

**EFFECT OF RECENT AUDITORY ENVIRONMENT ON NEURAL RESPONSE
IN ZEBRA FINCH AUDITORY CORTEX**

by LILLIAN YANG

A Thesis Submitted to

The Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the degree requirements

for the degree of

Master of Science

Graduate Program in Psychology

written under the direction of

David Vicario, Ph.D.

And approved by

New Brunswick, New Jersey

May, 2012

ABSTRACT OF THE THESIS

Effect of Recent Auditory Environment on Neural Response in Zebra Finch

Auditory Cortex

By LILLIAN YANG

Thesis Director:

David Vicario, Ph.D

Songbirds provide a model for studying human vocal learning due to many similarities between the two systems, e.g. a critical period for vocal learning, hemispheric lateralization, and sensory-motor integration for vocal imitation. One area of study relevant to human communication is short term plasticity in auditory cortex as a function of recent auditory experience. Changes in auditory processing in adulthood have been observed in the caudo-medial nidopallium (NCM), a higher auditory area that is known to respond selectively to conspecific vocalizations. To test how recent auditory experience with sounds of another species affects selectivity, adult male zebra finches were housed for 9d in different auditory environments. Two groups of birds were isolated as individuals and received playback of either recorded zebra finch (CONENV; n=9) or canary (HETENV; n=11) aviary. A third group remained in the general zebra finch aviary (Aviary; n=8). On day 9, electrodes placed bilaterally in NCM of these awake

restrained birds recorded extracellular multi-unit activity in response to presentation of novel conspecific(ZFStim) and heterospecific (CANStim) songs, and pure tones. We assessed differences in absolute response magnitude, stimulus-specific adaptation, and tuning between exposure groups, stimulus types, hemispheres, and at different depths in NCM.

Both CONENV and HETENV had higher responses overall compared to Aviary. Absolute responses were stronger to ZFStim than to CANStim across all exposure conditions. Preferential responding to conspecific song was greater in the right hemisphere than the left and greater in dorsal than ventral regions of NCM overall. CONENV birds showed higher absolute responses and higher rates of stimulus-specific adaptation in the right hemisphere for both ZFStim and CANStim. HETENV birds had higher absolute responses in the left hemisphere and showed no hemispheric difference in adaptation rates. Exposure to a completely novel auditory environment alters auditory processing of natural stimuli in a lateralized, but not stimulus-specific, manner. The reversal in lateralized processing suggests that the two hemispheres exhibit plasticity in different ways when confronted with the challenge of a new acoustic feature space and are likely to play different roles in the maintenance or revision of perceptual filters and stimulus categories.

Acknowledgement

Zebra finch data discussed in this thesis was analyzed in part by Tara Tegelci.

Assistance and support were provided by members of the Vicario lab.

Table of Contents

I.	Page ii	Abstract
II.	Page iv	Acknowledgement
III.	Page v	Table of Contents
IV.	Page 1	Introduction
V.	Page 13	Materials and Methods
VI.	Page 19	Results
VII.	Page 25	Discussion
VIII.	Page 35	Appendix
	a. Page 35	Figure 1. Anatomy of the Bird Song System
	b. Page 36	Figure 2. Stimulus Specific Adaptation(SSA)
	c. Page 37	Figure 3. Recording Apparatus and Histology
	d. Page 38	Figure 4. Effect of Recent Experience on ARMs Trials 2-6
	e. Page 39	Figure 5. Main effect of Stimulus Class on ARMs 2-6
	f. Page 40	Figure 6. Effect of Auditory Env on ARMs to ZF Stimuli
	g. Page 41	Figure 7. Effect of Auditory Env on ARMs to CAN Stimuli
	h. Page 42	Figure 8. Effect of Auditory Env on Adaptation Rates
	i. Page 43	Figure 9. Effect of Depth on ARMs 2-6 and 6-25
	j. Page 44	Figure 10. Effects of Depth and Stimulus Class
	k. Page 45	Figure 11. Effect of Hemisphere and Stimulus Class
	l. Page 46	Figure 12. Effects of Recent Auditory Env on Tuning Width
IX.	Page 47	References

Introduction

The auditory system must form an accurate internal representation of the external acoustic environment in order to instruct behavior. The peripheral auditory system decomposes the composite incoming signal into its component frequencies along the basilar membrane, and this parameter is represented in tuning functions of auditory neurons which are largely hardwired (Mann and Kelley 2011). Tuning functions are preserved in the pathway to the auditory forebrain, where sounds are represented in a tonotopic map. Studies in many species show that the tonotopic map favors a range of salient sounds that are species-specific (Suga et al., 1997; Maier and Scheich, 1987; Portfors et al., 2009; Ehret 1987). In order to do so efficiently, perceptual filters must be tuned to a class of commonly heard, salient stimuli, e.g. the native tongue for humans or conspecific vocalizations for other species. However, the origin of such conspecific biases is unclear. Derived features, e.g. sound localization and pitch, emerge through computational processes in the ascending auditory system (Kandel et al., 2000) and may be subject to developmental influences (Miller and Knudsen, 2001). More complex percepts; e.g. rhythm and timbre (Schreiner 1995; O'Connor et al., 2010; Woolley et al., 2009) that contribute to the recognition of auditory objects, may be subject to experiential effects both during development and in adulthood. Many researchers have focused on genetic and early developmental components that contribute to patterning of the auditory system, while neglecting to address how auditory experience in adulthood continues to maintain or modify perceptual filters.

The extent of plasticity in auditory cortex during adulthood impacts daily functioning of the organism and becomes relevant in humans when addressing concerns such as the acquisition of a second language. Foreign languages learned in adulthood never quite acquire the same level of native fluency as languages learned during the critical period in early development. These deficits could be due to many factors including but not limited to: inability to perceive phonemic categories of the non-native language, difficulties in motor production, interference from the native language, etc. However, perception of the non-native language can be improved through training as evidenced by adults who successfully acquire a second language. Thus the study of neural substrates that govern the modifiability of auditory perceptual filters is important for understanding normal and pathological processes that underlie speech perception.

While numerous studies have been done on language perception and acquisition in adults, human studies are limited by the types of experimental manipulations that can be performed and by the spatial and temporal resolution of current imaging technology; thus leaving us with a general understanding of neural circuitry involved in sound processing but only a vague notion of the underlying neurophysiological changes that accompany processing of new categories of sounds in the auditory environment. In order to study auditory processing on a neurophysiological level, we need a robust animal model.

Why the songbird is a good model of auditory processing

The bird song system is a popular animal model for vocal learning due its numerous similarities with the human auditory system and stages of language acquisition (Doupe and Kuhl, 1999). Bird songs have a complex acoustic structure, often containing a large repertoire of syllables that vary along spectral and temporal dimensions as well as in their rhythm and syntax, similar to complexities found in human speech. The auditory system in both humans and songbirds must be able to not only process basic acoustic features such as pitch and temporal duration, but also be able to distinguish between complex acoustic structures and sequences of acoustic elements. For example, European starlings, a type of songbird, are capable of classifying song sequences containing recursive syntax, i.e., song syllables inserted in the center of a longer song bout (Gentner et al., 2006). Like humans, song birds perceive and utilize information contained in syllable order and syntax. In addition to similarities in acoustic characteristics between birdsong and human speech, the auditory system in the two species also share commonalities in stages of development, functionality, and homologous or analogous anatomy. Although the avian brain does not contain a laminar neocortex, recent work suggests that the parallels between forebrain processing circuits for audition and vision may be more similar than previously believed (Wang et al, 2010).

Songbirds are one of only a handful of animals, aside from humans, that are capable of communicating using a system of learned vocal signals. Other documented vocal learners include dolphins, parrots and hummingbirds but the songbird system is the most accessible and well-defined of these. Similar to the

critical period for language acquisition in infants, normal song development in song birds requires exposure to conspecific vocalizations during an early critical period. In zebra finches, male song is learned from an adult male tutor during a sensory receptive period when exposure to species-typical song is necessary for normal development of song (Marler and Peters, 1977). Following early exposure to the tutor's song, song learning occurs in stages that parallel stages of language acquisition in human infants (Brainard and Doupe, 2002). Young males begin to produce modulated vocalizations at 30-40d of age, the beginning of the sensory-motor period. During this stage, the song is highly variable and error prone. As the juvenile zebra finch practices and refines its song, it comes to better approximate that of its adult tutor. This stage is analogous to babbling by human infants. Around 90 days of age, each zebra finch male sings a "crystallized song" with syllables and syllable sequence fixed for life (Brainard and Doupe, 2002).

Lateralization of Song

Another similarity between song and speech is lateralization for both production and processing of vocal signals. Early studies in songbirds revealed that the motor production pathway is lateralized for song. Unilateral severing of the left syringeal nerve led to a much greater loss of song syllables than cuts of the right syringeal nerve, implicating the pathway from the left hemisphere that controls the left syringeal nerve in canaries as dominant for song (Nottebohm, 1976). Zebra finches, another species of songbird, are lateralized for song as well, though in the opposite manner. In zebra finches, cutting the right syringeal

nerve affects more song syllables than cuts of the left syringeal nerve. The lateral difference is not as strong as in canaries, and different aspects of the song are affected depending which side is cut, which suggests separate roles for the left and right motor pathway (Floody and Arnold, 1997; Wild et al., 2000).

Both humans and song birds also show differential auditory responses to conspecific vocalizations in left vs. right hemisphere, possibly reflecting processing of different aspects of the auditory signal in each hemisphere (Hickok and Poeppel, 2007; Zattore, 2001; Tommasi, 2008)). Recent evidence shows that sensory processing of song is lateralized in zebra finches, (Phan and Vicario, 2010), consistent with some earlier observations (Voss et al., 2007; Poirier et al., 2009). Electrophysiological measures in zebra finches showed larger auditory responses in a cortical auditory area (NCM, see below) in the right hemisphere than in the left. In that area, auditory responses show stimulus-specific adaptation to sounds, and the speed of adaptation is faster in the right hemisphere. In addition, these lateral differences were affected by early auditory experience. Exactly how these differences may relate to the lateralization of motor production, and to vocal learning more generally, is unclear. However, the idea that left and right hemispheres parse separate characteristics of the auditory environment is not a novel one. In fact, lateralization for sensory and motor processing is most widely studied in humans using a class of sounds with special characteristics – human speech (Vouloumanos and Werker, 2004; Stevens, 1980).

