the time from P1 to P-1. Rising Slope (light blue) is the slope from P-1 to P0. Peak Amplitude (navy) is the amplitude at P0.

RESULTS

A total of 131 single units were analyzed; 57 from birds exposed to Canary songs, 38 from birds exposed to Zebra Finch songs and 36 from birds exposed to Silence. Total STRF change scores represent the total number of pixels that differed between pre and post STRFs in either an positive or negative direction. Exposure to Canary songs caused more total STRF change than hearing ZF songs ($D= 0.351$, $p<0.005$, **Figure 18**). In addition, Total STRF change was greater for ZF song than for silence as expected from pilot tests ($D= 0.336$, $p< 0.023$, **Figure 18**), and greater for Canary song than for Silence ($D= 0.513$, $p<0.0001$, **Figure 18**). An ANOVA on STRF change with Hemisphere and Stimuli as factors showed a significant main effect of Stimuli ($F(2, 125)=7.157$, $p<0.001$, **Figure 19**), driven mostly by Canary stimuli causing greater total STRF change than either ZF or Silence (Bonferroni post-hoc: $p<0.012$; $p<0.001$, respectively); there was no main effect of hemisphere and no interaction.

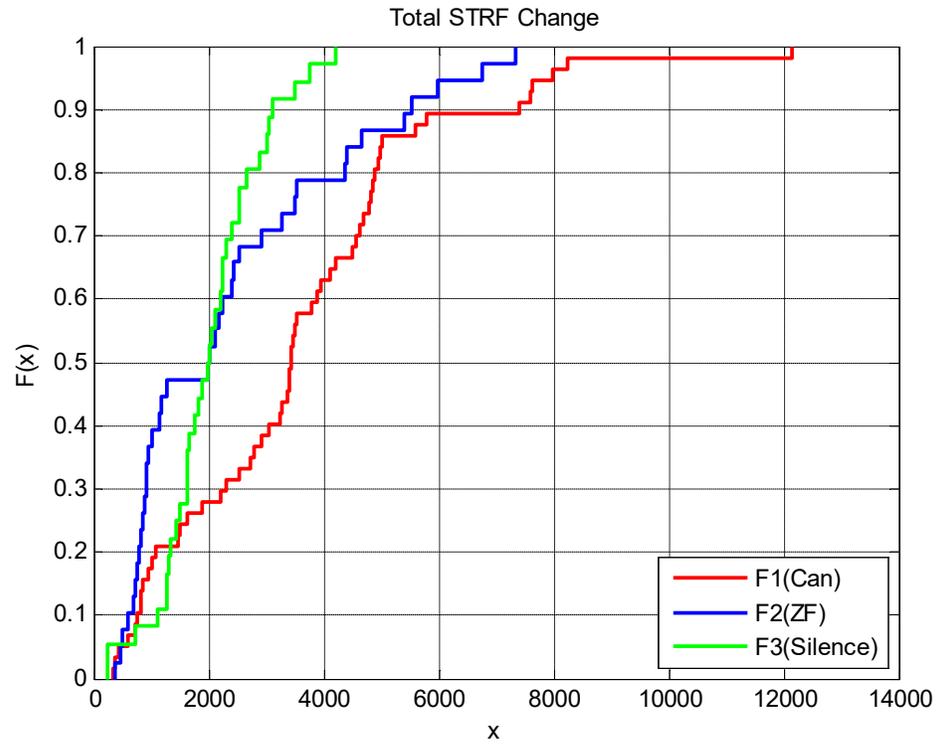


Figure 18. Cumulative frequency distribution of total STRF change between Pre and Post STRF for single units exposed to silence (green line), zebra finch songs (blue line) and canary songs (red line). X-axis represents the number of pixels that changed between Pre and Post STRF. Y-axis represents percent cumulative frequency.

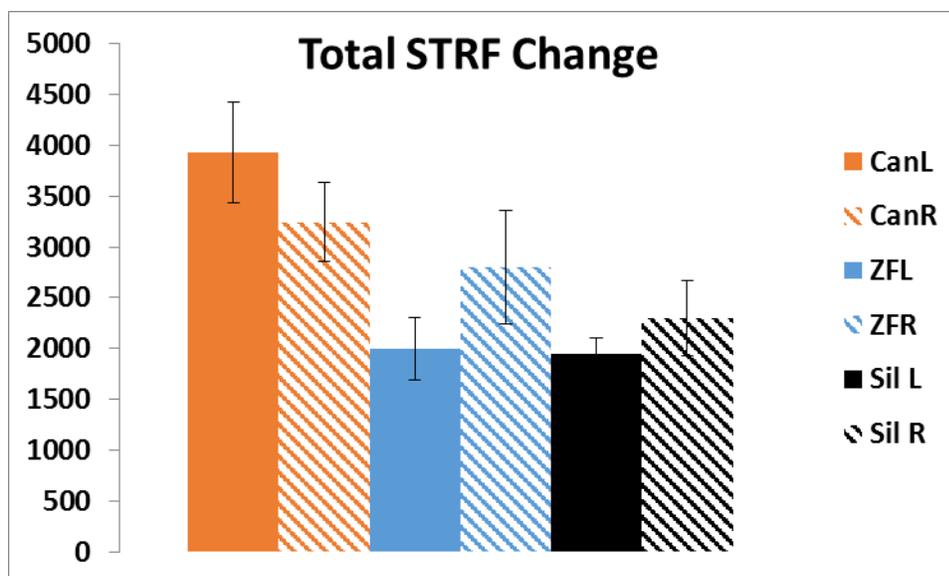


Figure 19 Total STRF change for the left (solid) and right (striped) hemispheres in three different stimulus sets as the intervening adaptation set, canary song (orange), zebra finch song (blue), and silence (black). Y-axis is the total number of bins that changed between the Pre and Post STRF. Error bars represent standard error of the mean (SEM).

To better define the characteristics of STRF modifications, we analyzed increases and decreases in responsive cells in the STRF separately. PosSum represents a sum of all positive cell values obtained after subtracting the Pre STRF from the Post STRF. Pos Sum quantifies regions that increased in response relative to the normalized mean of the STRF after exposure to testing stimuli. Following a similar pattern to Total STRF change, Pos Sum was showed a trend towards greater change for Canary stimuli than for ZF stimuli but the difference between the distributions was not significant ($D= 0.237$, $p= 0.133$, n.s., **Figure 20**). However, both Canary ($D= 0.412$, $p< 0.001$, **Figure 20**) and ZF ($D= 0.335$, $p<0.024$ **Figure 20**) stimuli evoked significantly more positive changes than Silence. An ANOVA on Pos Sum as the dependent variable, and Stimuli and Hemisphere as factors, showed a main effect of stimuli ($F(2, 125)=4.007$, $p=0.021$, **Figure 21**).

