

1. DEVELOPMENTAL EXPERIENCE SHAPES ADULT FMRI and ERP RESPONSES.

1.1. Developmental auditory experience and vocal learning

In the previous chapters we set the foundation for obtaining auditory brain responses in the zebra finch. Here we use these techniques to ask more interesting questions: Does the shaping of auditory responses to vocal sounds depend on early song learning experience? If so, what sort of early experience, auditory, vocal or social, is required for shaping response selectivity? And if developmental auditory, social, and song learning experiences are reflected in these responses in the adult bird, are the responses from each technique complementary?

Developmental vocal learning is normally a social experience - vocal repertoire is acquired during early life by imitating adult conspecifics from the social group (Catchpole and Slater, 2008; Marler and Tamura, 1964;) - that depends on auditory experience during development (Konishi, 1965). Song development is associated with changes in auditory responses: single neurons in the song system and auditory nuclei develop stronger responses to conspecific vocalizations over a sensitive period for vocal learning (Doupe, 1997; Doupe and Solis, 1997; Nick and Konishi, 2005; Volman, 1993). In addition, motor vocal activity is essential for development of song preferences in males, as muted male zebra finches are not able to develop behavioral preferences for conspecific song, in contrast to normal birds (Pytte and Suthers, 1999).

A common goal in research of human and zebra finch vocal learning is to determine the quantity and quality of auditory, vocal, and social experience required for converting sensory input into motor output (i.e. vocal learning). However, in humans the sensory environment (and many additional factors) cannot be controlled during development; being able to control the auditory and social environment of the songbird enables us to examine the role of early auditory experience in shaping neural correlates of perception in a vocal learner.

Both fMRI and ERP approaches were used to test how early auditory experience shapes responses – in brain space and in time – in the auditory forebrain of the adult bird. We quantified the consistency in auditory responses to conspecific songs, calls, and syllables, comparing birds that varied in their early experience: from birds who had rich social and acoustic experience during development, through birds that had only minimal song learning experience, and birds that had no social or song learning experience during the sensitive period for song learning. We were interested in the possibility of coupling between the sensory-motor song learning period and the shaping of stimulus-specific auditory response patterns to conspecific sounds, because sensory plasticity might guide the development of motor skills, and vice versa. The zebra finch female provides us with an interesting contrast: as opposed to males, the females do not sing. However, females select mates by judging quality of male songs (Riebel, Smallegange, Terpstra, Bolhuis, 2002): what sort of early experience is required for females to develop their taste to songs?

1.2. Experiment 1: Auditory brain responses to songs and calls in the adult male bird with normal social and auditory experience during development

1.2.1. Rationale

The natural environment of zebra finches is full of acoustic and social opportunities. Male zebra finches, who learn to sing, are exposed to female calls, multiple conspecific songs: of their father's, nearby adult males, and developing song of siblings in the nest, in addition to the various heterospecific songs and environmental sounds they may hear (Zann, 1996). As well, there are plenty of opportunities for social interaction in the wild: zebra finches are very social animals; they form small or medium size groups, spend much time together, and are monogamous, often throughout their life (Zann, 1996). Eventually, each male learns a single song, sometimes by copying his father's song, and in other cases by copying songs from adult male with whom he is interacting – a *male tutor*) but may improvise with aspects of siblings' songs, and songs of nearby adult males (Williams, 2001). Presumably, auditory and song learning experience during development affect the perception of the bird, which might be mirrored by alteration of forebrain auditory responses, particularly at large scales. Using physiological auditory responses and brain imaging we look at sensitivity of adult brains of bird's who have had a rich auditory and social experience during development. We ask whether this experience contributes to distinct patterns of auditory responses to different classes of sounds – calls and songs.

1.2.2. Methods

Experimental groups: Male birds raised in the City College zebra finch colony are raised in a pseudo-natural environment (see **Chapter 3 - Birds**). These birds are raised by their natural parents with siblings in a single cage among many cages of reproductive pairs, groups of males, and groups of females in a room designated for zebra finches. They are kept on a 12:12 photoperiod and receive food and water daily from caretakers, but otherwise during development are unobserved and undisturbed (by humans). Colony-raised birds develop in a relatively rich auditory and social context: direct social contact with parents and siblings and indirect (vocal, acoustic, and visual) social contact with numerous birds, in an intense acoustic environment: males directing song to females; males directing song to males; males practicing song (undirected); female, male, and juvenile calls, and varying stages of juvenile plastic (developing) song. In order to learn song in this environment - establish an auditory template of the tutor song, as well as modify one's own vocalizations to match the tutor - birds may require fine perceptual or sensory tuning during development. We observed auditory brain responses to naturally-produced calls and songs in adult male colony birds (ages 9-24 months). The fMRI and ERP responses to calls and songs in these birds will give us a foundation of "normative" data with which we can compare brain responses of birds raised manipulated auditory and social contexts.