A specialized forebrain area for processing communication signals: NCM

Histologically and functionally distinct song nuclei correspond to structures in human auditory cortex. The auditory thalamus in songbirds, known as nucleus Ovoidalis (Ov), homolog of mammalian medial geniculate nucleus (MGN), projects to forebrain region L2, thought to be homologous to thalamo-recipient layers III-IV of mammalian cortex (Wang et al., 2010). For further processing of auditory stimuli, Field L2 sends projections to adjacent areas, Field L1 and L3, which in turn project to the caudo-medial nidopallium (NCM) and caudal mesopallium (CM) (Vates et al., 1996) (Fig 1). NCM could be a secondary auditory area receiving inputs from primary auditory cortex Field L2 (Theunissen et al., 2000) although others suggest it analogous the superficial layers of mammalian A1 (Wang et al., 2010). NCM outputs are less well defined and involve possible reciprocal connections with CM (Vates et al., 1996), thus further reinforcing its role as higher auditory cortex. Despite its somewhat uncertain connections, several properties of NCM suggest that it plays an important role in processing complex species-specific vocalizations.

Conspecific Bias is Demonstrated Both Genomically and Electrophysiologically

Studies in songbird NCM reveal genomic and electrophysiological response biases for conspecific sounds (Chew et al 1995; 1996; Mello et al, 1992; Stripling et al., 2001). Induction of ZENK, an immediate early gene associated with learning and memory in the mammalian hippocampus, is much higher in NCM when subjects are presented with conspecific song vs. heterospecific song. ZENK expression is lower in response to heterospecific

song and no ZENK expression was observed in response to pure tones (Mello et al, 1992). This region of the avian auditory forebrain appears to be selectively tuned for conspecific songs.

Electrophysiological studies further support an auditory response bias to conspecific songs. Neurons in NCM respond more robustly to conspecific songs compared to non-species specific sounds such as heterospecific songs or white noise. Memory for conspecific songs, as measured by the persistence of adaptation for a specific stimulus, lasts much longer than memory for other acoustic stimuli, >20 hours vs. 6 hours respectively (Chew et al., 1995). Adaptation rate is measured as the slope of the regression line over successive presentations of a given stimulus divided by the average ARM over the same trials to normalize the rates for differences in absolute response size between recording sites. The rate represents the percentage drop in response amplitude per stimulus repetition (Chew et al, 1995; Phan et al., 2006) and provides a measure of familiarity to or memory for a specific stimulus (Fig 2). A shallow slope or low adaptation rate represents familiarity with or memory for a stimulus while a steep slope or high adaptation rate indicates novelty or “forgetting”. Adaptation rates remain low at 20h post stimulus presentation for conspecific songs but are as high as those for novel stimuli at 20h for heterospecific stimuli (Chew et al., 1995), signifying a prolonged memory for conspecific but not heterospecific stimuli. Thus, both genomic and electrophysiological evidence suggest a response bias for conspecific vocalizations. This response bias may

be the result of perceptual filters in higher auditory cortex that selectively respond to categories of familiar or salient sounds in the auditory environment.

Genetic and Early Developmental Effects on Perceptual Filters

There is some evidence that conspecific preference for song is genetically hard-wired in perceptual filters which are innately selective for conspecific vocalizations. Juvenile white-crowned sparrows exposed to both conspecific and heterospecific song chose to copy only the conspecific song (Marler and Peters, 1977). Fledgling white crown sparrows naïve to song showed a behavioral preference for conspecific vocalizations over heterospecific songs (Whaling et al, 1997). Male and female zebra finches raised in isolation showed a subsequent behavioral preference for conspecific song when given the choice to listen to conspecific or heterospecific song (Braaten and Reynolds, 1999). Additionally, a fMRI study showed that normally reared juvenile male zebra finches had stimulus-specific responses to conspecific song while isolation raised juvenile males did not. Interestingly, juvenile female zebra finches raised in isolation did show conspecific preference for song (Maul et al. 2010), suggesting an innate conspecific preference in females but not males. One caveat with these studies is that what appears to be an innate bias 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.

Early developmental effects are demonstrated in a 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). The removal of the behavioral conspecific bias through cross-fostering suggests that conspecific bias may be a result of early auditory experience, or at least that early developmental effects are able to override an existing genetic bias. However, since this was a behavioral study, whether or not the removal of the conspecific bias occurs at a neurophysiological level in auditory cortex remains to be seen.

Adult Plasticity in Auditory Cortex

Although study of developmental plasticity in the auditory system has yielded important basic results, adult plasticity may also make a significant contribution to auditory processing. Developmental plasticity in the auditory system appears to represent fine tuning of the developmental program in light of the organism's early experience, as described for "critical periods" in vision as well. Ongoing auditory plasticity in adults is less studied, and less well understood. Is the map of auditory features static in adulthood, or does it change in order to accurately represent changes in the external environment? For example, humans that emigrate or travel to a different country might be exposed to a language with different acoustic features than the one they are accustomed to. In these situations, where the stimulus statistics of the external world change, it might be useful for the auditory system to fine tune perceptual filters to become

more receptive to sounds in the new environment that may have behavioral significance.

Adult plasticity is the phenomenon that allows for updating of the auditory map and modification of perceptual filters. Studies of adult mammals and songbirds demonstrate auditory cortex plasticity in response to both passive and operant presentations of auditory stimuli. Researchers selectively increased the area of representation in the auditory map for a particular stimulus by manipulating the behavioral salience of that stimulus. In an operant conditioning task, rats trained to attend to a stimulus frequency had increased cortical representation of the target frequency while rats trained to attend to a specific stimulus intensity had increased cortical representation of the target intensity with no change in the frequency map (Polley et al, 2006). Auditory cortex was remapped to represent the relevant external feature space, and similar modification of cortical maps has been described in other sensory systems as well (de Villers-Sidani and Merzenich, 2011). The mechanism of this process was further investigated by pairing electrical stimulation of nucleus basalis (a major source of excitatory neurotransmitter acetylcholine) with presentation of a 4 KHz tone increased the excitation-inhibition (E:I) ratio of the paired frequency in auditory cortex (Froemke, Merzenich and Schreiner, 2007). Stimulation of nucleus basalis most likely increases salience of the paired tone, resulting in a shift of the E:I balance towards excitation. The change in E:I balance suggests a rapid modification of the tonotopic map to reflect recent stimulus statistics and is a possible mechanism for plasticity. In a songbird study, starlings operantly

conditioned to select a reinforced conspecific song in a two-choice paradigm showed increased neural response to the reinforced stimulus (Gentner and Margoliash 2003). In the above studies, manipulating the salience of some parameter of acoustic space by increasing attention to a specific stimulus in that space induced plastic reorganization in adult auditory cortex.

Passive exposure to a unfamiliar distribution of acoustic stimuli may also be sufficient to alter the auditory map. In adult rats, short term noise exposure is sufficient to re-open the critical period and enable reorganization of the tonotopic map in auditory cortex to favor a passively presented tone (Zhou et al, 2011). In a cross-housing study with zebra finches and canaries, passive short term exposure to heterospecific song revealed effects of environment on the width of tuning curves in higher auditory cortex (Terleph et al., 2008). Housing with an unfamiliar species, whose song has very different acoustic features, led to a narrowing of tuning curves while birds housed in isolation had a widening of tuning. Broadening of tuning in birds exposed to silence for a week suggests acoustic stimulation from conspecific sounds is required for maintenance of the status quo. Furthermore, narrowing of tuning after exposure to foreign heterospecific song may reflect learning and remapping of auditory cortex to accommodate the new feature space that now includes a new category of sounds. Both these studies suggest that passive exposure or immersion in an auditory context is sufficient to bring about changes in adult auditory cortex that move towards a better neural representation of the space.

While numerous studies have demonstrated that the brain is sensitive to changes in makeup of the external environment even in adulthood, most studies used artificial tones which have low behavioral salience and lack the syntax and rhythmic structure characteristic of species with learned vocal communication. Furthermore, studies have focused on using conspecific stimuli which contain similar clusters of features, rather than heterospecific stimuli which might reveal more interesting effects of category learning. In the Terleph et al., 2008 study, a change in tuning width was observed in both crossed-housed adult zebra finches and cross-housed adult canaries. However, how these changes in tuning affect and interact with responses to natural conspecific and heterospecific songs was not tested. Furthermore, cross-housing introduces a confounding social stress variable on the cross-housed individual because it is unable to communicate or engage in social interactions with its new neighbors.

Our present study followed procedures outlined in Terleph et al., 2008 with three differences: 1) we attempted to eliminate effects of social interaction by presenting the auditory context through speakers to a socially isolated bird, and 2) we tested with natural conspecific and heterospecific songs, in addition to tones, to quantify effects of cross-environment on the conspecific bias; and 3) we compared responses in the two hemispheres for differences in lateralization.

Materials and Methods

Subjects

28 adult male zebra finches were obtained from various commercial aviaries or bred in our facility. At the start of the experiment, subjects were assigned to one of three groups. Subjects assigned to Groups 1 (HETENV) and 2 (CONENV) were removed from the general group housing in the aviary and individually housed in soundproof isolation boxes for 9 consecutive days at the start of the experiment. Subjects in Group 3 (Aviary) remained in general aviary housing for the 9 day duration of the experiment and received no additional manipulation until testing. All subjects were maintained on a 12/12 light cycle with ad libitum access to food and water. All procedures conformed to a protocol approved by the Animal Care and Use Committee at Rutgers University.