Bonferroni post-hocs showed a significant difference between the Canary and Silence group ($p < 0.021$) and a trend towards a difference between the Canary and ZF groups ($p = 0.113$, n.s.). However, ZF and Silence were not statistically different.

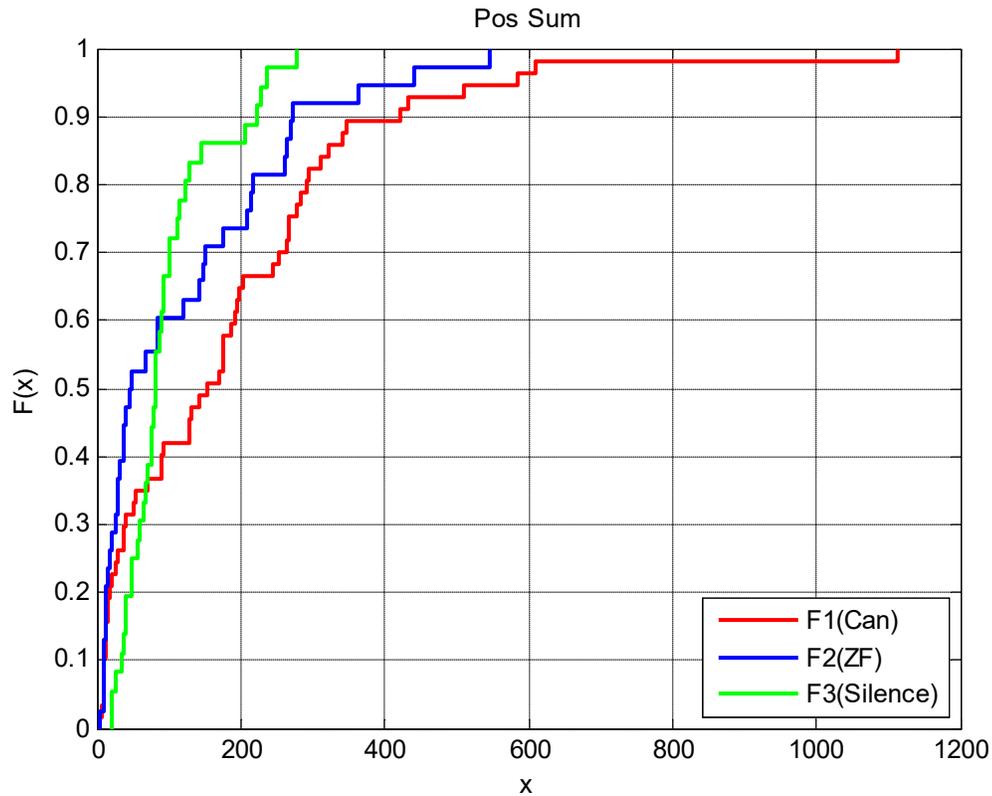


Figure 20 Cumulative frequency distribution of the summed magnitude of positive changes between Pre and Post STRF for single units exposed to silence (green line), zebra finch songs (blue line) and canary songs (red line). X-axis represents total increase in excitation between Pre and Post STRF, summed across the Difference STRF. Y-axis represents percent cumulative frequency.

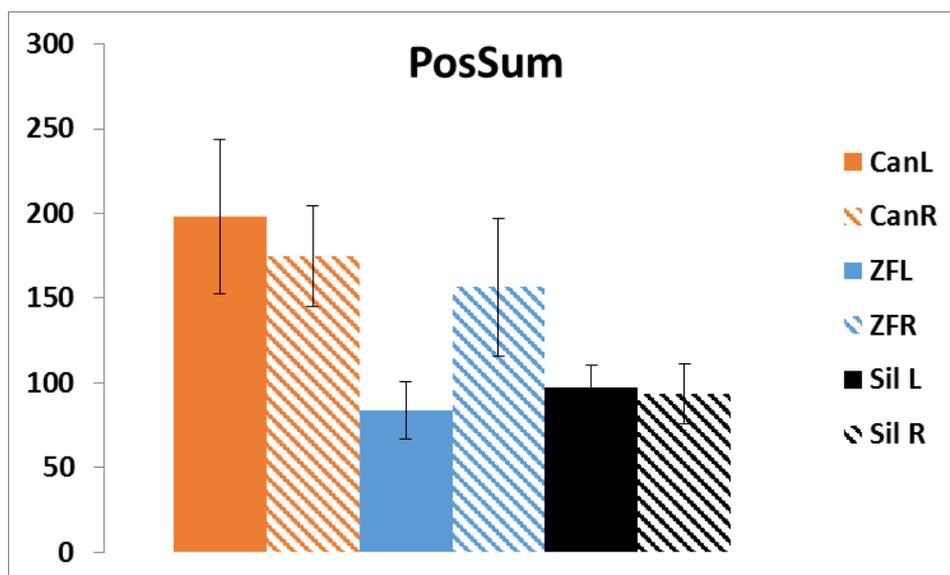


Figure 21 Sum of positive differences for the left (solid) and right (striped) hemispheres in three different stimulus sets as the intervening adaptation set, canary song (orange), zebra finch song (blue), and silence (black). Y-axis is the sum of positive values in the Difference STRF obtained after subtracting the Pre STRF from the Post STRF. Error bars represent standard error of the mean (SEM).

We also tested the effects of ZF or Canary stimuli on the Neg Sum (the sum of all negative cell values after subtracting the Pre STRF from the Post STRF). Negative cell values would mean that a region was more responsive in the Pre STRF than in the Post STRF, thus showing a decrease in response after song exposure. Canary songs caused greater decreases in response than ZF songs ($D=0.999$, $p < 1.963e-21$, **Figure 22**), and both ZF songs and Canary songs caused more inhibitory changes than Silence ($D=0.999$, $p < 1.374e-17$; $D=0.999$, $p < 1.963e-21$, **Figure 22**). An ANOVA on Neg Sum with Stimuli and Hemisphere as factors showed a main effect of Stimuli ($F(2, 125)=4.233$,

$p=0.017$, **Figure 23**) and a trend towards a hemisphere by stimuli interaction where the left hemisphere showed more change to Canary songs while the right hemisphere showed more change to ZF songs ($F(2, 125)=2.526$, $p=0.084$, n.s., **Figure 23**). Bonferroni post hoc on the main effect showed that Canary song exposure lead to significantly more change than ZF song ($p<0.030$) and a trend towards a difference between the Canary and Silence condition ($p= 0.070$, n.s.). ZF song was not significantly different from Silence in the Bonferroni.