Scanning, Recording and analysis: see Methods Chapters 2 & 3

1.2.3. Results

Our previous finding (see **Chapter 2 - Results**) showed that familiar and unfamiliar songs elicit strong BOLD activation in colony males. Interestingly, in the present study we saw strong BOLD activation to songs and weaker activation to repeated syllables, an example of response variation across stimulus type is seen in Figure 4-1, A. Responses differed significantly across stimuli (Figure 4-2 A), with the strongest activation to songs and weaker activation to calls and tones (1-way ANOVA, $p < 0.01$, $F = 4.5$, $n = 5$). Similar to the fMRI results, in ERP responses colony males had a strong rostro-caudal potential (note color gradient on rostro-caudal axis) to a male syllable but weaker rostral-caudal response to a female call (Figure 4-1, B) and overall these birds showed significant response heterogeneity (Figure, 4-2, B, 1-way ANOVA, $p < 0.01$, $F = 12.9$, $n = 5$).

Figure 1-1

Colony birds show differences in BOLD and ERP auditory responses to different stimuli. A. BOLD activation maps for bird's own song (BOS) and repeated syllable (SYLL1) in colony males shows stronger activation to BOS. Each map shows average activity in left and right hemisphere 0.5 mm from midline (sagittal slices 4 & 5). **B.** Rostro-caudal potential difference at stimulus onset (0-40 ms) varies by stimulus in colony males.

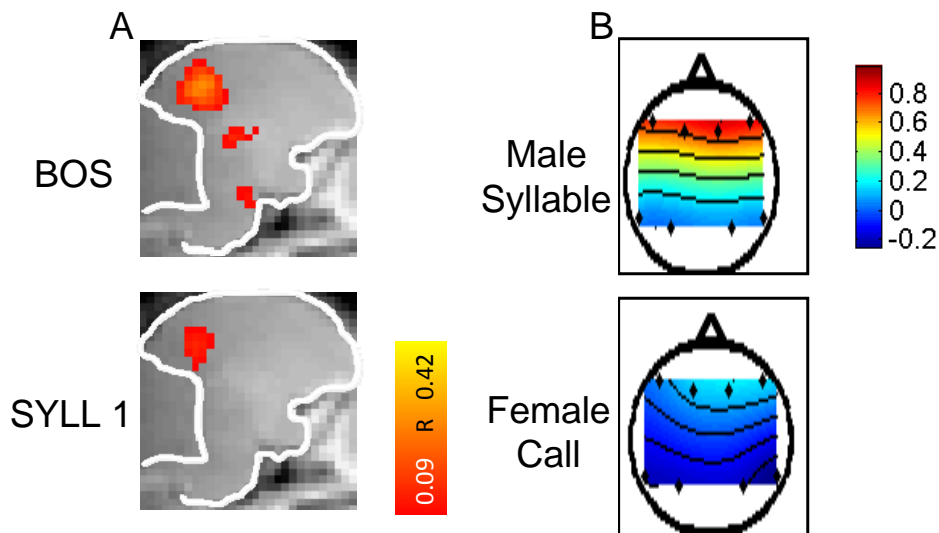
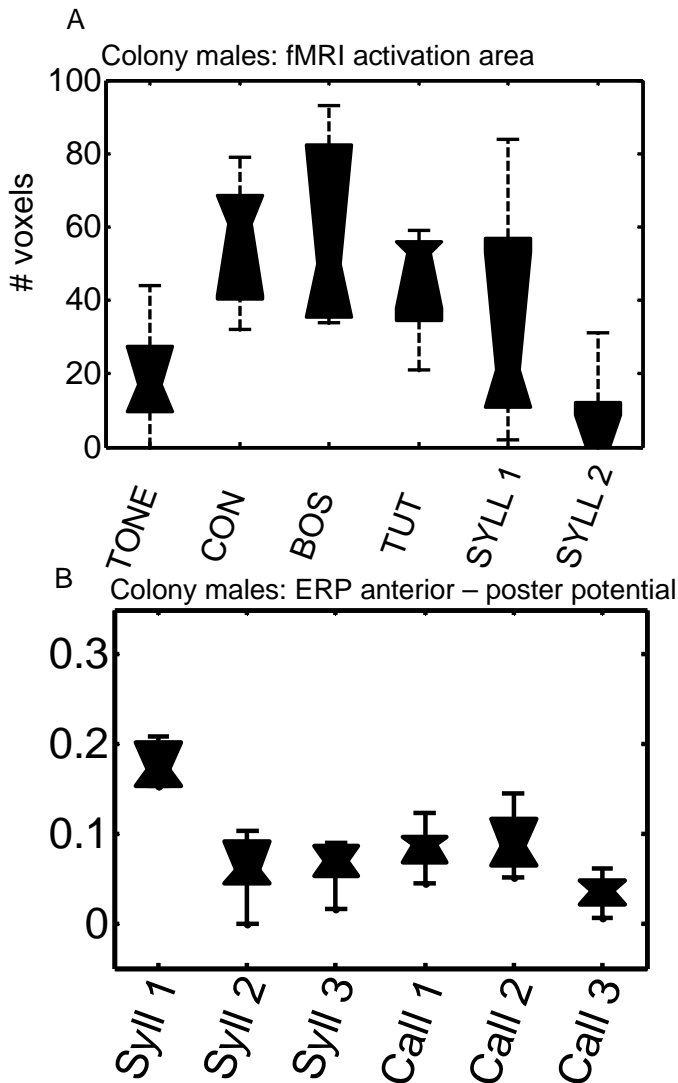