Housing and acoustic environment

During lights-on, individually housed birds received continuous 12h playback of sounds through a speaker in the isolation box. Birds in the HETENV group received an auditory diet of canary songs and calls (recorded in an aviary at Rockefeller University Field Research Station) which simulate a 'foreign' acoustic environment (cf. the "cross housed" condition of Terleph et al, 2008). Birds in the CONENV group received an auditory diet of zebra finch songs and calls (recorded in a zebra finch aviary at Rutgers University) to simulate their 'native' acoustic environment. The Aviary group remained in their original zebra

finch aviary housing for the duration of the experiment and received no additional manipulation, serving as a control group.

Testing Stimuli

Song stimuli consisted of 20 novel zebra finch (ZFStim) and 20 novel canary (CANStim) songs (duration: 800-1500ms; sampling rate: 40 KHz). Songs were grouped to form 4 testing sets, each containing 5 zebra finch and 5 canary songs. These allowed a set of novel stimuli of both stimulus types to be played at each site at 4 different sampling depths (see below). Each song testing set consisted of 25 repetitions of each of the 10 song stimuli presented in a random shuffled order with an 8ms inter-stimulus interval. Tone stimulus sets contained 20 pure tone stimuli ranging from 500Hz to 5000Hz at 250Hz increments (duration: 260ms; sampling rate: 40 KHz). The tone set consisted of 3 repetitions of each of the 20 tone stimuli in random shuffled order with a 6ms inter-stimulus interval. The tone set was presented after each set of song stimuli at each depth. All stimuli were equalized for root-mean-square (RMS) amplitude of the power spectra to eliminate differences in overall volume.

Procedure

On isolation Day 7, all subjects underwent surgery under isoflurane anesthesia (2.5% induction and 1.8% maintenance) to attach a metal pin to the skull with dental cement, which was also used to form a chamber for subsequent recording. This pin was used to immobilize the subject's head during subsequent testing. After surgery, subjects were returned to their respective housing

conditions and given 48 hours to recover while continuing to listen to their assigned acoustic environments until the day of testing. On day 9, awake subjects were tested electrophysiologically in a soundproof booth (IAC Inc., Bronx, NY). Subjects were restrained in a custom made plastic tube and head-fixed to the stereotaxic apparatus via the previously implanted head pin to reduce movement during testing. A multielectrode microdrive (Thomas Recording, Giessen, Germany) was used for the placement of sixteen tungsten micro-electrodes (Type ESI2ec, impedance: 2–4 M ohm, Thomas Recording) in NCM, bilaterally (8 in each hemisphere)(Fig 3). Stereotaxic 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).

All sixteen electrodes were placed at an initial depth of ~1000 microns from the dorsal surface. Depth of each electrode was adjusted while white noise stimuli with the amplitude envelope of canary song were played to identify the first responsive site along the penetration of each electrode. Once responsive sites were located on all sixteen electrodes, playback of the testing stimuli commenced. Multi-unit recordings of neural spike activity were taken simultaneously from all sixteen electrodes using Spike 2 software (CED, Cambridge, England). Recorded activity was amplified (x19,000) and band-pass filtered from 0.5-5kHz. Auditory stimuli were presented in randomized order through a speaker centered 30cm in front of the subject. Presentation of each set of song stimuli was followed by presentation of tone stimuli to obtain tuning

curves and best frequencies for each recording site. After presentation of both song and tone stimuli, all electrodes were lowered 300 microns 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 songs were played and recordings made from 4 depths in NCM spaced 300 microns apart along the dorsal-ventral axis. Upon completion of testing, 3 small electrolytic lesions (10 μ A for 10s) were made in each hemisphere for histological verification that recording sites were within the boundaries of NCM.

Histology

Subjects were sacrificed with Nembutal overdose 3 days post-test and transcardially perfused with saline followed by 4% paraformaldehyde. Brains were removed and 50um sagittal sections taken through NCM for both left and right hemispheres. Slices were stained with cresyl violet and visualized under light microscopy to identify recording sites. Sites determined to be outside of NCM were excluded from the analyses.

Data Analysis

Absolute Response Magnitude: Trials 2-6 and 6-25

To quantify responses to song stimuli, the absolute response magnitudes (ARMs) on each trial for each stimulus presentation was obtained by subtracting the root mean square (rms) of neural activity of the control period (500ms prior to stimulus onset) from the rms of the response period (stimulus duration plus 100ms), following established methods in the laboratory (Phan et al, 2006; Phan

and Vicario, 2010) (Fig 3). ARMs were averaged for trials 2-6 (early ARMs) of each song stimulus and further averaged by stimulus category (ZFStim or CANStim) to yield a conspecific ARM average and a heterospecific ARM average for each recording site. Each recording site constituted one independent sample. The same analysis was repeated for trials 6-25 (late ARMs). Repeated measures ANCOVA was performed for main effects and interactions of Auditory Environment (CONENV, HETENV) and Hemisphere (Left, Right) with Stimulus Class (ZFStim, CANStim) as the repeated measure and Depth (D1, D2, D3, D4) as the covariate. Repeated measures ANCOVA was performed for all three dependent variables: ARMs 2-6, ARMs 6-25, and Adaptation Rates 6-25. A separate non-repeated measures ANCOVA was performed on tuning data for main effects and interactions of Auditory Environment and Hemisphere, with Depth as the covariate, to quantify tuning width and peak throughout NCM.

Adaptation Rate: Trials 6-25

Additionally, adaptation rates were measured for trials 6-25 (late adaptation). Adaptation rate is the slope of the regression line over successive presentations of a given stimulus divided by the average ARM over the same trials to normalize the rates for differences in absolute response size between recording sites. The rate represents the percentage drop in response amplitude per stimulus repetition (Chew et al, 1995; Phan et al., 2006) and provides a measure of familiarity to or memory for a specific stimulus. Low adaptation rates signify familiarity or memory for a stimulus while high adaptation rates signify novelty and unfamiliarity with the stimulus. ANOVA for main effects and

interactions of auditory environment, hemisphere, and stimulus category was performed to identify significant differences in adaptation rate between groups. A separate analysis was performed for main effects and interactions of auditory environment, depth and stimulus category.

Tuning

To quantify the phasic ARM of a given site to tone stimuli, the control rms (500ms prior to stimulus onset) was subtracted from the phasic response (the 10-60ms interval after stimulus onset) and averaged across the 3 repeats of each stimulus frequency per recording site, following established methods in the laboratory. Peak frequency and tuning width per recording site were calculated using an algorithm in Excel (Terleph et al., 2008). Tuning width is defined as the contiguous frequency range over which phasic responses are at least 2 SD above baseline. ANOVA on tuning width and tuning peak was performed for effects of housing environment, hemisphere and depth. LSD post hoc tests were performed for all significant effects to further identify differences between groups.

Results

In all, 499 recording sites (164 sites across 8 Aviary controls, 187 sites across 11 HETENV birds, and 148 sites across 9 CONENV birds) were histologically verified to be in NCM and used for ARMs and adaptation rate analysis. ARMs for trials 2-6, 6-25, and Adaptation Rate for trials 6-25, were computed.

Effect of Recent Auditory Environment on ARMs

Repeated measures ANCOVA, with depth as a covariate (depth effects will be described in a separate section below), was performed for both ARMs from trials 2-6 and 6-25. In each case, there was a significant main effect of Auditory environment ($F=12.075$, $p<0.000008$ for trials 2-6; $F=12.474$, $p<0.000005$, for trials 6-25). However, LSD post hocs showed that this effect was driven solely by the Aviary control group having much lower absolute responses than either the CONENV or HETENV groups (Fig 4).

Absolute responses did not differ between the CONENV and HETENV groups. The lowered aviary response may be due to the general stress of transfer from the aviary to an isolated testing condition. In contrast, both CONENV and HETENV birds were transferred from isolation boxes to testing, thus having been acclimated to social isolation. Another explanation could be that the 9d of social isolation increased sensitivity to all stimuli in the CONENV and HETENV groups, leading to an enhanced response to all auditory stimuli during testing. Absolute response of CONENV and HETENV groups were

comparable to magnitudes seen in other isolation box reared birds (Phan and Vicario, 2010), while the Aviary group had comparatively lower responses. We suspect that the difference in ARMs is due to differences in acclimation for the Aviary group and thus we only included the CONENV and HETENV groups, which received identical treatment in all further data analyses.

Both CONENV and HETENV had significantly higher responses to conspecific stimuli than heterospecific stimuli during testing ($F=34.246$, $p<0.0000001$ for trials 2-6; $F=51.660$, $p<0.0000001$ for trials 6-25), in agreement with previous findings (Chew et al., 1996)(Fig 5). Thus, short term exposure to a foreign acoustic environment, does not seem to enhance absolute responses to foreign stimuli in NCM and lack of exposure to conspecific stimuli for 9 consecutive days is not sufficient to reduce the response bias for conspecific stimuli.

However, there was a significant environment by side interaction ($F=5.440$, $p<0.02$) for ARMs trials 2-6, though this effect only trends towards significance in ARMs trials 6-25 ($F=2.879$ $p=0.09$, n.s)(Fig 6 and 7). Birds in the CONENV group had larger right hemisphere responses to all stimuli, in agreement with previous findings that the right hemisphere in zebra finches is lateralized for complex stimuli such as songs (Phan and Vicario, 2010). Interestingly, in the HETENV group, the left hemisphere had higher responses to both conspecific and heterospecific stimuli, suggesting that short term exposure to a foreign environment increases activity in the left hemisphere, causing a reversal of the lateralization pattern. A bird by bird analysis for lateralization

patterns reveal that 8 out of 11 birds in the HET environment condition had stronger early ARM responses in the left hemisphere, while only one out of 9 CON environment birds and 2 out of 8 aviary birds trended towards left dominance. Nonparametric Chi-square analysis of these frequencies in the CONENV and HETENV groups revealed a significant difference between groups ($p < 0.005$).