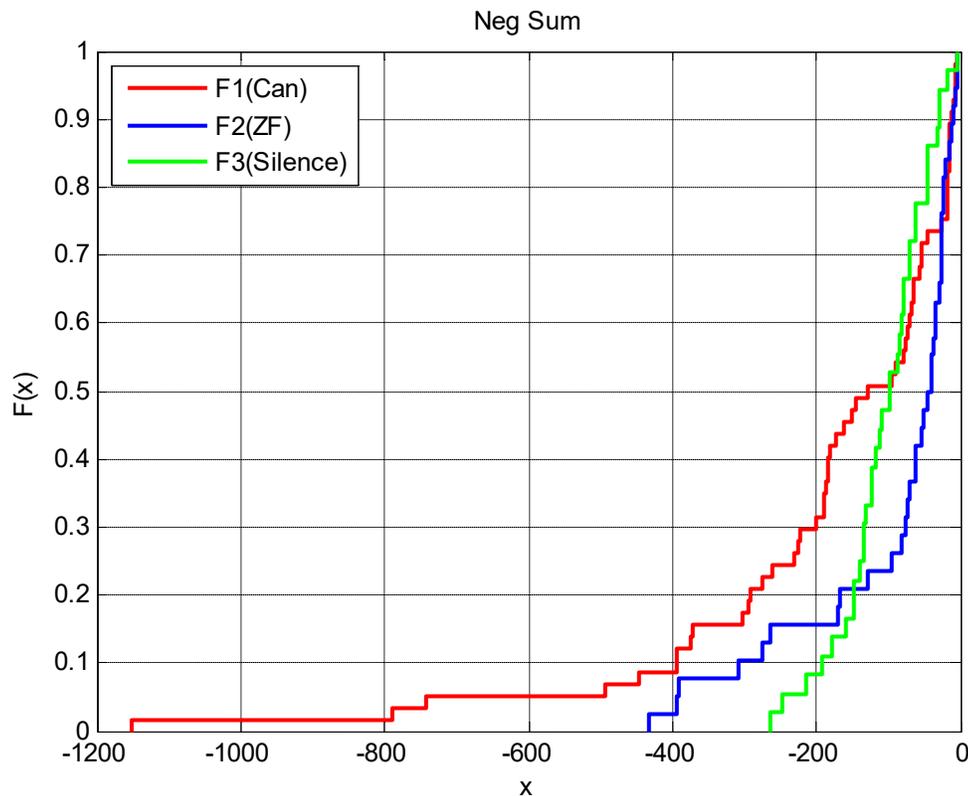


Figure 22 Cumulative frequency distribution of the summed magnitude of negative changes between Pre and Post STRF for single units exposed to silence (green line), zebra finch songs (blue line) and canary songs (red line). X-axis represents total decrease in excitation between Pre and Post STRF, summed across the Difference STRF. Y-axis represents percent cumulative frequency.

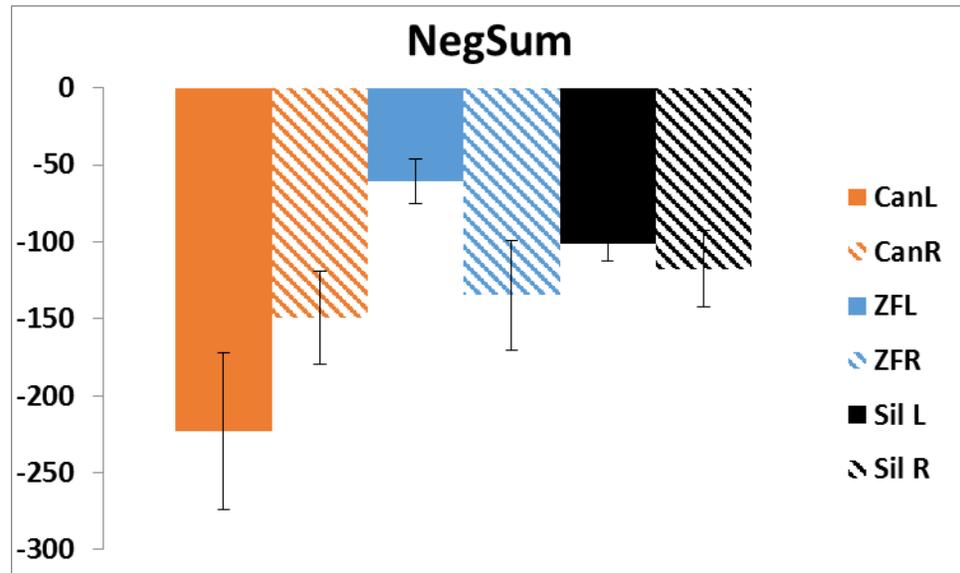


Figure 23 Sum of negative differences in the Difference STRF for the left (solid) and right (striped) hemispheres in three different stimulus sets as the intervening adaptation set, canary song (orange), zebra finch song (blue), and silence (black). Y-axis is the sum of negative values in the Difference STRF obtained by subtracting the Pre STRF from the Post STRF. Error bars represent standard error of the mean (SEM).

Analysis of the predicted relationship between depth of adaptation in spike rates and STRF change did not reveal any significant relationship to Total strf change, pos sum, or neg sum for single units exposed exposed to either zfstim or to canstim (**Figure 24-26**).

