

1. DISCUSSION

1.1. General overview of the results and potential to complement human studies

1.1.1. *Overview*

This dissertation focuses on the influence of early experience on auditory responses to vocal sounds, attempting to complement the knowledgebase of speech development by experimenting with auditory and social environment during song development in birds. In particular, we wanted to test if and how vocal imitation during early life might affect auditory responses. Changes in auditory responses and perception might, in turn, affect vocal development. Inspired by the success of using imaging technology in humans to assess neural correlates of auditory discrimination and perception (Kuhl and Rivera-Gaxiola, 2008), we developed fMRI and ERP methods to look at global brain responses (i.e. far-field potentials and widespread hemodynamic changes) to auditory stimuli in the songbird. With these techniques we asked how spatial and temporal structure of auditory responses is shaped by song learning, in controlled sensory and learning environments. We explored auditory responses to songs in male birds raised in different social and acoustic environments, and compared these to responses in female birds. Male birds kept in isolation (isolates) during the sensitive period for song learning lack consistency and selectivity in responses to different auditory stimuli. However, a few minutes of daily song playbacks during development were sufficient to induce stimulus-specific auditory responses to a variety of unfamiliar sounds. This sensory plasticity is related to the process of song imitation: in females, who do not sing, stimulus-specific auditory responses appear even in complete acoustic isolation and with no exposure to song.

1.1.2. *Technical progress*

fMRI

Several technical difficulties were encountered in attempts to perform fMRI scanning in such small animals. The solution to those problems included diverse methods such as designing fMRI hardware for use on birds, including custom-made radiofrequency coils and reducing the scanner noise heard by the bird by building a custom-made sound insulation box. Another challenge was to properly immobilize the bird, with minimal stress to the bird and minimal movement artifacts, without completely changing the behavioral state of the bird with anesthesia. The result of these efforts is the ability to detect strong BOLD responses to songs in auditory brain areas of mildly sedated birds.

ERP

Using ERP methods in zebra finches required modifying existing procedures. Auditory Brainstem Responses have been recorded and compared in a number of vertebrates (Corwin, Bullock, Schweitzer, 1982; Lucas, Freeberg, Krishnan, Long; 2002; Kenyon, Ladich, Yan, 1998) and Middle and Long-latency evoked responses have been measured in small animals and in some songbirds (Dooling and Walsh,

1976; Espino et al., 2003; Knight, et al., 1985). However, no attempts have been made to capture *response patterns* to vocal sounds in the songbird forebrain. Procedures used in previous research were modified in this study to 1) increase the number of electrodes placed on the skull (relative to the ABR long-latency potential experiments); 2) increase the stability of electrodes and length of time electrodes adhere to the skull; 3) determine minimum number of trials adequate to produce response in average of trials; 4) develop stimuli and a stimulus design in which auditory responses would reflect experience of the bird; 5) develop a protocol for implanting chronic epidural electrodes in juvenile zebra finches.

Electrodes were configured to form an epidural electrode cap that remains on the adult birds for >3 months and consistently provides a stable signal at intermittent recording intervals. This electrode cap does not appear to harm the bird or negatively affect his health or behavior and birds can live years with the electrodes cap in place. Further, the signal from these electrodes is highly stable and in adult birds, song recorded prior to, 24 hours post-, 4 weeks post-implantation is indistinguishable. In terms of time and cost, this is a feasible method for obtaining auditory evoked brain potentials from a large sample of birds (we aimed at 20-25 per experiment) and for obtaining multiple recordings from each bird – both for reducing measurement noise, but potentially also for longitudinal studies.

The ERP data acquisition system was constructed using an industry standard analog data acquisition card, professional sound card, custom-made amplifier (SA Instruments, San Diego) with variable filter and gain settings, and Matlab programming environment. The independent hardware and software components make the system flexible enough to accommodate different experimental designs by expanding the hardware filter and modifying the software. In addition to long-latency evoked potentials we briefly explored Auditory Brainstem Responses, Mid-latency evoked potentials such as the 'sensory gating' potential (human P50 component), EEG signals, and the novelty response (i.e. response elicited by an infrequent change in a repetitive stimulus).

1.1.3. Remaining technical challenges

Our goal is to use both of these techniques in zebra finches across development. In this respect we face the same challenges as researchers using these techniques in human infants and children: 1) the brain is structurally and functionally immature; 2) the immature brain is changing dynamically, and there may be greater individual variability between immature developing brains, than between adult brains; 3) the first two factors can lead to greater signal variability within a subject, and across subjects; 4) the juvenile bird and infant may have less stable behavioral state changes. For example, in scanning adult birds we determined a dose of diazepam that produced the same effect in most adult birds; in initial attempts to scan juvenile zebra finches, we have found that the effect of sedative is more variable across juvenile birds than adults. Challenges in obtaining and analyzing fMRI data in juveniles are to determine and stabilize the levels of sedation across birds and determine whether data reflect the same behavioral state in juveniles and can be compared to images acquired in adults. In addition, analysis and interpretation of fMRI images in juveniles will require additional imaging techniques to measure and

account for developmental structural changes, such as vascularization, that may influence the BOLD response.