Effects of recent auditory exposure on familiarity to a class

When Adaptation rates for trials 6-25 were analyzed, there was again a main effect of Auditory Environment ($F = 11.353$, $p < 0.0008$), this time driven by birds in the CONENV condition having much faster adaptation rates than birds in the HETENV groups (Fig 8). Further analysis revealed an Auditory Environment by Hemisphere interaction ($F = 4.038$, $p < 0.045$) which was driven specifically by the right hemisphere in the CON group having faster adaptation than all other groups (Fig 8). Post hoc comparisons show that the right hemisphere in the CONENV group has significantly higher adaptation rates than left hemisphere in the CONENV group. However, in the HETENV group, there is no difference in adaptation rates between left and right hemispheres. Thus, the CONENV data agree with previous findings in normal experience birds (exposed to only conspecific vocalizations), where the right hemisphere has faster adaptation rates as well as greater ARMs than the left (Phan and Vicario, 2010) but the HETENV data suggests that something changes in birds that are exposed to a “foreign” auditory environment.

Interactions of Stimulus class with Location in NCM on conspecific bias: Depth

Previous electrophysiological studies on conspecific bias in zebra finches have only explored dorsal NCM. In our study, we recorded from 4 different depths along the dorsal-ventral axis of NCM to better characterize conspecific bias throughout the entirety of NCM. We found a significant main effect of Depth: ARMs for trials 2-6 and 6-25 were stronger in dorsal NCM and decreased as recording sites moved more ventral ($F=20.838$, $p<0.000007$ ARMs 2-6; $F=17.784$, $p<0.00003$ ARMs 6-25)(Fig 9).

In addition, there was a Stimulus Class by Depth interaction in ARMs 2-6 and 6-25 ($F=9.324$, $p<0.002$; $F=14.249$, $p<0.0001$)(Fig 10) indicating that the decrease in ARMs occurred for both ZFStim and CANStim but not in the same manner. LSD posthoc comparisons show that ARMs on trials 2-6 and 6-25 to ZFStim decrease significantly from Depth 1 to Depth 3 but is only significant between Depth 1 and Depth 4 to CANStim. This shows that ZFStim responses drop out faster than CANStim responses as penetrations go from dorsal to ventral NCM (Fig 10). A further look at the posthoc result shows that ZFStim response is significantly higher than CANStim at Depth 1 and Depth 2 but not higher at Depth 3 and Depth 4 (Fig 10). Our manipulation of the auditory environment does not appear to reverse or reduce the conspecific bias. However, there is an interesting localization of the conspecific bias to more dorsal regions of NCM, with the ventral regions showing less conspecific preference.

Interactions of Stimulus class with Location in NCM on conspecific bias:

Hemisphere

Furthermore, there is a Hemisphere by Stimulus Class interaction for ARMs trials 2-6 but not for ARMs trials 6-25 ($F=6.231$, $p<0.01$; $F=2.421$, $p=0.12$, n.s.). In ARMs trials 2-6, posthoc LSD shows a greater difference between ZFStim response and CANStim response in the right hemisphere (Fig 11).

In summary, an analysis of the data by location in NCM reveals a greater ZFStim vs. CANStim response difference in dorsal regions of NCM. Ventral regions in NCM have a smaller difference between ZFStim response and CANStim response regardless of housing condition. This pattern of interactions was the same in both CONENV and HETENV conditions. Furthermore, the bias towards higher ZFStim responses is more prominent in the right hemisphere than in the left. These regional differences in level of conspecific response bias may indicate divisions in NCM with specialized roles for processing of conspecific vs. heterospecific stimuli.

Tuning and conspecific bias

In a previous study, short term cross-housing of adult male zebra finches in canary aviary resulted in a narrowed tuning width in NCM (Terleph et al., 2008). In our data, there was also a significant effect of environment on tuning width ($F=5.723$, $p<0.018$) where birds in the HETENV condition had narrower tuning than those in the CONENV condition (Fig 12). Furthermore, there was a significant effect of depth with narrower tuning widths at ventral sites ($F=10.86$,

$p < 0.001$) and significantly narrower tuning in right hemisphere than left ($F = 4.839$, $p < 0.029$). Analysis of tuning peak, defined as the best response frequency, confirmed a tonotopic organization in NCM with dorsal depths responsive to lower frequencies and ventral depths responsive to higher frequencies ($F = 4.392$, $p < 0.038$). There was also a main effect of environment where birds exposed to HETENV had overall higher tuning peaks than birds in ZFENV ($F = 7.423$, $p < 0.007$). This may be a result of 9d exposure to canary songs in the HETENV which have substantial power at higher frequencies compared to zebra finch songs which contain most of their power in the lower frequencies.

Discussion

Canary songs differ from zebra finch songs in a number of acoustic parameters. Nine days of exposure to these novel combinations of sounds represent a significant change in the distribution of acoustic features to which the isolated bird is exposed. This change in stimulus statistics of the auditory environment induces neurophysiological changes in adult avian auditory cortex that include a narrowing of tuning for simple sounds and loss, or even reversal, of the normal pattern of lateralization for song stimuli, although there is no change in the qualitative neuronal preference for conspecific sounds.

We observed that birds exposed to a foreign sound environment (HETENV) showed the same narrowing of tuning that was seen in an earlier study in the laboratory (Terleph et al., 2008), and the tuning width observed in our CONENV group resembles the tuning widths of normally housed birds in that study. When Terleph et al. exposed birds to a heterospecific aviary, it included many visual and social, as well as acoustic cues. We achieved similar tuning effects, even though our birds were socially isolated when they heard the HETENV sounds. Thus, changes in NCM tuning do not depend on visual and social effects of physically being in the “foreign” environment. Instead, continuous auditory stimulation that simulates the acoustic environment of a heterospecific aviary is sufficient to induce plastic changes in tuning. However, the sudden removal of social interaction i.e., transfer of a subject from general aviary to isolation, either in the isolation box or the recording booth, may be a stressor and

require a period of acclimation before testing, thus accounting for the oddly attenuated neural responses in our aviary controls.

Recent experience with spectral and temporal features of heterospecific song did not alter response magnitudes to either class of stimuli although it did increase the peak frequency of tuning. Responses to conspecific stimuli remained higher than to heterospecific stimuli in all conditions, regardless of previous exposure. Likewise, adaptation rates remained the same for conspecific and heterospecific stimuli, as expected, because all ZFStim and CANStim testing stimuli were novel. In earlier work, there was no conspecific bias in adaptation rate; however, differences were seen in the duration of the adapted state to each type of stimulus. Responses to conspecific song remained adapted for over >20h, while heterospecific song responses only stayed adapted for 6 hours, an apparent difference in neuronal memory duration (Chew et al, 1995). However, long-term memory was not tested in the present set of experiments. Since our manipulation did not eradicate the response bias for conspecific song, selectivity for conspecific vocalizations may reflect hard-wired circuit properties and/or may be due to early developmental experience with conspecific vocalizations (of both caregivers and self) that tune perceptual filters to select for a biologically relevant set of sounds. Hence the conspecific bias appears relatively fixed in adulthood, although we cannot exclude the possibility that longer periods of exposure to foreign sounds may reduce the conspecific bias.

Although recent exposure to a set of foreign heterospecific stimuli caused no change in conspecific selectivity overall, there was a distinct change in the

pattern of lateralization both in terms of absolute responses and adaptation rate. Previous work showed differential responses to novel conspecific sounds in the left and right hemispheres in normally housed birds, with the right hemisphere exhibiting both higher absolute responses and faster stimulus-specific adaptation rates for trials 6-25 (Phan & Vicario, 2010). This form of lateralization depended on the bird's exposure to complex sounds in development and was seen for song stimuli but not for tones. This distinction appears to reflect different roles for the two hemispheres in processing sounds with spectral and temporal features that are familiar from ontogeny.

The previously observed patterns of lateralization were replicated in our CONENV group who had both higher ARMs and faster adaptation rates on the right. There was a significant interaction of auditory environment and hemisphere for adaptation rates on trials 6-25. In HETENV birds, this faster adaptation on the right was lost, resulting in the interaction ($p < 0.045$). Our CONENV birds maintain faster right side adaptation for both stimulus types, perhaps because the new heterospecific stimuli are both novel as individual songs, and novel as a class of sounds. However, the conspecific testing stimuli should be familiar as a class and thus have slower adaptation than the heterospecific stimuli that are novel as both a class and as individual songs. A possible explanation lies in unpublished data (K. Lu, personal communication) that suggests that varying stimulus statistics within a set, either by randomly ordering ZF and CAN or by varying the inter-stimulus interval, will influence the amount of adaptation to a target song. Thus by intermixing a class of native

sounds with a class of completely novel foreign sounds (as in the shuffled stimulus sets used in the present study), we may be increasing the novelty of our testing set as a whole. This might produce the steeper adaptation slope indicative of a novelty response in the CONENV group, even though as a class these conspecific testing stimuli are already familiar.

In the HETENV group, one can speculate that the class of CAN stimuli, though still novel as individual songs, are already somewhat familiar as a class due to 9d days exposure, and hence not as surprising to the bird when intermixed with originally class familiar conspecific stimuli, leading to overall a shallower adaptation slope indicative of familiarity to both CANStim and ZFStim.

Results regarding the change in pattern of lateralization for ARMs can be interpreted in several ways. The increase in absolute response in the left hemisphere for HETENV subjects may indicate more plasticity in the left hemisphere than in the right in adulthood. Indicators of conspecific selectivity, such as higher ARMs to conspecific songs and a significant Stimulus class by Hemisphere interaction ($p < 0.01$), both indicate that the right hemisphere has a greater conspecific preference, and distinguishes between CANStim and ZFStim to a greater extent than the left hemisphere. The right hemisphere seems to express more of a bias towards conspecific vocalizations, and this bias may be fixed, either innately or due to early experience.