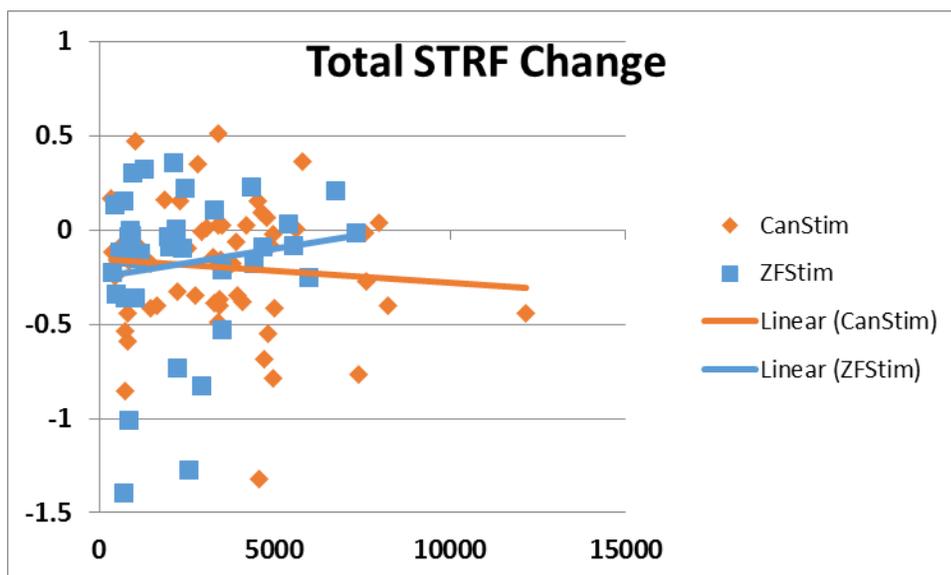


Figure 24 Correlation of adaptation rates with Total STRF Change. X-axis is total STRF change and Y-axis is adaptation rate. Each point represents a single unit that was exposed to either canary songs (orange) or zebra finch songs (blue). The best fit regression line is plotted for each group. R-squared is not significant for either group.

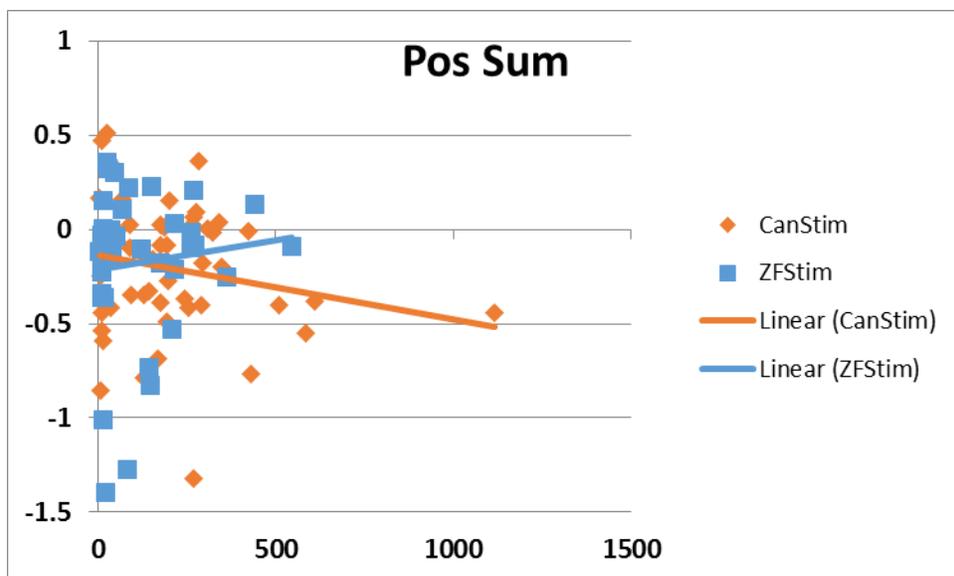


Figure 25 Correlation of adaptation rates with Pos Sum. X-axis is Pos Sum and Y-axis is adaptation rate. Each point represents a single unit that was exposed to either canary songs (orange) or zebra finch songs (blue). The best fit regression line is plotted for each group. R-squared is not significant for either group.

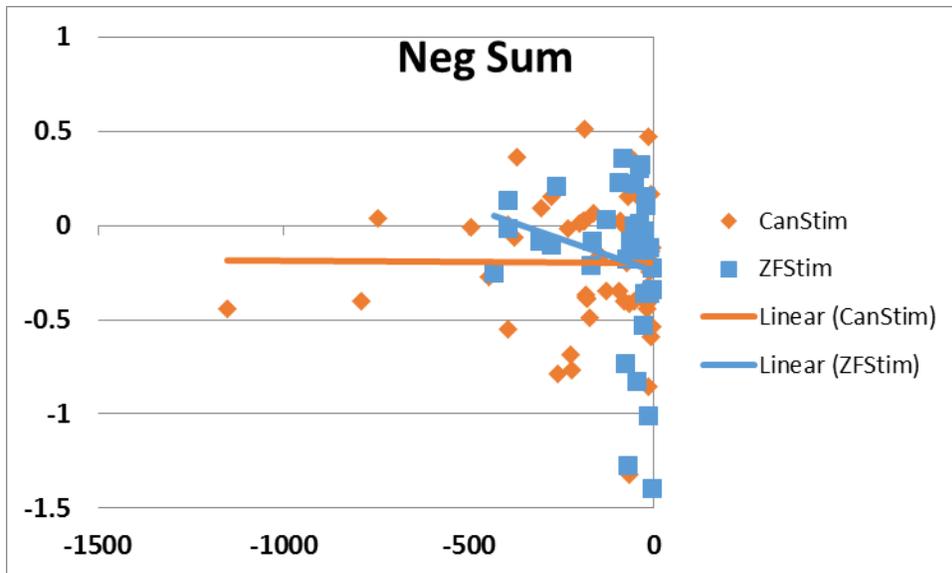


Figure 26 Correlation of adaptation rates with Neg Sum. X-axis is Neg Sum and Y-axis is adaptation rate. Each point represents a single unit that was exposed to either canary songs (orange) or zebra finch songs (blue). The best fit regression line is plotted for each group. R-squared is not significant for either group.

Single units in NCM showed both positive and negative changes in their STRFs following the presentation of a set of auditory stimuli, suggesting a convergence of excitatory and inhibitory inputs onto a given cell. Approximately half of the neurons in NCM are inhibitory, and half are excitatory. Therefore, there are likely two (or more) subtypes of neurons in NCM that process and adapt to incoming stimuli to varying degrees. When spikes were clustered into two groups (see **Figure 17**) based on spike width (**Figure 27A**) using k-means clustering, narrow and wide spikes differed on other characteristics. Narrow spikes tended to have a steeper slope from the first minimum to the peak of the spike (**Figure 27B**). Narrow spikes also tended to have higher peak amplitudes than wide spikes (**Figure 27C**) and spikes in the right hemisphere had higher peak amplitudes than the left. This difference in peak amplitude between the two hemispheres may contribute partially to the right side higher ARMs observed in the multi-unit data, although other factors such as spike rate and number of neurons activated are likely to also affect multi-unit ARMs. Given the separation of cell types based on spike shape, we proceeded to investigate whether these two cell types were functionally distinct as well.