Figure 1-2

A. Box plot summarizing BOLD activation across stimuli in colony males. Area of activation varies significantly across stimuli with greater activation to songs (ANOVA $p < 0.01$). Stimuli: 2 kHz pure tone, conspecific song (CON), bird's own song (BOS), tutor song (TUT), song syllables (SYLL 1, SYLL 2). All conspecific songs were produced by unfamiliar, colony raised birds. **B.** Box plot summarizing ERP rostro-caudal potentials across stimuli in colony males. Potentials vary significantly across stimuli (ANOVA $p < 0.01$). Stimuli: syllables (SYLL 1, SYLL 2, SYLL 3) and female calls (CALL 1, CALL 2, CALL 3).



1.2.4. Conclusions

Results in colony birds that have had rich auditory and social experience during development indicate that, as a group, the colony birds show stimulus specific responses in both fMRI and ERP: in general, responses tended to be stronger to vocal sounds compared to a tone, and responses to songs, and song syllables tended to be stronger than responses to calls. We found it encouraging

that fine perceptual tuning to types and features of natural stimuli - songs vs. calls and complexity and duration of calls, can be captured by both fMRI and ERP (two complementary approaches). While these global measures of brain response, i.e. measuring the entire activity of the posterior auditory region, or potential differences in anterior and posterior brain regions, give some information about auditory organization and sensitivity at the organismal (rather than cellular) level, the findings in colony birds raise the question if and how the responses we see in brains of “normal” adult male zebra finches relates to their early experience? Is song learning experience in isolation sufficient? Is vocal learning experience necessary? The experiments below attempt to answer these questions.

1.3. Experiment 2: Does the shaping of auditory responses to vocal sounds depend on a rich social and auditory experience during development?

1.3.1. Rationale

To examine the role of developmental experience in shaping adult auditory responses we controlled the auditory and social experience of the birds. Without exposure to song, isolated male zebra finches develop an abnormal, loosely structured song. Several seconds of song playback per day over development is sufficient to induce song learning and shape normal song structure in male birds that are otherwise acoustically and socially isolated (Tchernichovski, 1999). We wondered if song tutoring by playbacks in isolation is sufficient to induce response patterns as observed in the colony males. *Box trained* birds were raised by the mother alone (who does not sing) from 7-29 days post hatch, isolated from song until training (see **Chapter 3 – Birds** for details). From day 30-100 (during song development) birds were kept individually in sound-attenuated chambers and were trained with thirty seconds of song playback every day, starting from day 43 (*box-trained*). This design allows us to disentangle social factors from song learning in the question of how early experience affects adult auditory responses. We compare isolated birds that did learn a song from song playback, and colony birds, who were raised in a socially and acoustically rich environment.

1.3.2. Results

In box-trained birds heterogeneity of responses was still apparent (Figure 4-3, A). Across stimuli, differences in responses were significant and similar to the pattern observed in colony males, with strongest responses to songs (Figure 4-4, A, 1-way ANOVA $p < 0.05$, $F = 2.7$, $n = 5$). As with fMRI, minimal exposure to song playbacks in box-trained males was sufficient to elicit stimulus-specific responses, Figure 4-3, B shows two example responses (anterior-posterior potential) to biologically different stimuli: female call and male syllable (Figure 4-4, B $p < 0.05$, $F = 5.2$, $n = 9$).

Figure 1-3

Box-trained birds show differences in BOLD and ERP auditory responses to different stimuli.
A. BOLD activation maps for bird’s own song (BOS) and repeated syllable (SYLL1) in colony males

shows stronger activation to BOS ($p < 0.05$). **B.** Rostro-caudal potential difference at stimulus onset (0-40 ms) varies by stimulus in box-trained males.