However, if we interpret faster adaptation rates and higher ARMs in the right hemisphere of CONENV birds as a marker of novelty, and take reduced

ARMs and slower adaptation rates in the left hemisphere to be indicative of familiarity, then the right hemisphere responds and adapts to all novel stimuli, both ZF and CAN songs, more vigorously than the left. Thus, the right hemisphere may play an important role in discriminating between stimuli with different acoustic characteristics, rather than have any prior knowledge as to what is conspecific.

On the other hand, slower adaptation and lower ARMs to both ZF and CAN stimuli can indicate either a lack of response or an adapted response, where a stimulus is recognized as belonging to a familiar class. In this view, the left hemisphere, may be responsible for responding to sounds a class that is familiar, and thus for classifying new categories of sounds, such as those in our HETENV recent experience paradigm. Our CONENV group showed the typical low ARMs and slow adaptation rates in the left hemisphere but our HETENV manipulation produced an interesting reversal. Higher ARMs in the left hemisphere for our HETENV birds might be a product of learning new stimulus categories (canary) over the 9d exposure. In this view, a change in the stimulus statistics of the environment would evoke reorganization of categorical space in the left hemisphere, leading to heightened responses to both ZF and CAN stimuli. Studies of bilingual humans corroborate the idea that left hemisphere is plastic and responsive to changes in categorization of languages into adulthood. A meta-analysis of found that second languages acquired before age 6 had bilateral activation, whereas bilinguals who acquired their second language beyond age 6 had left hemisphere activation for both the native and second

language (Hull and Vaid 2007), similar to the effect we observed in our HET group where left hemisphere responses to both ZF and CAN songs were enhanced.

One other speculative explanation for a shift towards stronger responses in the left hemisphere in our HETENV group can be found in the spectral and temporal characteristics of CON and HET songs. Conspecific zebra finch songs contain broad-band harmonically structured notes that are seldom repeated, while canary songs contain harmonically-structured notes, trills and whistles in a narrower frequency range with fast repetitions (Terleph et al., 2006). Canaries are left dominant for vocal production (Nottebohm 1976), although lateralization of their sensory system is unknown. If the sensory system is also in some sense “left dominant”, it is possible that the spectro- temporal features of canary vocalizations are better processed in the left hemisphere, while the acoustic features of zebra finch songs are better processed in the right. Similarly, in human studies, the left hemisphere is better at processing rapidly modulated sounds such as the phonetic contrasts that contribute to understanding of human speech; while the right hemisphere is better suited to perceive slower modulations and encodes the prosody or tone/mood of speech (Telkemeyer et al., 2011). Short term exposure to vocalizations that stimulate the left hemisphere more so than the right may lead to a more active left hemisphere such as in our HETENV birds. Whether or not the change in lateralization pattern is due to spectro-temporal features of the recent acoustic environment can be determined by exposing adult zebra finches to songs of a different species that contain

features processed in the right hemisphere, or by performing the same experiment described above in canaries.

Aside from hemispheric differences, ventral and dorsal NCM differ in conspecific selectivity and overall responsiveness. As one records more ventrally in NCM, there is a distinct loss of response to CON song, and a more modest loss of response to HET song. It is not clear why absolute responses and CON vs. HET selectivity decreases in ventral NCM. These two properties may be related by a floor effect where smaller absolute responses to CAN stimuli reduce the amount of possible difference between ZFStim and CANStim ARMs. However, our electrophysiology results may be consistent with a study that showed reduced ZENK expression to conspecific song in ventral NCM (Sanford et al., 2010). If conspecific selectivity, seen most prominently in dorsal NCM, is due to weaker responses to heterospecific stimuli, there could be a role for inhibitory inputs in dorsal NCM which originate in other parts of the song system, possibly deeper in NCM. Such an inhibitory network would require a different population of neurons to respond to selectively to heterospecific stimuli. There are large numbers of GABA-ergic neurons within higher auditory areas NCM and CMM, as well as primary auditory area, field L (Pinaud et al., 2004) that may play an important role in this balance of excitation and inhibition. Furthermore, NCM neurons that are both GABA-ergic and express ZENK in response to song stimuli have been identified in double-labeling studies and mapped to regions of NCM (Mello, unpublished data). Double-labeled cells that responded to heterospecific songs were primarily found in more ventral regions of NCM. If these neurons

project dorsalwards and form inhibitory synapses on more dorsal neurons, they could reduce responses to heterospecific stimuli at dorsal sites, resulting in a greater conspecific selectivity there, as was observed. At the same time, ventral multi-unit recordings include both excitatory and inhibitory activity, and this might underlie the weaker conspecific responses recorded in ventral NCM. This idea is somewhat complex and will require further testing.

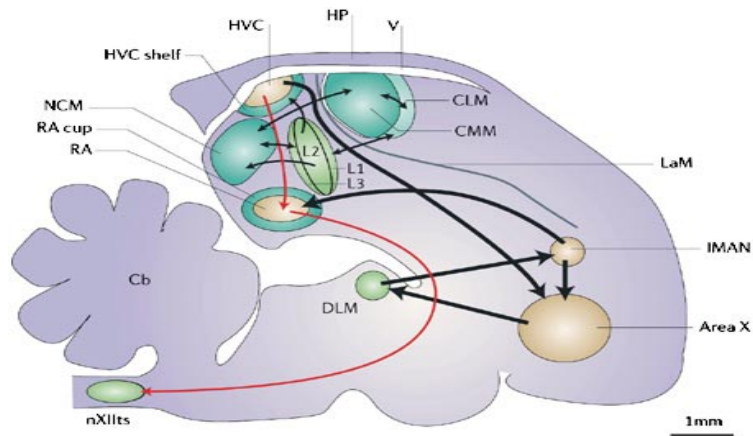
Overall, although we did not observe a reduction or reversal of conspecific selectivity in our HETENV experimental group, we have ample evidence that the auditory system in zebra finches remains labile into adulthood. We speculated that short term adult experience of a new distribution of sounds, such as those encountered on a trip to a foreign country or, in our zebra finches, sounds heard in the HET environment, may trigger reorganization in higher auditory cortex that may serve to better encode a new set of acoustic parameters. It is interesting that this reorganization, reflected in part by the narrowing of tuning width, occurs without major effect on the conspecific response selectivity. It is even more intriguing that this reorganization occurs differently in the two hemispheres, with the suggestion that heightened responsivity, which may help to encode new stimulus parameters, occurs only in the left hemisphere. Our experiment utilized completely passive exposure to heterospecific song, which did not explicitly change the salience of either type of song, and did not affect selectivity for conspecific songs. However, powerful operant conditioning paradigms that reinforce discrimination, e.g. between heterospecific songs, might overcome the conspecific preference, whether innate or acquired early. Furthermore, it is

important to note that, while a conspecific bias in neural activity remains, this does not mean that the bird failed to modify its perceptual filters for heterospecific stimuli since we did not test for effects of recent experience on ability to discriminate between heterospecific songs. This awaits discrimination training and testing in an operant paradigm. Data from our study show that short term exposure to foreign auditory stimuli modifies properties of NCM in a lateralized manner, which is likely due to differentiation of function between hemispheres in the avian auditory forebrain.

In right-handed monolingual humans, the native language is left lateralized. However, results on lateralization in bilingual humans have mixed results and interact with the level of proficiency in the second language as well as the age at which the second language was acquired (Tao et al., 2011; Aladdin et al., 2008; Peng and Wang, 2011; Evans et al., 2008). Although our birds are not capable of learning a second language or a second song in adulthood, we observe a change in both tuning width and tuning peak, as well as a change in the pattern of lateralization. Changes in perceptual filters may also be occurring in the human brain as we are exposed to a foreign language in adulthood. These changes may be different in an adult brain that has already learned a native language vs. a child's brain that is still in the sensitive period. If adulthood plasticity in auditory cortex occurs in a different manner and involves different mechanisms than developmental plasticity, i.e., a reorganization of lateralization in left and right hemispheres in the mature brain, this could explain why

acquisition of a second language in adulthood is more difficult and follows a different course than in childhood.

Appendix



Copyright © 2006 Nature Publishing Group
Nature Reviews | Neuroscience

Figure 1. Anatomy of the Bird Song System. Primary auditory area Field L2 projects to Field L3 and L1 which then project to higher auditory areas caudo-medial nidopallium (NCM) and caudal mesopallium(CMM).

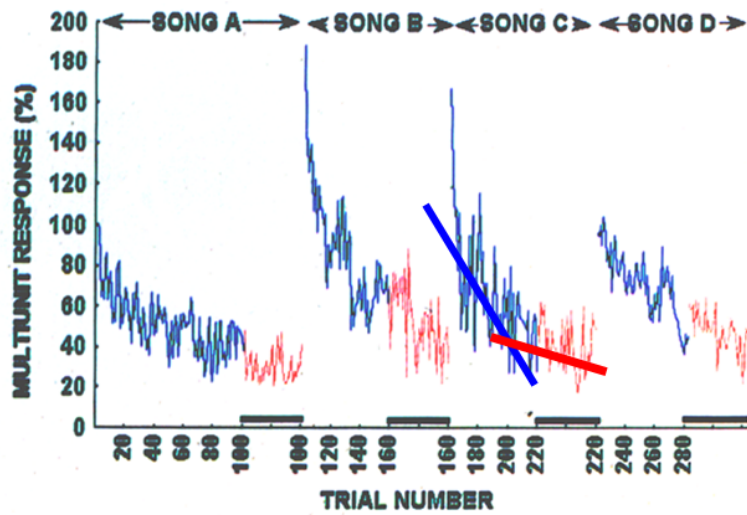


Figure 2. Stimulus Specific Adaptation(SSA). Auditory responses decrease with repeated presentation of the same stimulus, but remain robust to novel stimuli. Blue traces represent initial repetitions of stimuli, red traces represent re-testing with the now familiar stimuli after initial adaptation. Adaptation rate is the slope of the regression line over successive presentations of a given stimulus divided by the average ARM over the same trials to normalize the rates for differences in absolute response size between recording sites. Blue line is representative of fast adaptation rates to novel stimuli and red line is representative of slower adaptation rates to familiar stimuli.