In ERP recordings the primary challenge is structural, the zebra finch skull undergoes a process of pneumatization during development: the extremely thin and soft single layered skull (< 0.5 mm) calcifies and develops into a double-layered air-filled structure with small bony supports (spicules) between the layers (this process starts around 50 days post-hatch). Studies of chronic recording from single and multiple units over development have focused on singing-related firing patterns in song nuclei during the sensorimotor phase of development (approximately 55-80 days post-hatch) (Crandall, Aoki, Nick, 2007). Attempts to record chronically from auditory nuclei in juveniles < 50 days old, as they are acquiring their sensory template (e.g. 35 post-hatch day; Amin et al., 2007), through the sensorimotor phase are generally acute preparations. Our approach to developmental recordings is to obtain all vocalizations (starting with subsong) and all potential global sensory changes throughout song development, and relate these on a developmental time-scale. In addition to modifying electrodes and implantation technique for juveniles, we foresee that the ERP signal may be more variable over time and birds, than the adult responses we have looked at so far.

1.1.4. Comparing responses across vocal sounds of different biological function

FMRI

The BOLD response to vocal sounds was detected mostly at the posterior auditory forebrain. More interestingly, strong differences in BOLD responses were detected in response to different stimuli. Sounds that differ in their biological function, BOS versus TUT, show different BOLD responses. Such differences could potentially reveal brain areas involved in song template (TUT) and in imitation (BOS, assessing auditory feedback), and some of the common activation areas might be involved in sensory-motor integration.

Evoked Response components

Looking at averaged response traces, distinct temporal components were detected in the Auditory Evoked Response to simple and complex stimuli (single click pulse, zebra finch calls, and zebra finch song syllables). We did not attempt to relate zebra finch response components to specific known AEP components in humans, for example “obligatory” cortical evoked components indicate a general auditory response to acoustic stimulation (Luck, 2005). In humans these components are seen as a small positive peak at 50 ms, a large negativity at 100 ms and a large positive peak around 200 ms (P1-N1-P2 complex). Although we did not believe that time scales would be homologous across brains of such diverse architecture and size we do see the general morphology of positive-negative positive (in most electrodes) in a shorter time window (P1: ~30 ms, N1: ~ 50 ms, P2: ~ 100 ms). Perhaps we can interpret this consistent pattern (seen in response to clicks and complex stimuli) as the zebra finch parallel to the obligatory cortical evoked responses in humans. Future studies could compare response

components in songbirds by testing stimulus designs established in humans and mammals to study “low-level” maturation: For example, systematically changing rate of presentation, intensity and frequency of stimuli; recording evoked potentials to song units larger than the syllable or call.

Using complex stimuli (calls and song syllables) we found that Evoked Responses are sensitive to stimulus type: significant differences in the onset response, subtle (though not significant) differences in amplitude of main peak (130-250 ms), and offset response that coincides with stimulus duration. Taken together, these results suggest a link, or coupling, between the sensory-motor song learning period and the shaping of stimulus-specific auditory response patterns to conspecific sounds.

The current findings on birds with different developmental auditory experiences suggest that early auditory experience has a major role in shaping auditory perception. It will be interesting to determine whether there are critical moments of developmental song perception and how these relate to critical moments of song development (e.g. emergence of syllable clusters). Longitudinal fMRI and ERP studies combined with analysis of song development could show if and how sensory plasticity might guide the development of motor skills.

Combining Techniques

These findings suggest that Evoked Potentials in the zebra finch are sensitive to acoustic differences in stimuli and that this method could be a useful tool to study metrics of song and song syllables, and perhaps perception. Rather than look directly at within subject discrimination of sounds, we compared group biases due to auditory experience. Group biases were reflected in the *response pattern* seen across stimuli; i.e. variations in amplitude and anterior-posterior gradient for different stimuli. The methods developed here, e.g., using the F-statistic as a yardstick for estimating stimulus specificity in response patterns, could be used in future comparative studies, or in available ERP data obtained from human.