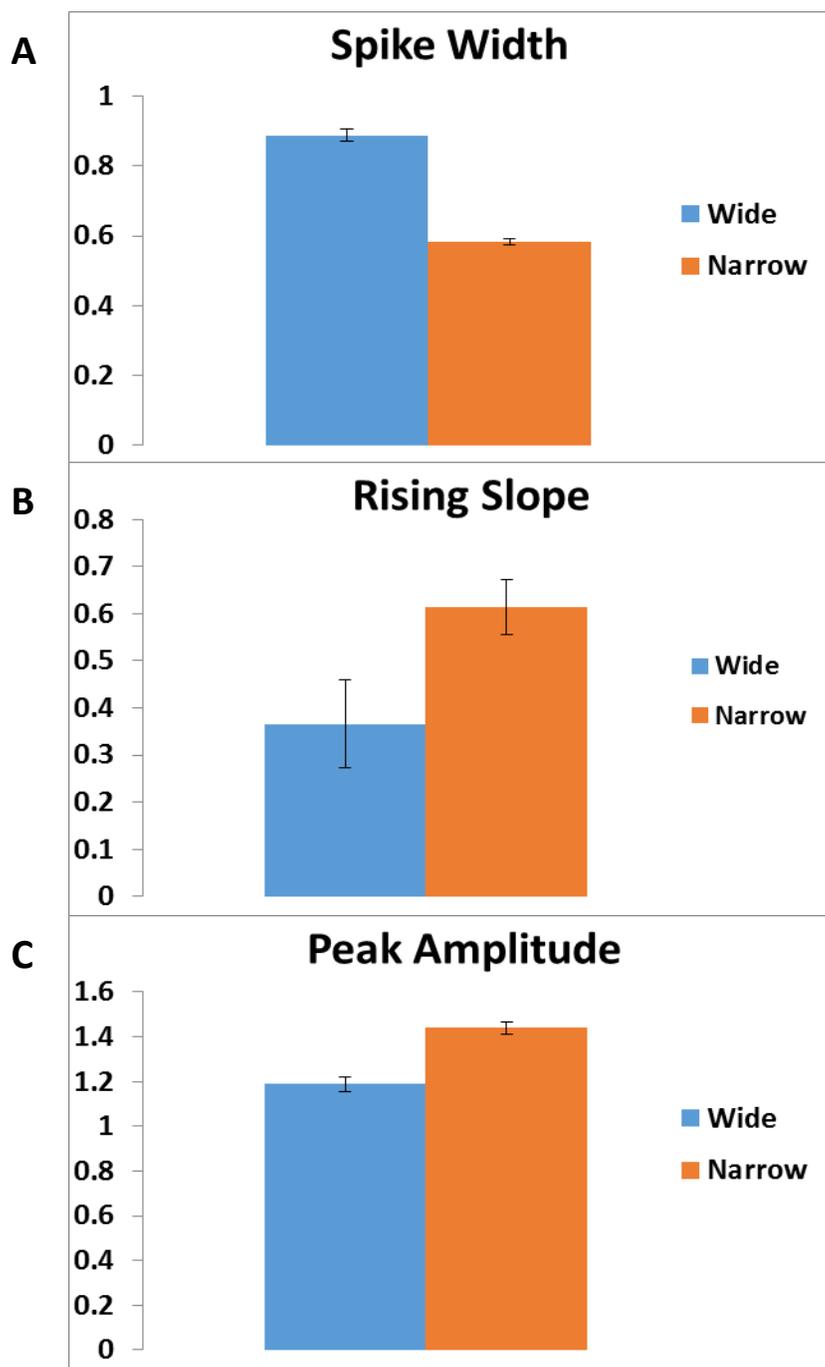


Figure 27 Spike characteristics A) Spike width of wide and narrow spikes. K-means clustering on spike shape yielded two clusters that differed significantly on spike width. Y-axis is the duration in milliseconds (ms) between the first and second minimum of the spike. B) Rising slope for wide and narrow spikes. Y-axis is the slope from the first minimum to the first maximum of the spike. Rising slope was significantly steeper for narrow spikes (orange) than for wide spikes (blue). C) Peak amplitude of wide and narrow spikes. Y-axis is the amplitude (Volts/ 19,000 amplified gain) at the first maximum of the spike. Peak amplitude is significantly greater in narrow spikes (orange) compared to wide spikes (blue). Error bars represent standard error of the mean (SEM).

Thus, we investigated whether the amount of modification to the STRF differed between narrow and wide cells after auditory experience. An ANOVA with hemisphere and width as the factors showed no main effect of hemisphere but a significantly larger amount of total STRF change in narrow spikes ($F(1, 127)=13.625, p=0.0003$, **Figure 28A**).

Furthermore, narrow spikes also showed greater increases and decreases in responsive regions of the STRF compared to wide spikes ($F(1, 127)=9.2882, p=0.003$; $F(1, 127)=13.046, p=0.0004$, respectively, **Figure 28B, C**). Interestingly, wide and narrow spikes did not differ in their adaptation rates ($F(1, 127)=0.565, p=0.454$) or in their spike rates ($F(1, 127)=0.678, p=0.412$). However, there was a trend towards faster spike rates in the right hemisphere ($F(1, 127)=3.322, p=0.071, n.s.$), possibly related to right side higher ARMs that we observe in multi-unit data in control birds.