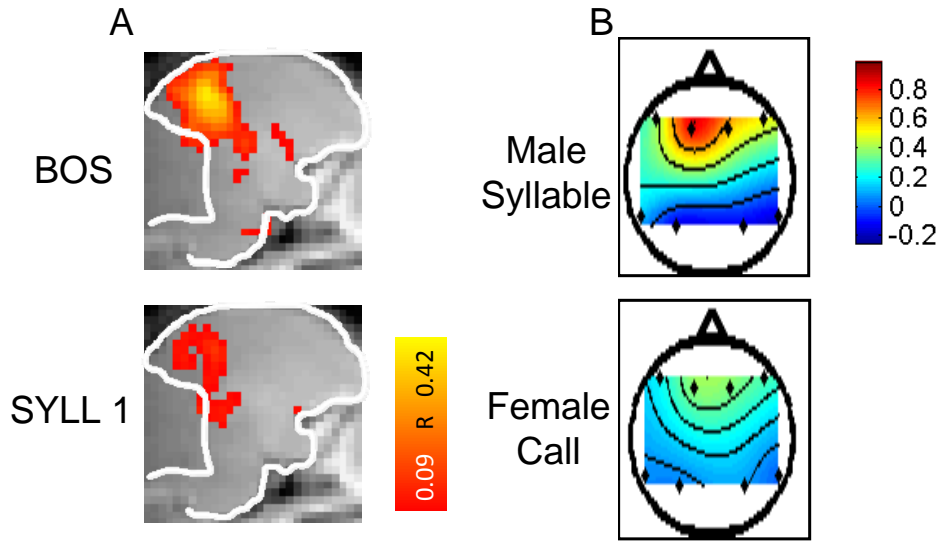
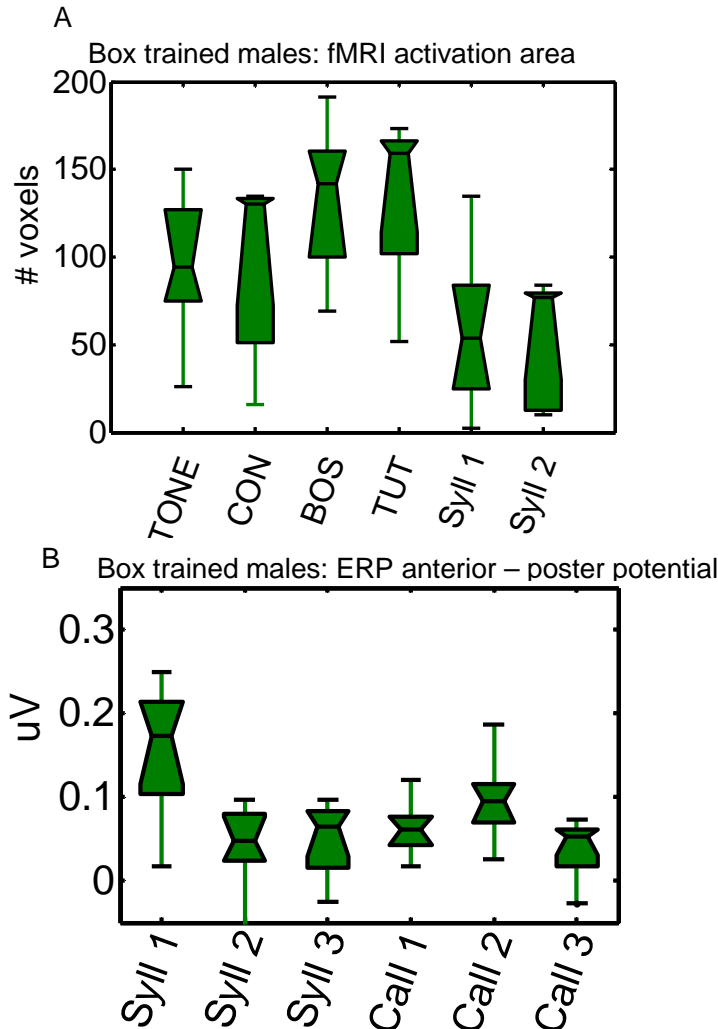


Figure 1-4

A. Box plot summarizing BOLD activation across stimuli in colony males. Area of activation varies significantly across stimuli with greater activation to songs ($p < 0.05$). **B.** Box plot summarizing ERP rostro-caudal potentials across stimuli in box-trained males. Potentials vary significantly across stimuli (ANOVA $p < 0.05$).



1.4. Experiment 3: Is exposure to isolate song (untutored, self-produced song) and isolate song learning sufficient to shape auditory experience in the adult male zebra finch?

1.4.1. Rationale

A minimal amount of song exposure (~ 30 seconds per day) is sufficient for isolated male birds to learn a song, but without such exposure isolated male zebra finches develop an abnormal, loosely structured song. Isolate songs are acoustically very different from each other, and from conspecific song. Experiment #2 showed us that minimal exposure is sufficient to shape the auditory responses, in addition to the vocal production, of isolated birds. We wondered whether any exposure

to an external song is necessary in order to see the stimulus-specific auditory response patterns in an adult singing male. Ultimately, completely isolated birds who are not exposed to, and do not learn from an external song model, still *do* develop song, albeit song that is distinctly different, less-structured than song produced by males that learned from a live tutor or tape playback . Perhaps this abnormal song, and the very act of hearing and modifying one's own song - is sufficient to shape auditory responses in the adult isolated male.