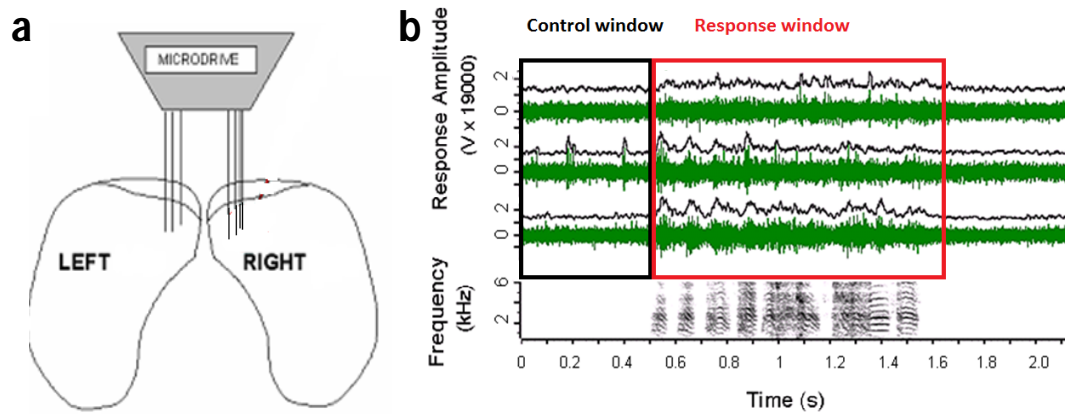


Figure 3. Recording Apparatus and Histology. (a) Sixteen electrodes placed bilaterally in NCM. (b) Multiunit recording of neural activity in NCM. ARMs are computed as RMS of response window (stimulus on) minus RMS of control window (500ms before stimulus on).

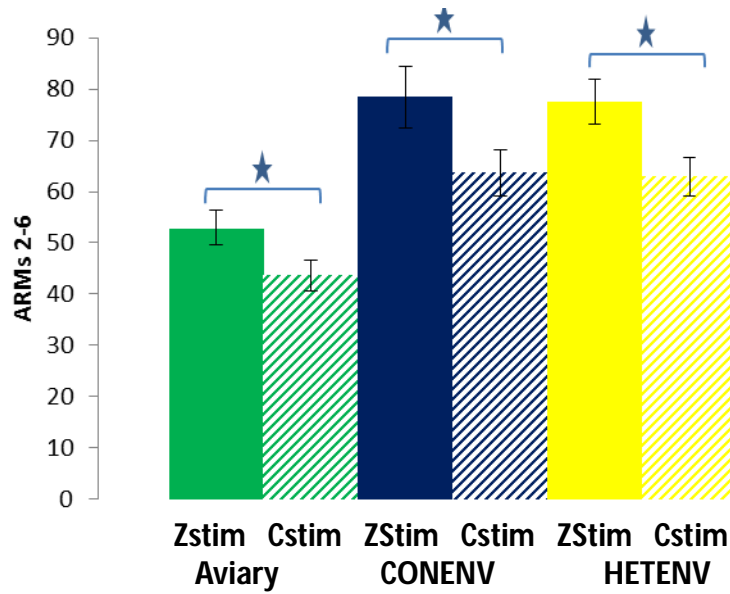


Figure 4. Effect of Recent Experience on ARMs Trials 2-6. Significant main effect of Auditory Environment ($p < 0.000008$) driven by ARMs in the Aviary condition being lower than either CONENV or HETENV. ZFStim also elicits a higher response than CANStim across all conditions ($p < 0.0000001$).

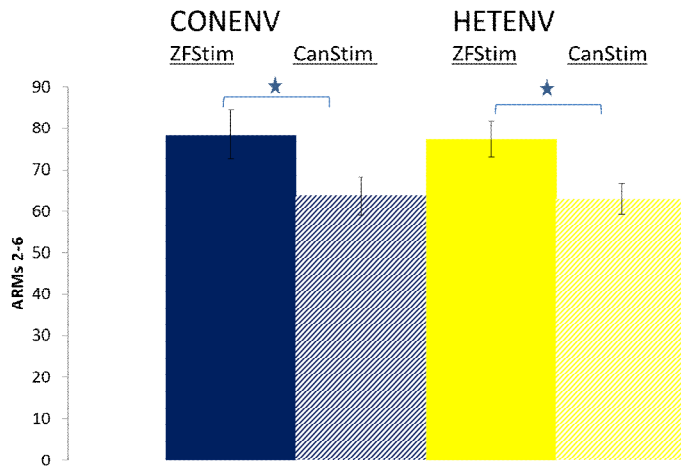


Figure 5. Main effect of Stimulus class on ARMs 2-6 in CONENV and HETENV. Solid bars represent responses to CANStim and solid bars represent responses to ZFStim. Responses to ZFStim are significantly higher than responses to CANStim across auditory environments. ($p < 0.0000001$).

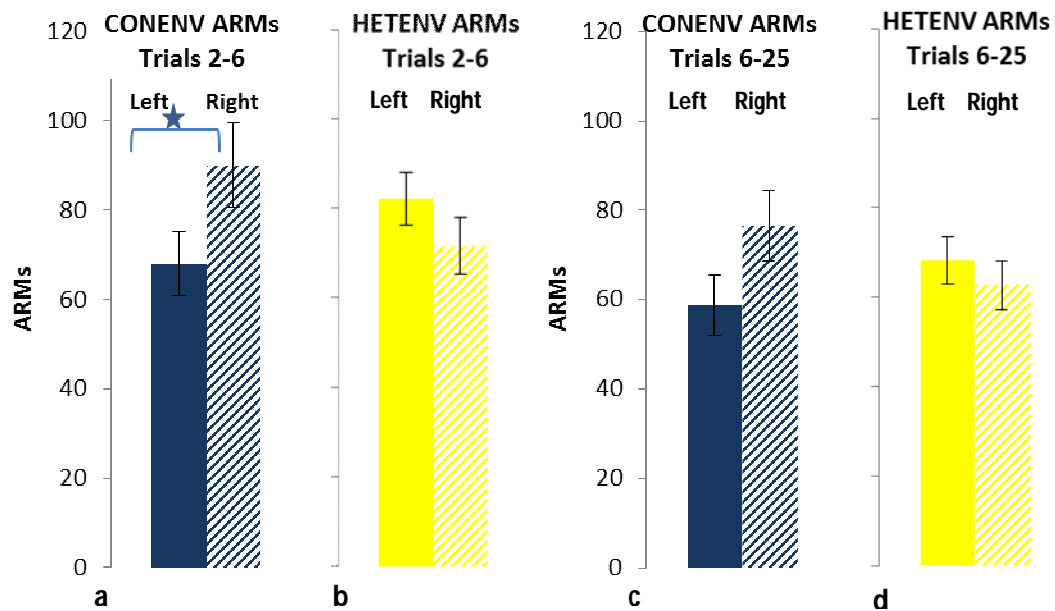


Figure 6. Effect of Auditory Environment on Absolute Response Magnitude to ZF Stimuli.

Environment by Hemisphere interaction for Trials 2-6 ($p < 0.02$) and Trials 6-25 ($p = 0.09$ n.s.).

Striped bars represent RIGHT hemisphere averaged responses and solid bars represent LEFT hemisphere responses. (a) Trials 2-6 Responses of CONENV birds to ZF Stimuli in the left and right hemispheres. (b) Trials 2-6 Responses of HETENV birds to ZF Stimuli in the left and right hemispheres. (c) Trials 6-25 Responses of CONENV birds to ZF Stimuli in the left and right hemispheres. (d) Trials 6-25 Responses of HETENV birds to ZF Stimuli in the left and right hemispheres.

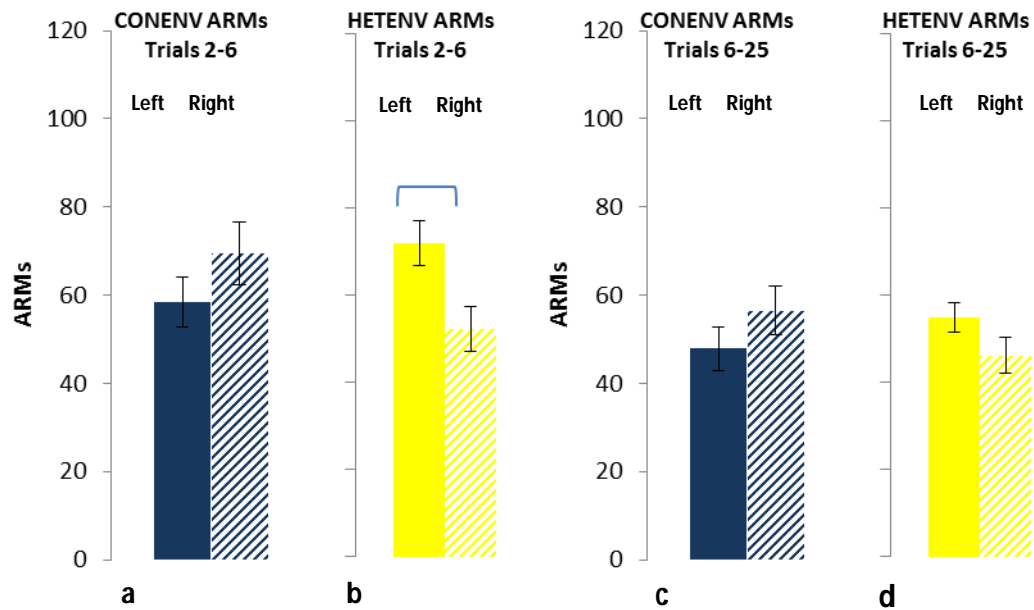


Figure 7. Effect of Auditory Environment on Absolute Response Magnitude to CAN Stimuli.

Environment by Hemisphere interaction for Trials 2-6 ($p < 0.02$), and Trials 6-25 ($p = 0.09$ n.s).