Lateralization

Overall BOLD amplitude was comparable in medial areas of left and right hemispheres. Stimulus-dependent differences however were only seen in the right hemisphere; BOLD effect was significantly. Stimulus discrimination in the right-hemisphere suggests lateralization of song perception; which complements previous findings of left- or right-sided dominance (depending on songbird species) of vocal function in songbirds (Floody & Arnold, 1997; Nottobohm, 1971; Williams, Crane, Hale, Esposito, & Nottebohm, 1992;). Right-side lateralization for song sensory stimulation has been noted in electrophysiological studies in starlings: right-hemisphere Field L and NCM neurons respond more strongly to biologically relevant stimuli (George, Cousillas, Hausberger, 2005; George, Vernier, Richard, Hausberger, Cousillas, 2004). Most recently right-side lateralization of song discrimination in BOLD responses has been shown in the midbrain of the zebra finch (Poirier, Boumans, Verhoye, Balthazart,

Van der Linden, 2009). Although it is in the opposite hemisphere, another obvious example of lateralization of vocal learning function is the left-hemisphere dominance for language in humans.

1.1.5. Comparing to humans

Can we now compare human and birdsong fMRI and ERP data as a result of this study? No, we cannot directly and usefully compare fMRI activation plots of zebra finch and human brains, but we have taken steps toward a more direct comparison between perceptual development in two groups of vocal learners. ERP responses that are associated with perception and attention in humans and non-human mammals may be easier to compare between humans and zebra finches. For example, obligatory ERP components and an auditory memory component can reflect varying speech perception skills in groups of people with different auditory experiences (Kelly, Purdy, Thorne, 2005).

In sum, we have established methods for recording auditory responses to vocal sounds in the adult zebra finch; the next step is to use these techniques across the lifespan of the animal and detect the emergence of song and feature specific perception in the zebra finch; research that is already underway in humans using these techniques. Together fMRI and ERP provide spatial and temporal information about global brain responses and both techniques can be used for longitudinal studies (multiple times in the same animal) – something that would be difficult to do with humans, and without the level of control on the auditory and social environment that is possible in birds. Cross-fertilization between human and birdsong research could work both by implementing our approach to recording and segmenting vocalizations and observing general response patterns in the human ERP data, and by using human/mammalian approach on songbirds, to detect low-level maturation of responses. In other words, the human speech development research could perhaps gain from more generic analysis of differences in responses to sounds, and the birdsong research could gain from more low level investigation of auditory responses.

1.2. Limitations

Our approach suffers from both technical and conceptual limitations, starting from generic limitations of fMRI and ERP approaches in characterizing neuronal activity at the population level, the difficulties in binding spatial dynamics to temporal dynamics, and the difficulties in relating behavioral development, which occurs over multiple time scales, to the measurements taken in this study on adult birds. The major motivation of this study is to prepare the ground for longitudinal studies – little of this has been done as of yet, and it is still questionable if we will succeed to overcome difficulties involved in both recording and interpreting developmental data at the fMRI and ERP levels. For example hemodynamic response depends not only on neuronal activity but also on arterioles and capillary anatomy and physiology – both might change over development.

The obvious technical limitation of non-invasive recording of brain responses is the lack of microscopic localization of brain activity at the smallest time scales. We see ERPs and fMRI as providing

complementary information to the body of literature that exists in the songbird field on the level of single-unit and multicellular activity, circuitry, and dynamics in the song system.

We hope that simultaneous recording of ERP and fMRI signal can eventually provide an appropriate complement of (non-invasive) spatial and temporal responses, but our current electrode hardware does not allow for Evoked Potential recording in the fMRI scanner. Recently developed silicon electrodes could perhaps solve this problem (Kruggel, Wiggins, Hermann, von Cramen, 2000). Evoked potential response components, and potential brain sources have been well-studied and well-established in humans, and some components have been studied in the small animals (rats – sensory gating); the zebra finch brain is extremely small relative to the human brain and even rat brain, and it is not clear how the brain size interacts with electrode size and location. Because this is an exploratory study with a new technique in this species, we do not have a reference for source-localization, component generators, and additional response component characteristics (amplitude, latency).

We measured the BOLD response in the fMRI studies; changes in hemoglobin are only one type of physiological change that accompanies neural activity; we did not measure Cerebral Blood Flow, Cerebral Blood Volume, or use contrast agent for more detailed anatomical images (Song, Huettel, McCarthy, 2004). In addition, the current 3.0 Tesla machine was designed for human research and does not provide the highest spatial resolution; greater magnet strength in a designated animal scanner would improve spatial resolution of activation.