1.4.2. Results

Isolated male birds showed activation to all sounds – two examples are high activation to BOS and a repeated syllable (Figure 4-5, A), but responses were highly variable across birds and equally strong to socially relevant (songs) and less relevant stimuli (e.g. tone). In isolated male birds auditory responses did not differ significantly across stimuli (Figure 4-6, A, 1-way ANOVA $p=0.9$, $F=0.2$, $n=5$). In the ERP responses to the syllable and call were similar, i.e. the anterior-posterior potential did not vary between these two stimuli in this group, Figure 4-5, B.

Figure 1-5

Male isolates do not show differences in BOLD and ERP responses to different stimuli. A. BOLD activation maps for bird's own song (BOS) and repeated syllable (SYLL1) in isolated males shows no difference in activation to between BOS and a repeated syllable. **B.** In isolated males rostro-caudal potential difference at stimulus onset (0-40 ms) is nearly identical in response to the Male Syllable and to the Female Call.

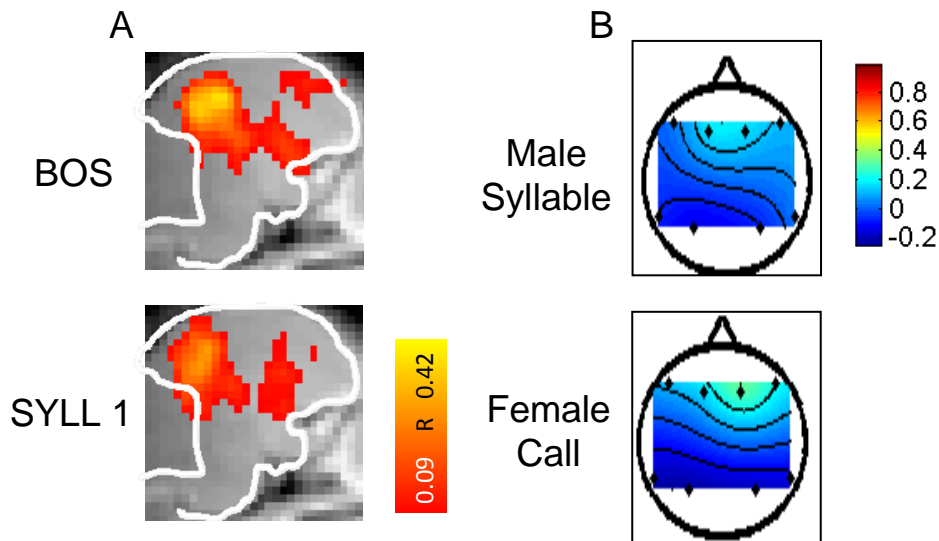
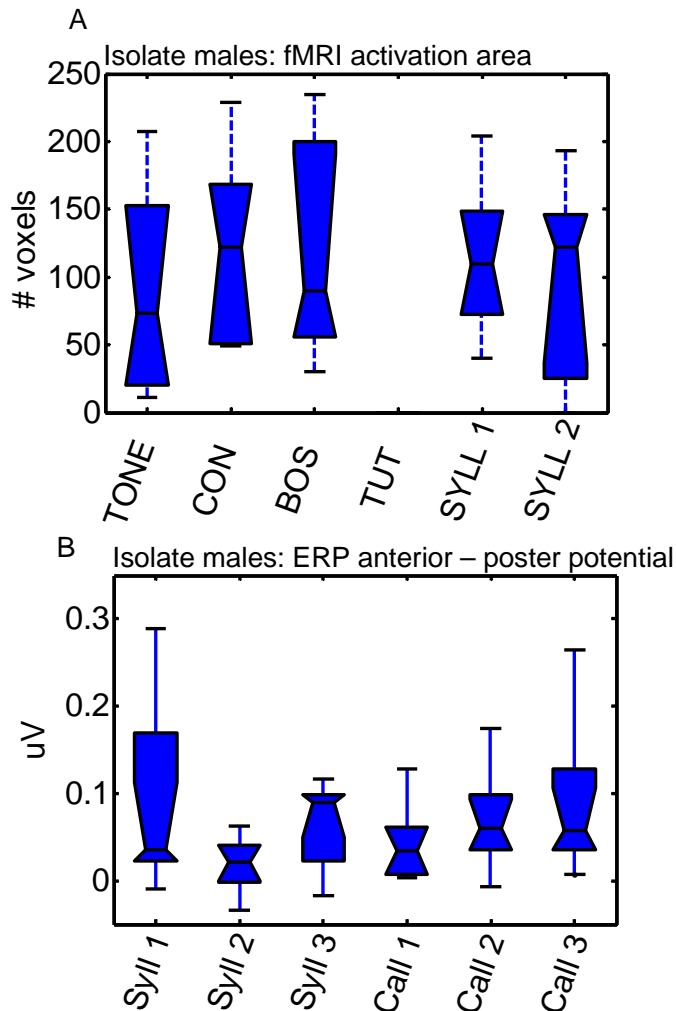