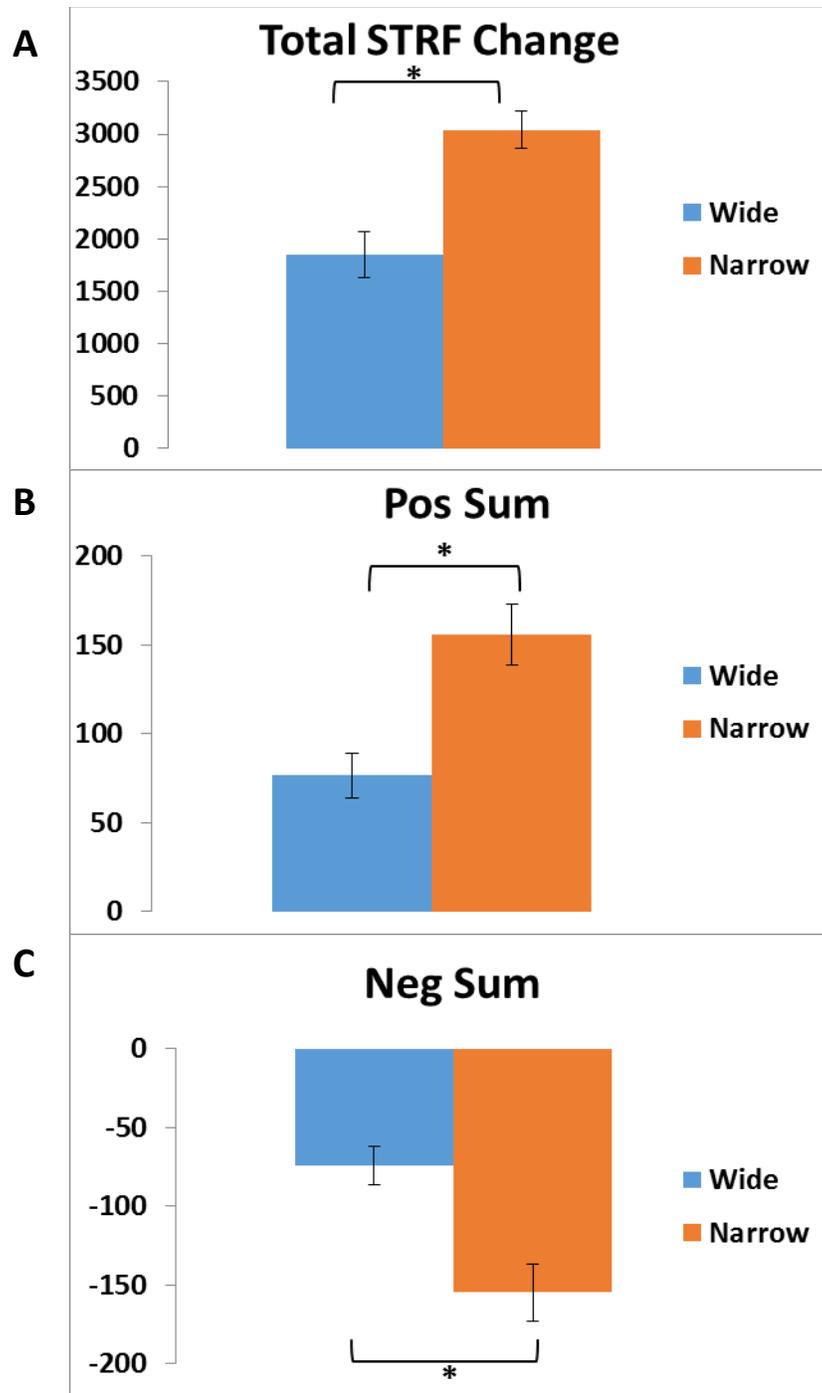


Figure 28 Strf change in narrow and wide spikes A) Total STRF change was significantly greater in narrow spikes (orange) than wide spikes (blue). Y-axis is the total number of cells that differed between Pre and Post STRF. B) Pos Sum was significantly greater in narrow spikes (orange) compared to wide spikes (blue). Y-axis is the sum of all positive values in the difference STRF. C) Neg Sum was significantly greater in narrow spikes (orange) compared to wide spikes (blue). Y-axis is the sum of all negative values in the difference STRF. Error bars represent standard error of the mean (SEM) and asterisks denote significant differences.

DISCUSSION

The results show that spectral-temporal receptive fields of neurons in secondary auditory cortex, NCM, can be modulated by auditory experience on a short time-scale. Changes in STRFs occur after less than 30 minutes of song presentation and the magnitude of these changes varies depending on the type of song stimulus presented (conspecific, heterospecific, or silence). If auditory cortex contains a set of perceptual filters that were formed based on exposure to a lifetime of sounds with the distribution of zebra finch song, presentation of a novel zebra finch song may adjust that prior slightly by adding a new exemplar of conspecific song. Exposure to silence would not be expected to cause any STRF change since it does not update the distribution of historically heard sounds. However, exposure to a heterospecific canary song that is both novel as a stimulus, and belonging to a novel category far from the mean distribution of typical zebra finch sounds, would be expected to change STRFs most drastically. These are in fact the results that we observe. Single unit recordings from neurons exposed to canary or zebra finch stimuli (between ripple tests) had significantly greater STRF changes in all three measures (Total STRF change, Pos Sum and Neg Sum) than units exposed to silence. STRF changes were generally greater in units exposed to canary stimuli than those exposed to zebra finch stimuli, although this difference was not significant in Pos Sum, where zebra finch and canary stimuli caused about equal amounts of increases in the STRF response.

In addition to differences in amount of STRF change between the three groups, we also expected to see a correlation between adaptation rate and STRF change. We hypothesized that if adaptation was a selective sharpening of the response, then the

underlying STRFs might also become more finely tuned. However, we did not see any correlations between amount of STRF change and adaptation rate on any measure. Our result may be due to the heterogeneity of neural responses. STRF change and adaptation rate could be decoupled if some neurons reduced their firing rate indiscriminately to all elements of the song. A unit like this would have a steep adaptation slope with very little STRF change. On the contrary, other units sampled could have a shallow adaptation slope while having large changes in the STRF if firing rate increased selectively to specific elements of the song, and decreased to other elements. In this case, the net change in firing rate would be close to zero. Our suspicions that our single units represented a heterogeneous population of cells in NCM were confirmed when we clustered single units by spike width.

Neurons were classified by their shapes as either narrow or wide spikes, and these subtypes had different characteristics. Narrow spikes had higher peak amplitudes and steeper rising slopes than wide spikes. Furthermore, narrow and wide spikes showed differing amounts of total STRF change, Pos Sum change and Neg Sum change. Narrow spikes had more total STRF change, as well as more increases and decreases in the Post - STRF. Neither Wide nor Narrow spikes showed any correlation between adaptation rate and STRF change possibly due to the heterogeneity of adaptation rates within the categories of Wide and Narrow spikes. It is likely that several (at least 2) functionally different subtypes of neurons exist in NCM. Some neurons may be less plastic and are responsible for maintaining perceptual filters formed during the early critical period i.e. response bias for conspecific songs while other subtypes may be more plastic and are recruited to process novel sound categories should they arise. Currently, we have

identified two possible subtypes of neurons in NCM based on differences in spike shape and plasticity. However, this is just one of many ways neurons in NCM could be categorized. Other modes of classification, i.e. by STRF shape, connectivity, etc., may reveal even finer distinctions between neuronal subtypes in NCM.