Striped bars represent RIGHT hemisphere averaged responses and solid bars represent LEFT hemisphere responses. (a) Trials 2-6 Responses of CONENV birds to CAN Stimuli in the left and right hemispheres. (b) Trials 2-6 Responses of HETENV birds to CAN Stimuli in the left and right hemispheres. (c) Trials 6-25 Responses of CONENV birds to CAN Stimuli in the left and right hemispheres. (d) Trials 6-25 Responses of HETENV birds to CAN Stimuli in the left and right hemispheres.

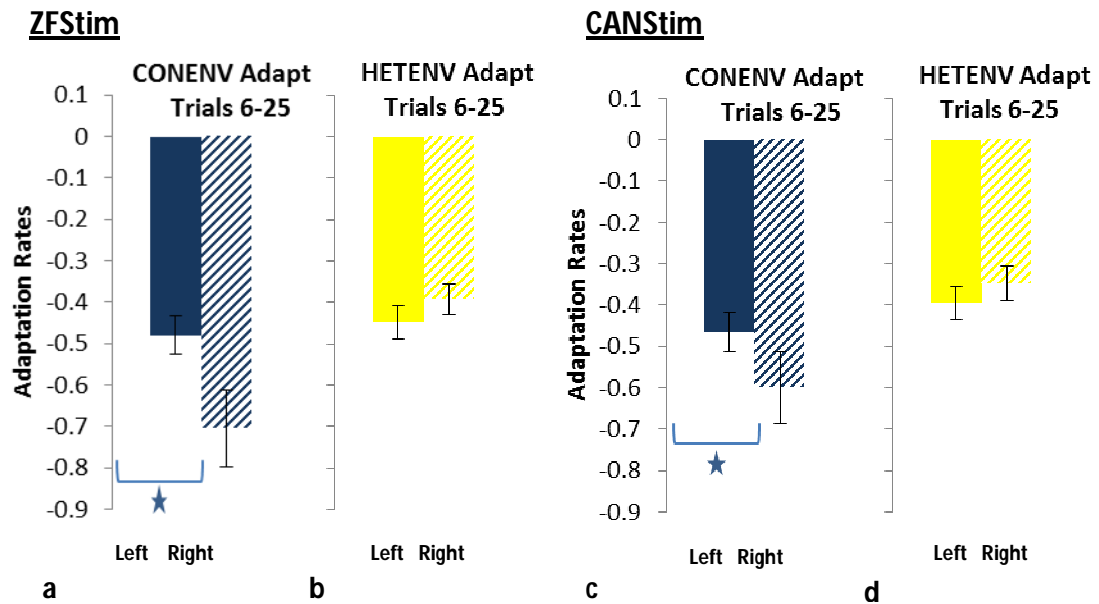
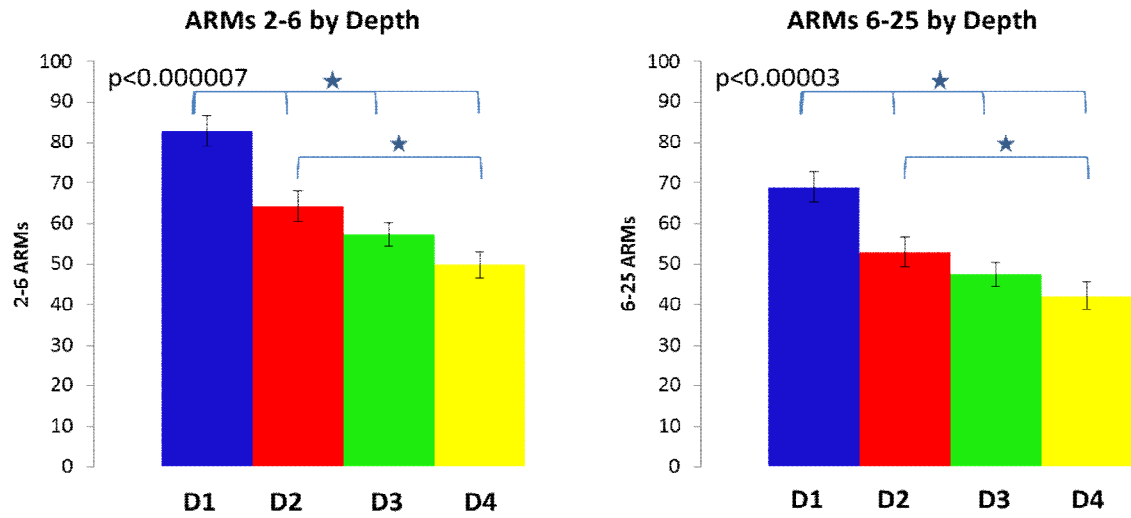


Figure 8. Effect of Auditory Environment on Adaptation Rates – Environment by Hemisphere Interaction – Trials 6-25. Striped bars represent RIGHT hemisphere averaged responses and solid bars represent LEFT hemisphere responses. (a) Adaptation rates to ZF Stimuli for CONENV birds. (b) Adaptation rates to ZF Stimuli for HETENV birds. (c) Adaptation rates to CAN Stimuli for CONENV birds. (d) Adaptation rates to CAN Stimuli for HETENV birds. Significant interaction of Auditory Environment and Hemisphere ($p < 0.0001$).



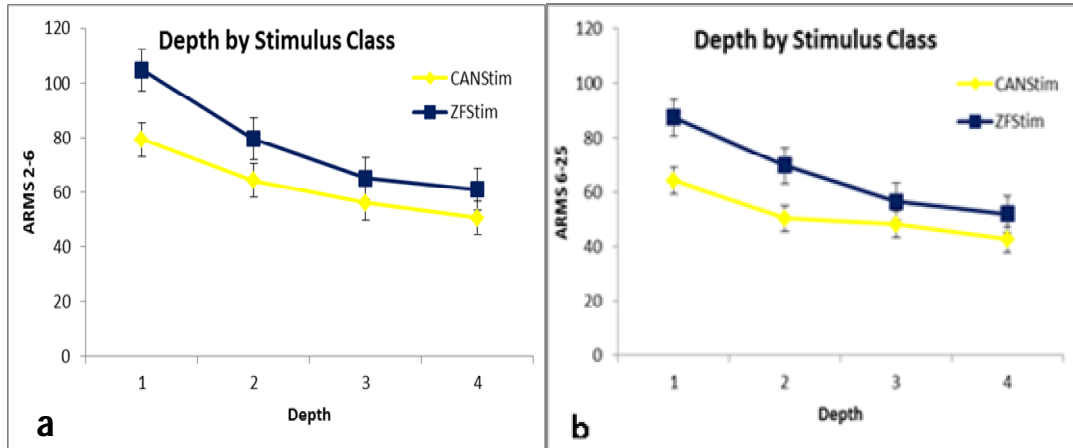


Figure 10. Effects of Depth and Stimulus Class on ARMs 2-6 and 6-25. Blue line is response to ZFStim and yellow line is response to CANStim. Depth is on the x-axis and ARMs is on the y-axis. (a) Significant Depth by Stimulus Class interaction for ARMs 2-6 where ZFStim response is significantly higher than CANStim response at Depths 1 and 2 but not at Depths 3 and 4. (b) The above effect is also seen in ARMs 6-25.

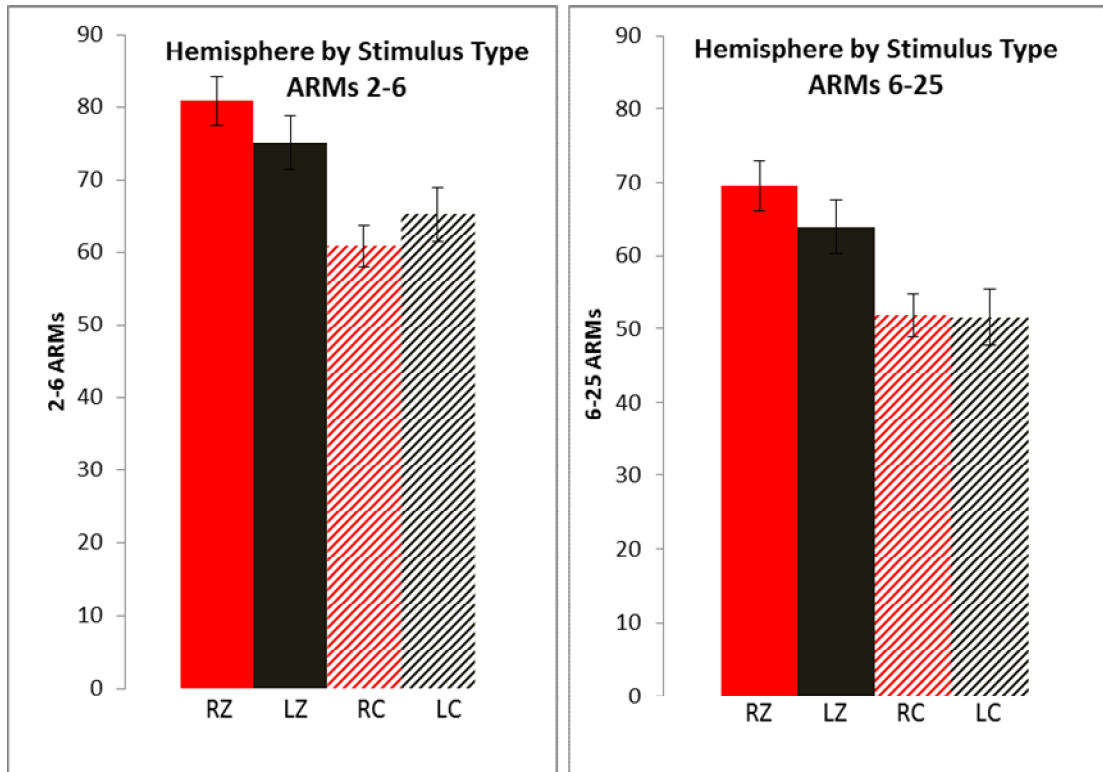


Figure 11. Effect of Hemisphere and Stimulus class on ARMs 2-6 and 6-25. In ARMs 2-6, interaction of Hemisphere with Stimulus Class ($p < 0.01$) driven by right hemisphere having higher ZFStim response than CANStim response. Left hemisphere shows no significant difference between ZFStim and CANStim responses. ARMs 6-25, trend towards interaction of Hemisphere with Stimulus Class ($p = 0.12$, n.s.)