Figure 1-6

A. Across all stimuli Isolated males show no stimulus-specific response patterns in BOLD activation ($p=0.9$), as seen in this summary boxplot. Stimuli are the same as for colony and box-trained birds, except that there is no tutor song. **B.** Box plot summarizing ERP rostro-caudal potentials across stimuli in isolated males: potentials do not vary significantly across stimuli (ANOVA $p<0.7$).



1.5. Experiment 4: Does the shaping of auditory responses to vocal sounds depend at all on auditory and song learning experience during development? Is developmental auditory experience required to shape auditory responses in the adult female zebra finch?

1.5.1. Rationale

The zebra finch female provides us with an interesting contrast: as opposed to males, the females do not sing. Song nuclei are not developed in the female (some reports indicate existence of very small nuclei) and lack functional connections (Williams, 1985). However, females do show song preference and discrimination and more importantly they select their mates by judging song quality of

males around them. We wondered what sort of early experience is required for females to develop their taste to songs.

1.5.2. Results

The isolated females in this experiment do not hear any song and therefore experience even greater auditory deprivation than isolated males, who hear their own song during development. Despite this, and in contrast to adult isolated males, adult isolated females showed significant differences in responses across stimuli in both the fMRI and ERP. In this way, female responses look much like those of adult males that have learned a song. Figure 4-7 shows differential BOLD activation and intensity between the SONG and repeated syllable stimulus (Figure 4-7, A). Also in the ERP anterior posterior potential gradient, there is a marked difference in response to male syllable and female call, Figure 4-7, B. In female, just as in the box-trained and colony males, both the fMRI and ERP responses are significantly different across stimuli. This is remarkable given that the isolate males, who do have auditory exposure to their own song, do not show response patterns.

Figure 1-7

Isolated female birds show differences in BOLD and ERP auditory responses to different stimuli. **A.** BOLD activation maps for bird's own song (BOS) and repeated syllable (SYLL1) in colony males shows stronger activation to BOS ($p < 0.05$). **B.** Rostro-caudal potential difference at stimulus onset (0-40 ms) varies by stimulus in box-trained males.

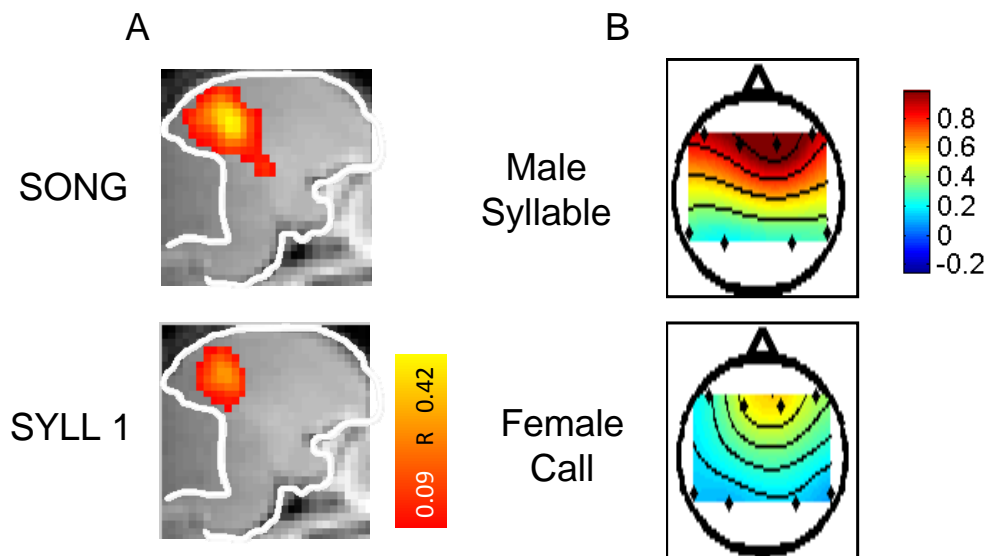
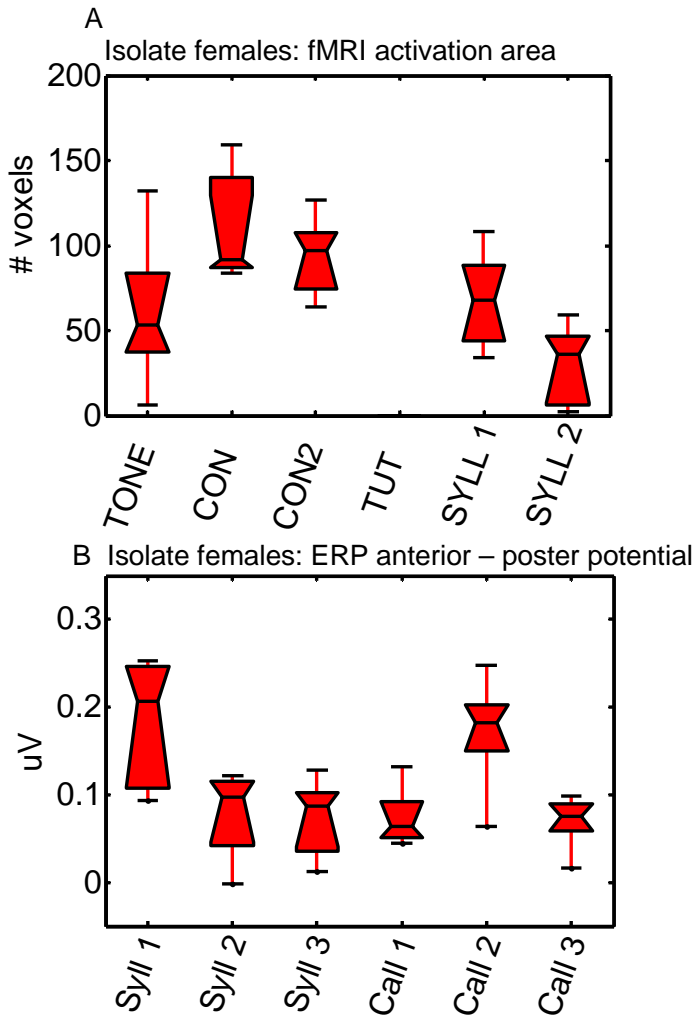