Chapter 5

General Discussion

Passive exposure to auditory stimuli is sufficient to cause reorganization

Passive exposure alone is sufficient to induce significant plasticity in the properties of NCM auditory responses. Patterns of lateralization, tuning width, and STRFs all change after exposure to a novel category of sounds. Some studies show no changes in adult auditory cortex to passively presented stimuli; however, these studies often present passive stimuli as probe trials in an operant conditioning paradigm (Bieszczad and Weinberger, 2010), while in other studies, the passive exposure control group is exposed to stimuli for equal lengths of time as subjects undergoing operant training (de Villers-Sidani et al., 2007). These studies differ from ours in two ways. First, the duration of passive exposure may not be long enough to induce plasticity. Changes in neural response induced by passive exposure may require a prolonged period (Zhou et al., 2011), compared to durations typically used in operant training paradigms (Bieszczad and Weinberger, 2010, Polley et al., 2006, de Villers-Sidani et al., 2007). Furthermore, if statistical learning about the underlying probability distribution of sounds is the factor driving cortical plasticity, then a sufficient number of exemplars must be presented from which to deduce the probability distribution of novel sounds, or to form a novel category of sounds. If only a few passive stimuli are presented sparsely in time, then subjects may not receive a sufficient quantity or variety of stimuli to trigger a reorganization of perceptual filters in auditory cortex.

Prolonged passive exposure to novel category improves discrimination

Our data show that a prolonged period of passive exposure to a novel stimulus category improves behavioral discrimination of novel exemplars from that category, even though they were not part of the passive stimulus set. In humans, a similar effects of passive exposure can be seen during perception of foreign accented speech. Speech perception requires the mapping of a noisy acoustic signal to phonemic categories, a task made even more difficult when listening to a speaker with a foreign accent (Kleinschmidt and Jaeger, 2015). Previous exposure to a speaker with Mandarin-accented English improved comprehension of Mandarin-accented speech across multiple speakers (Baese-Berk et al., 2013). Passive exposure to Dutch words with ambiguous final consonants along the /f/-/s/ continuum shifted the categorical boundary in both L1 and L2 speakers of Dutch. Furthermore, the shift in perceptual boundary induced by accented speech in a foreign language were shown to persist for words in the native language (Reinisch et al., 2013). These studies demonstrate that passive exposure to a novel category of sounds i.e., a novel foreign accent, could cause changes in categorical boundaries with the potential to alter perception of incoming stimuli, even those belonging to the native category.

Perhaps the retuning of categorical boundaries also contributes to the improvements in behavioral discrimination that we observe in our birds after passive exposure to a novel category. Our data show that passive exposure to novel heterospecific sounds does generalize to improve discrimination of two completely novel heterospecific songs from the previously heard category. Modifications of perceptual filters after exposure to a novel distribution of sounds could explain these long-lasting and wide-ranging effects on behavioral discrimination.

Our results also are consistent with data from one of the few human studies that show implicit learning of grammar in adults after passive exposure (Friederici et al., 2002). Perhaps both explicit and implicit forms of learning are occurring simultaneously during the second language acquisition process, although gains from passive immersion are slower to become apparent. Similarly, performance gains from passive experience in our paradigm also required a prolonged period of exposure.

Functional lateralization is labile and could indicate category learning

Processing of the phonetic and phonological components of language is often associated with the left hemisphere, while more slowly modulated prosody, such as tone of voice, is believed to be processed to a greater degree in the right hemisphere (Gandour et al., 2003). While this division of labor exists at baseline, and seems to facilitate overall comprehension of language, our results suggest that functional lateralization is more dynamic than previously believed. In fact, patterns of lateralization may change depending on the type of stimulus (native vs. non-native, simple tones vs. complex song) and the subject's prior experience. In our experiment, exposure to a novel acoustic space in the form of HETENV reorganized lateralization patterns temporarily. However, after prolonged exposure, lateralization patterns reverted to normal. The timing for the reversion for the return to normal patterns of lateralization was similar for that associated with improved operant discrimination of two heterospecific stimuli. Therefore, shifts in the pattern of lateralization appear to be not just an artifact of novel environment exposure, but instead, have functional significance in improving auditory discriminations. Similar changes in lateralization are seen in adults after brief exposure to words in a foreign language (Plante et al., 2015) and was correlated with increases in proficiency

after training in a foreign artificial grammar (Newman-Norlund et al., 2006). Thus, dynamic fluctuations in lateralization could serve as a metric for how well learned a category is.

If shifts in lateralization are indicative of learning and consolidation of a novel category, one future direction would be to predict performance on a task based on changes in lateralization. In addition, observing dynamic fluctuations in lateralization, or the lack thereof, could be a diagnostic tool in language impaired populations. Autistic and language impaired children show atypical lateralization at a greater rate than the normal population (Dawson et al., 1989). Although the traditional view is that the lack of lateralization is the cause of the deficit: no hemispheric specialization leads to slow or inefficient processing, an alternate interpretation is that the lack of functional lateralization reflects failure to form perceptual filters, or categories, for the native language. Our observation that dynamic fluctuations in lateralization coincides with better performance on an auditory discrimination task inspires further research about how tightly coupled this relationship is, and also suggests that monitoring shifts in lateralization patterns could be used as a diagnostic or predictive tool for language performance.

Conspecific bias is fixed during the early critical period

Despite changes in STRFs and lateralization, one measure that remained constant throughout our experiments was conspecific bias. Regardless of the auditory stimuli our birds heard as adults, responses to conspecific song was always greater than responses to heterospecific song. The conspecific bias is likely genetically hardwired, or perhaps

established during early auditory experience. From an ethological standpoint, the conspecific bias ensures that conspecific songs are copied by juvenile songbirds, and that females prefer conspecific song over heterospecific song to encourage within-species mating. Furthermore, in a natural setting, where conspecific vocalizations are the only relevant communication signals, and processing power is expensive both in terms of energy expenditure and cognitive resource allocation, efficiency can be gained by responding robustly to conspecific vocalizations while allocating less resources to the regions of acoustic space that define heterospecific vocalizations. Thus, stability in perceptual filters is beneficial if those filters are properly formed during development.