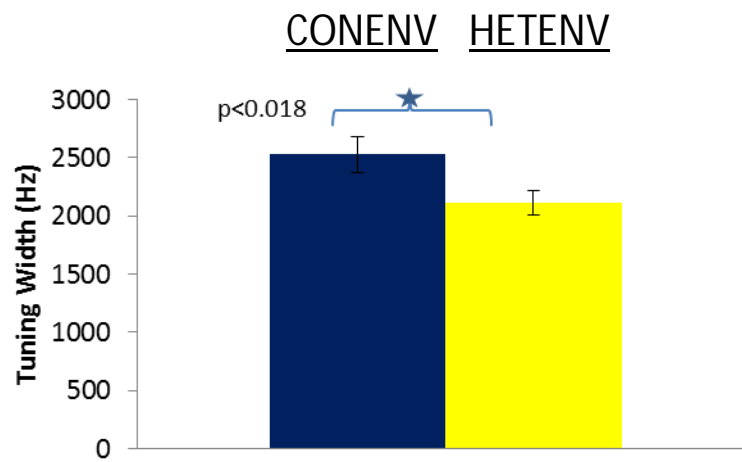


Figure 12. Effects of Recent Auditory Environment on Tuning Width. Subjects with short term exposure to HETENV had narrower tuning widths than those exposed to CONENV.

References

- Aladdin Y, Snyder TJ, Ahmed SN. Pearls & Oysters: selective postictal aphasia: cerebral language organization in bilingual patients. *Neurology*. 2008 Aug 12;71(7):e14-7.
- Brainard MS, Doupe AJ. What songbirds teach us about learning. *Nature*. 2002;417(6886):351-8. Review.
- Braaten RF, Reynolds K. Auditory preference for conspecific song in isolation-reared zebra finches. *AnimBehav*. 1999;58(1):105-111.
- Campbell DL, Hauber ME. Cross-fostering diminishes song discrimination in zebra finches (*Taeniopygia guttata*). *AnimCogn*. 2009;12(3):481-90.
- Chew SJ, Mello C, Nottebohm F, Jarvis E, Vicario DS. Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. *ProcNatlAcadSci U S A*. 1995;92(8):3406-10.
- Chew SJ, Vicario DS, Nottebohm F. A large-capacity memory system that recognizes the calls and songs of individual birds. *ProcNatlAcadSci USA*. 1996;93:1950–1955.
- Doupe, AJ & Kuhl, PK. Birdsong and human speech: common themes and mechanisms. *Annu. Rev. Neurosci*. 1999;22, 567-631.
- Ehret G. Left hemisphere advantage in the mouse brain for recognizing ultrasonic communication calls. *Nature*. 1987;325(6101):249-51.
- Evans J, Workman L, Mayer P, Crowley K. Differential bilingual laterality: mythical monster found in Wales. *Brain Lang*. 2002 Nov;83(2):291-9.
- Gentner TQ, Margoliash D. Neuronal populations and single cells representing learned auditory objects. *Nature*. 2003;424(6949):669-74.
- Hickok G, Poeppel D. The cortical organization of speech processing. *Nat Rev Neurosci*. 2007;8(5):393-402.
- Hull R, Vaid J. Bilingual language lateralization: a meta-analytic tale of two hemispheres. *Neuropsychologia*. 2007;45(9):1987-2008.
- Kandel ER, Schwartz JH, Jessell TM. *Principles of Neural Science*. McGraw-Hill New York; 2000 pp.590-613
- Maier V, Scheich H. Acoustic imprinting in guinea fowl chicks: age dependence of 2-deoxyglucose uptake in relevant forebrain areas. *Brain Res*. 1987 Jan;428(1):15-27.

- Mann ZF, Kelley MW. Development of tonotopy in the auditory periphery. *Hear Res.* 2011;276(1-2):2-15.
- Marler P, Peters S. Selective vocal learning in a sparrow. *Science.* 1977;198(4316):519-21.
- Maul KK, Voss HU, Parra LC, Salgado-Commissariat D, Ballon D, Tchernichovski O, Helekar SA. The development of stimulus-specific auditory responses requires song exposure in male but not female zebra finches. *DevNeurobiol.* 2010;70(1):28-40.
- Mello CV, Vicario DS, Clayton DF. Song presentation induces gene expression in the songbird forebrain. *ProcNatlAcad Sci.* 1992;89(15):6818–22.
- Nottebohm F, Stokes TM, Leonard CM. Central control of song in the canary, *Serinuscanarius*. *J Comp Neurol.* 1976;165(4):457-86.
- Palmer CV, Nelson CT, Lindley GA 4th. The functionally and physiologically plastic adult auditory system. *J AcoustSoc Am.* 1998;103(4):1705-21.
- Peng G, Wang WS. Hemisphere lateralization is influenced by bilingual status and composition of words. *Neuropsychologia.* 2011 Jun;49(7):1981-6. Phan ML, Pytte CL, Vicario DS. Early auditory experience generates long-lasting memories that may subserve vocal learning in songbirds. *ProcNatlAcadSci U S A.* 2006;103(4):1088-93.
- Phan ML, Vicario DS. Hemispheric differences in processing of vocalizations depend on early experience. *ProcNatlAcadSci U S A.* 2010;107(5):2301-6.
- Pinaud R, Terleph TA, Tremere LA, Phan ML, Dagostin AA, Leão RM, Mello CV, Vicario DS. Inhibitory network interactions shape the auditory processing of natural communication signals in the songbird auditory forebrain. *J Neurophysiol.* 2008;100(1):441-55.
- Pinaud R, Velho TA, Jeong JK, Tremere LA, Leão RM, von Gersdorff H, Mello CV. GABAergic neurons participate in the brain's response to birdsong auditory stimulation. *Eur J Neurosci.* 2004(5):1318-30.
- Polley DB, Steinberg EE, Merzenich MM. Perceptual learning directs auditory cortical map reorganization through top-down influences. *J Neurosci.* 2006 May 3;26(18):4970-82.
- Poirier C, Boumans T, Verhoye M, Balthazart J, Van der Linden A. Own-song recognition in the songbird auditory pathway: Selectivity and lateralization. *J Neurosci.* 2009;29:2252–2258. Sanford SE, Lange HS, Maney DL. Topography of estradiol-modulated genomic responses in the songbird auditory forebrain. *DevNeurobiol.* 2010 Feb;70(2):73-86.
- Schreiner CE. Order and disorder in auditory cortical maps. *CurrOpin in Neurobio.* 1995;5:489–496.

- Stevens KN. Acoustic correlates of some phonetic categories. *J Acoust Soc Am*. 1980 Sep;68(3):836-42.
- Stripling R, Kruse AA, Clayton DF. Development of song responses in the zebra finch caudomedialneostriatum: role of genomic and electrophysiological activities. *J Neurobiol*. 2001;48:163–18
- Suga N, Zhang Y, Yan J. Sharpening of frequency tuning by inhibition in the thalamic auditory nucleus of the mustached bat. *J Neurophysiol*. 1997;77(4):2098–2114.
- Tao L, Marzecová A, Taft M, Asanowicz D, Wodniecka Z. The efficiency of attentional networks in early and late bilinguals: the role of age of acquisition. *Front Psychol*. 2011;2:123.
- Telkemeyer S, Rossi S, Nierhaus T, Steinbrink J, Obrig H, Wartenburger I. Acoustic processing of temporally modulated sounds in infants: evidence from a combined near-infrared spectroscopy and EEG study. *Front Psychol*. 2011;1:62.
- Terleph TA, Lu K, Vicario DS. Response properties of the auditory telencephalon in songbirds change with recent experience and season. *PLoS One*. 2008;3(8):e2854.
- Theunissen FE, Sen K, Doupe AJ. Spectral-temporal receptive fields of nonlinear auditory neurons obtained using natural sounds. *J Neurosci*. 2000;20(6):2315-31.
- Vates GE, Broome BM, Mello CV, Nottebohm F. Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. *J Comp Neurol*. 1996;366(4):613-42.
- de Villers-Sidani E, Merzenich MM. Lifelong plasticity in the rat auditory cortex: basic mechanisms and role of sensory experience. *Prog Brain Res*. 2011;191:119-31.
- Voss HU, Tabelow K, Polzehl J, Tchernichovski O, Maul KK, Salgado-Commissariat D, Ballon D, Helekar SA. Functional MRI of the zebra finch brain during song stimulation suggests a lateralized response topography. *Proc Natl Acad Sci U S A*. 2007 Jun 19;104(25):10667-72. Vouloumanos A, Werker JF. Tuned to the signal: the privileged status of speech for young infants. *Dev Sci*. 2004 Jun;7(3):270-6.
- Wang Y, Brzozowska-Prechtel A, Karten HJ. Laminar and columnar auditory cortex in avian brain. *Proc Natl Acad Sci U S A*. 2010;107(28):12676-81.
- Whaling CS, Solis MM, Doupe AJ, Soha JA, Marler P. Acoustic and neural bases for innate recognition of song. *Proc Natl Acad Sci U S A*. 1997;94(23):12694-8.

- Wild JM, Williams MN, Suthers RA. Neural pathways for bilateral vocal control in songbirds. *J Comp Neurol.* 2000 Jul 31;423(3):413-26.
- Woolley SM, Gill PR, Fremouw T, Theunissen FE. Functional groups in the avian auditory system. *J Neurosci.* 2009;29(9):2780-93.
- Zhou X, Panizzutti R, de Villers-Sidani E, Madeira C, Merzenich MM. Natural restoration of critical period plasticity in the juvenile and adult primary auditory cortex. *J Neurosci.* 2011;31(15):5625-34.