Figure 1-8

A. Box plot summarizing BOLD activation across stimuli in colony males. Area of activation varies significantly across stimuli with greater activation to songs ($p < 0.01$); to balance stimuli, females heard two conspecific songs (CON1 and CON2) rather than BOS and TUT. **B.** Box plot summarizing ERP rostro-caudal potentials across stimuli in isolate females. Potentials vary significantly across stimuli (ANOVA $p < 0.05$).



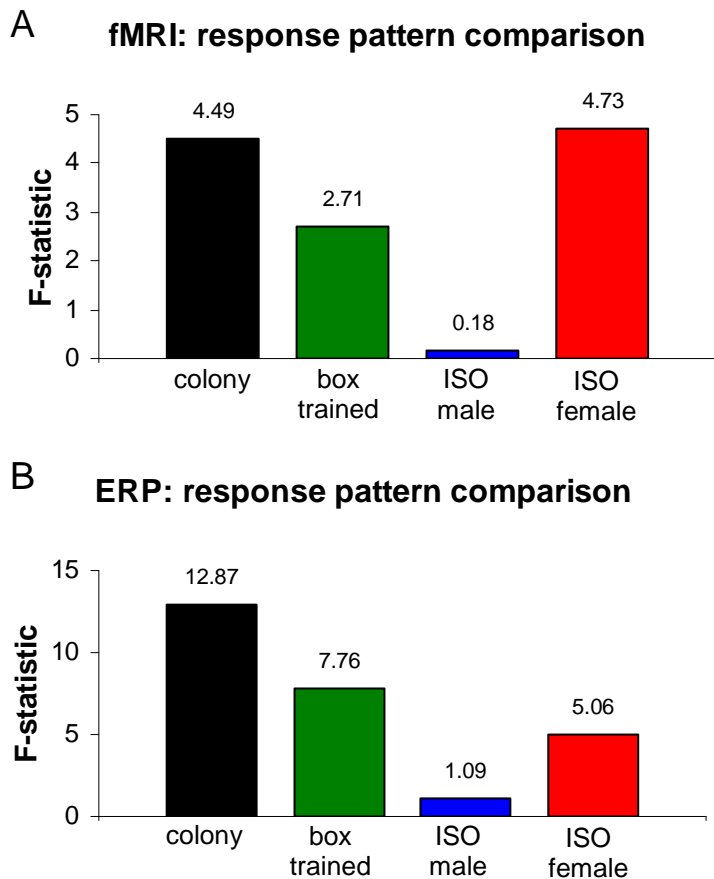
1.6. Analysis of Results across experiments 1-4

We summarize the findings across groups by using the F-statistic (for each group) as a yardstick of how variably each group responds to different stimuli. Namely, the F-statistic is a measure of the group tendency to respond more strongly to some sounds, and less strongly to other sounds (Figure 4-9). We refer to this measure as a *stimulus-specific response pattern*. As shown, in both the fMRI (Figure 4-9, A) and ERP (Figure 4-9, B) results, response patterns are strong in the colony males, weaker in the box-trained males and absent in the isolated males. In the isolated females response patterns are comparable to those found in the colony-trained males. To test directly if response patterns are significantly different

across groups we performed bootstrap tests, computing the F statistic for random selections of birds irrespective of their group (box-trained, isolated male, isolated females). We found significant differences in response pattern between boxed-trained and isolated males and also between isolated females and isolated males in both the fMRI and ERP responses ($p < 0.05$ for each comparison).