STRF changes as auditory memory

Although we do not find a direct correlation between rates of adaptation and magnitude of STRF change, the STRF changes we observe are a direct result of exposure to the song sets of canary and zebra finch stimuli that cause adaptation in most units. We observe much less STRF change in the silence condition. In NCM, repeated exposure to particular song stimuli both elicits long-lasting stimulus-specific adaptation, and also changes the underlying STRFs of neurons, measured with a neutral stimulus (ripple noise). The first effect can be measured as a neural memory for the stimuli heard, and the second is best described as an adaptive perceptual filter that changes responses to other future stimuli. In other words, memory and perceptual filter adaptation may be two different ways of describing the same neural phenomenon. Given that stimulus-specific adaptation occurs for both canary and zebra finch songs, and this adaptation has been shown to be long-lasting (Chew et al., 1995; Phan et al., 2006; Tsoi et al., 2014), one question that remains is the durability of the STRF changes that we observed on an

immediate time scale. If STRF changes support stimulus-specific adaptation, then we would expect the STRF changes to be long lasting. However, if we think of STRFs keeping a running sum of auditory experience, perhaps they are constantly changing in response to auditory input.

Neuronal subtypes in NCM support stability and plasticity of perceptual filters

In an auditory environment where new sounds are sometimes encountered, stability and plasticity must trade off to maintain precision in auditory processing. One way of maintaining filters for native/ conspecific sounds, while being receptive to changes in the distribution sounds (i.e., when one moves to a foreign country), is to have separate neuronal populations devoted to stability vs. plasticity. Our data hint at a division of labor between subtypes of neurons in NCM. Narrow-spike cells tended to show greater changes in STRFs than wide spike cells after brief exposure to novel stimuli. Although we cannot definitively classify narrow and wide spiking neurons as either excitatory or inhibitory cells, it is worth noting some similarities between narrow-spiking cells in our data and inhibitory interneurons in other systems. Recently, studies have highlighted the role of inhibitory interneurons in learning and memory (Kullmann et al., 2012). Inhibitory interneurons tend to have narrower spike widths (Swadlow 2003), and seem to drive cortical plasticity in other sensory systems (Kätzel and Miesenböck, 2014; Heindel et al., 2011, Chevaleyre and Piskorowski, 2014; Morishita et al., 2015), and may contribute to adaptive effects (Gainey et al., 2016). If inhibitory interneurons are driving cortical plasticity, then narrowing of tuning seen in Terleph et al., 2008 and in Experiment 1 of the current study could be explained by increased sideband inhibition from inhibitory interneurons. Narrowing of the tuning width increases frequency

selectivity of individual neurons, enabling finer discriminations that may contribute to improvements in behavioral discrimination (Experiment 2). If plasticity first occurs in inhibitory interneurons, then we would see greater changes in the short term in these cells. In fact, we did observe greater STRF changes in narrow spike cells after exposure to novel heterospecific and conspecific songs.

Conclusions

The experimental data provided answers to all three questions that were originally asked.

Experiment 1: Can exposure to a novel category of sounds in adult songbirds induce changes in response patterns reflecting neural plasticity? We have shown that passive exposure to a set of novel heterospecific sounds causes a reversal of the normal pattern of lateralization after brief periods of exposure, but a return to the normal pattern after prolonged exposure. We conclude that exposure to a novel category of sounds does induce neural plasticity in the form of transient shifts in lateralization over the course of exposure and that these changes reflect learning of the novel category.

Experiment 2: Can exposure to a new sound category improve behavioral discrimination of novel sounds? Our data indicate that prolonged (14d and 30d), but not brief (9d) exposure to a novel category of sounds improves behavioral discrimination of two novel exemplars from that category relative to naïve controls. The duration of passive exposure that was required for enhanced behavioral performance coincided with a return to normal patterns of lateralization, suggesting that assimilation of the novel category increased discriminability of category exemplars. We conclude that implicit learning of the underlying stimulus statistics of heterospecific sounds may generalize to

other novel heterospecific stimuli, leading to the enhancement in behavioral discrimination observed.

Experiment 3: Does exposure to novel sounds and sound categories have an immediate effect on receptive field organization? Our results show that exposure to complex auditory stimuli can change spectro-temporal receptive fields (STRF) of neurons in NCM on the order of minutes. These modifications of STRFs and underlying perceptual filters may reflect the initial stages of memory formation for novel auditory objects and unfamiliar acoustic categories. Furthermore, STRF changes were greater for heterospecific stimuli than conspecific stimuli, indicating that the brain detects and responds to differences between native and foreign categories.

The present study has shown how the adult brain processes incoming auditory stimuli using pre-existing perceptual filters and how these filters are modified by auditory experience. A better understanding of how the mature brain maintains stability of pre-existing perceptual filters while remaining open to novel auditory input, could provide valuable insights into processes that serve second language acquisition in adults.

Future Directions

Neural mechanisms of plasticity in adults

We have shown that STRFs undergo more change when novel stimuli are derived from a distribution of sounds that differs from the distribution of native sounds. Evidence that perceptual filters are modified in a stimulus and category-specific manner in adulthood raises questions about the underlying neural mechanisms supporting these changes. While there has been some progress in identifying the neural mechanisms

underlying changes in perceptual filters induced through operant training (Polley et al., 2006), mechanisms underlying short-term plasticity in STRFs after passive exposure are less clear. Operant training paradigms that take advantage of top down attentional control of perceptual filters, increasing the salience of a target tone, and likely increase cortical representation for the target tone through stimulation of acetylcholinergic pathways (Polley et al., 2006). Expansions and shrinkages in the tonotopic map in A1 that occur after training require several training sessions and happen over the course of days (Bieszczad and Weinberger, 2010). However, the changes we observe in STRFs of NCM neurons happen on time scales of less than an hour and may not involve the same top-down attentional mechanisms as those seen in operant conditioning. Instead, mechanisms underlying rapid changes in STRFs could be evidence of rapid statistical learning in adults (Saffran et al., 1996; Saffran et al., 1999; Gebhart et al., 2009; Newport and Aslin, 2004; Creel et al., 2004). Detection of altered distributions of sound in adults could occur through bottom-up processes and potentially use the same neural mechanisms that support distributional learning of speech in children.

Prolonged exposure to a novel distribution of sounds such as those found in HETENV also induced large-scale changes in NCM, in the form of shifts in the pattern of lateralization over the course of HETENV exposure. The question about mechanism applies equally here as above. We still need to identify the neural mechanism for how these changes in lateralization occur and whether they are an extension of the changes in STRFs that we see on a shorter time scale. It is possible that long term changes are an accumulation or consolidation of changes that occur on an immediate scale. However, the prolonged time course over which lateralization shifts occur also suggest slower acting

mechanisms such as incorporation of new neurons into NCM, a process that is biased towards the left hemisphere (Tsoi, et al., 2014). One future direction would be to study not just shifts in electrophysiological response, but also test for fluctuations in neurogenesis between the two hemispheres over the whole course of passive HETENV exposure.

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