1.3. Future Directions

1.3.1. *In birds*

Whole brain imaging techniques seek to establish global “neural correlates” of perception (or sensation) of the organism itself, rather than sensitivity of isolated neurons. These techniques, being non-invasive and non-terminal preparations, allow us to look at brain responses of the same bird multiple times, over development. Tracking production and auditory sensitivity on a global level in the zebra finch could shed light on the vocal learning process in general. For example, the juvenile zebra finch sings an unstructured and variable sub-song, which develops into a spectrally and temporally stereotyped song: “song crystallization” occurs (Konishi, 1965; Tchernichovski, 2000). Electrophysiological evidence suggests that single neurons become tuned to song features, but it is unclear whether a general “sensory crystallization” occurs in the brain. It is not known whether a sensory crystallization would precede vocal crystallization, effectively guiding the motor output, or follow vocal crystallization. Both fMRI and ERP approaches allow repeated measurements over development and should make it possible in future studies to look continuously at emergence of auditory responses over development. Further, it should be possible to examine side by side the development of song motor skills when songs becomes more and more structured, with the emergence of structured stimulus specific auditory responses. We could investigate vocal and sensory changes dynamically during development by performing continuous sound recording and analysis, and tracking the entire trajectory of song development. We could compare

changes in recorded song patterns with changes in auditory responses obtained by serial longitudinal fMRI scanning and ERP recordings at critical stages of song development. Stimuli for this would be the bird's own vocalizations produced at the corresponding time point in song development. For example, when a syllable type (cluster) emerges, responses to that syllable type could be compared to other (unclustered) vocal sounds produced at that time point. It would be useful to examine responses to the tutor's song and to non-vocal control sounds (pure tone), as well and in this way we could obtain quantitative correlations between vocal sound patterns and their auditory spatiotemporal (BOLD and bioelectrical) patterns.

1.3.2. *In humans*

Despite many gross differences between human speech and birdsong, in the crudest behavioral description, development of vocal production appears to follow a basic principle of organization, from unclustered to a clustered distribution, and involves changing motor patterns of vocal production for better approximation of an acoustic target. We are not aware of any parametric documentation of vocal development in human that can compare to what we have in the zebra finch. The speech signal is very difficult to segment, vocal samples are collected in various contexts using various methods, and there is no database or analytic framework for presenting a continuous image of pre-lingual vocalizations to speech sounds and words in an individual (reviewed in Hsu, Fogel, Cooper, 2000). Techniques have been developed for analyzing the entire vocal development of songbirds, so as to track vocal changes (e.g., emergence of syllable types) in nearly real-time (Tchenichovski, 2000). These techniques could easily be used to track and quantify infant vocal development on an acoustic level. Near continuous recordings of infant vocalizations in ecological conditions (i.e. day-care, home environment) are feasible to obtain; the automated processing and visualization techniques (used in analysis of song development) would allow for observation of dynamic changes in infant vocalization in nearly real-time. Quantification of vocal development on a large scale would complement what is known about infant speech perception, and would provide insight to the relationship between perception and production in human vocal learning in the first year of life.

1.3.3. *In techniques*

In this study fMRI and ERP were used as complementary techniques but were not performed simultaneously, more complex stimuli were used to elicit fMRI BOLD responses (songs and song syllable stimuli) than ERP responses (song syllables and call stimuli), and the relationship between the temporal signal of ERP and spatial signal of BOLD was not deeply examined (likewise, temporal component of BOLD and spatial component of ERP were not closely examined). We observed similar trends in response patterns of groups of birds with both measures, but being an initial attempt to combine techniques, we did not explicitly study the ERP and BOLD responses as they relate to one another. A number of research directions could be pursued from this point: 1) combine electrophysiological recordings and fMRI simultaneously; 2) combine electrophysiology and fMRI, not simultaneously but, with

standard stimuli, and ability to scan bird with electrode implants (not feasible in current project); 3) improve scan quality and examine additional parameters of fMRI scan: Cerebral Blood Flow, Cerebral Blood Volume; 4) examine additional parameters of electrophysiological activity: source localization of ERP and simultaneous Local Field Potentials.

1.4. Summary

The zebra finch has been used as the animal model for human speech learning because of the shared behavior of vocal learning. Despite the commonalities in behavior it is very difficult to make clear and detailed comparisons between song learning in birds and speech learning in humans. Studying songbirds presents the unique opportunity to look at both perception and production development side by side in a controlled environment, which is not possible in humans. The research described in this dissertation required the successful development of new research methods in the zebra finch and our results included identifying vocal-learning related auditory plasticity in male and female zebra finches. This is of broad interest, because it could be a key to understanding how sensory plasticity contributes to the development of motor skills, and vice versa. This current study should make it possible (in future studies) to combine the tracking of vocal changes (techniques already available) with frequent assessments of sensory plasticity using non-invasive methods. The goal we achieved in this dissertation was to investigate how developmental experience shapes auditory responses in the adult zebra finch. Future studies can now look at this process dynamically and analyze auditory and vocal development side by side. Applying non-invasive neuroimaging technology that is used extensively in human speech research to developing songbirds would allow a closer comparison between neurophysiology of song development, speech & language development, and learning disorders and could facilitate true cross-fertilization between birdsong research and speech development fields.