Figure 1-9

Stimulus-specific response patterns. By using the F-statistic for each group, group tendency to respond more or less to different stimuli can be compared across fMRI and ERP responses. **A.** F-statistic is a measure of variability across responses: colony, box-trained and female isolated birds have relatively large F-values, corresponding to *stimulus-specific response patterns*. BOLD responses of male isolated birds are relatively uniform across stimuli. F-values vary significantly ($p < 0.05$) between male isolates and each other group of birds. **B.** Comparisons of ERP F-values across groups are similar to fMRI results, showing that all groups but isolated males exhibit *stimulus-specific response patterns* ($p < 0.05$ males compared to each other group).



1.7. Anterior Forebrain Activation

In addition to posterior activity we found activation in the song learning area in some isolate male birds. In contrast, in birds that had learned a song from a tutor or model, box trained and live-tutored

birds, showed only a single cluster of activity, centered at Field L and NCM and no consistent activation of the anterior song learning nuclei. We find it remarkable that several minutes of song playbacks over the entire song development were sufficient to nearly eliminate AFP activation. In addition, scans in juvenile male birds learning song from a live-tutor showed that on average there is significant activation of the most lateral part of area X in these birds (ages 39 – 56 day post-hatch).

These preliminary results suggest that closure of the sensitive period for song learning involves a decrease in activation of the song learning area, area X. The anterior learning pathway has a central role in song imitation, and these results could be interpreted as “gating by learning”; namely, that the decreased sensitivity of AFP to sounds might be the neuronal mechanism of closing the sensitive period for song learning.

1.8. Discussion

The current study reveals some interesting aspects of the process of auditory experience shaping auditory responses in vocal learners: we found that exposure to playback of a single song (with no variability) is sufficient to shape auditory responses to a variety of unfamiliar stimuli in a manner that appears comparable to that seen in animals raised in a rich social and auditory environment. The ERP technique revealed that responses were sensitive to different syllables and calls, whereas the fMRI showed differences in responses to songs, calls and tones. Males that learned song during a sensitive period of development showed stimulus-specific response patterns in adulthood, whereas males that did not learn song, did not show such patterns. Non-singing females showed response patterns, with a strong preference for songs, in adulthood despite not hearing songs during development. Taken together, these results suggest a link between the sensory-motor song learning period and the shaping of stimulus-specific auditory response patterns to conspecific sounds. In the female, such responses are likely to be hardwired. An unlikely alternative possibility is that in females only, auditory responses are shaped by exposure to female calls before day 30. In either case, evidence suggests that although auditory responses patterns are similar in males and females zebra finches, the timing, and possibly the mechanism, of shaping those responses is very different across genders.

It is important to note that claims made here about female response patterns being “hardwired” may apply only to the coarse shaping of auditory responses toward broad vocal categories. Female songbirds show strong behavioral discrimination across songs, and those are likely to be acquired early in life. For example, females show behavioral preference towards songs of older males and toward “local dialect” songs (Lauay, Gerlach, Adkins-Regan, & Devoogd, 2005; Reibel, 2000; Terpstra, Bolhuis, Riebel, van der Burg, & den Boer-Visser, 2006). However, the effect of early experience on female’s song preference, might develop on the foundation of generic hardwired tuning toward conspecific songs. Investigating the development of such preferences would probably gain from the higher resolution fMRI animal scanners which are now becoming available.

In the male, the stimulus-specific response patterns we observed might be shaped by the saliency of the song template (sensory hypothesis), or by vocal practice (sensory-motor learning hypothesis). A third possibility, supported by the similarity in response patterns across isolated females and trained males, is that the stimulus specific response patterns observed in isolated females is the default (perhaps hardwired) state. According to this hypothesis, in the male there is a delay in the maturation of response patterns so as to facilitate perceptual plasticity during vocal learning.

Some features of the adult male zebra finch responses might mirror maturation whereas other features might relate more specifically to the song playbacks. Although this study does not provide enough data to attempt a distinction between the two, both fMRI and EEG approaches allow repeated measurements over development and should make it possible in future studies to look continuously at how response patterns emerge during development. Further, it should be possible to examine side by side the development of song motor skills (when song becomes more and more structured) and the emergence of structured stimulus specific response patterns.