1. EVENT RELATED POTENTIALS IN THE ZEBRA FINCH

1.1. Why use far-field electrophysiology?

The vast majority of measurements of auditory responses in the songbird brain have been obtained by recording spiking activity of neurons using sharp electrodes. It is sometimes possible to isolate activity from single neurons (single-unit recording), but often, spikes recorded are mixture of activity originating from a few neighboring neurons (multi-unit recording). As technology improved over the last few years, single unit recording in awake behaving birds has become more common, whereas multiunit recordings are considered (at least by some researchers) substandard. However, both single and multiunit recording share a significant limitation: sampling micro-scale activity of one or a few neurons cannot capture large scale patterns of network dynamics. Recording with sharp electrodes can detect neural activity at very high spatial and temporal resolution, but may not capture the global activity elicited by various sounds. Quite often, behavior and perception correlate with global neural activity, as measured by local field potentials (Logothetis, 2002; Stark & Abeles, 2007), scalp or epidural electrodes (Keil, Müller, Ray, Gruber, Elbert, 1999; Rodriguez, et al., 1999).

In humans, non-invasive techniques are often the only option, and therefore methods for recording global network activity in behaving humans has progressed rapidly. As noted, in songbird research, sharp electrode recording techniques have evolved rapidly, facilitated by the accessibility of the song system nuclei for such recordings. On the other hand, recording global brain activation has not been considered necessary, and is potentially problematic due to small brain size (a major issue with fMRI), and nucleated nature of the nidopallium (as oppposed to laminar structure of mammalian cortex). Neurons in nucleated brain structures may be aligned orthogonally in which case synchronous activity of these neurons could cancel locally in which case there would not be a signal for the far-field electrodes to detect.

As with fMRI, the motivation to use the far-field sensors and measure Event Related Potentials is the notion that a comprehensive view of auditory processing and representation might require both short time-scale localized activity (single unit neurophysiology) and summated patterns of activity that emerge at longer-time scales ('global' activity). A comprehensive view of neural activity would also take into account activity patterns that can be correlated with attention and perception (Eggermont, 2001; Makeig, 2002). The current work attempts to complement the vast studies on auditory neurophysiology in the zebra finch (primarily single and multi-unit activity) by recording long-latency and large scale auditory responses in the zebra finch. We use auditory Event Related Potentials (Auditory Evoked Potentials - AEPs) to examine and characterize temporal and spatial features of auditory responses to biologically relevant stimuli (song syllables and calls) and less relevant stimuli (clicks).

1.1.1. *Temporal scales of brain activation*

Single Unit recordings

A highly localized signal that presumably captures the activity of a distinct neuron is called a single-unit recording. A number of techniques can be used to record intracellular activity (whole-cell patch, coltage clamp) and properties of individual ion channels on the membrane (variations of patch-clamp) (Matthews, 1986). But the most feasible and commonly used method of recording single-unit activity *in* vivo is with a sharp (3-10 μm), high-impedance micro-electrode placed in extracellular space close to the neuron cell body (Boulton, Baker, Van der Wolf, 1990; Logothetis, 2003;). Such electrodes record extracellular potentials from one (possibly several) neurons in the immediate vicinity of the electrode tip; these potentials reflect intracellular action potentials, but are much smaller in amplitude.

Despite the excellent spatial resolution in terms of whole-cell activity, single-unit recordings present several technical and theoretical disadvantages. Single-unit activity can be more difficult to isolate and identify than multi-unit activity; spikes from neighboring cells are often detected and adequate interpretation of the signal requires complex post-processing (spike-sorting). Signal from a single unit (or several units) is generally less stable and cannot be recorded for long periods of time (> several hours). Single-unit activity does not give information about integrative processes (i.e. sub-threshold changes in potential), can be biased towards larger cells: large cells are easier to identify and isolate (i.e. large cells: 20-30 μm diameter generate potentials up to 100 microvolts), and subsequently biased towards specific cell types (i.e. large pyramidal cells that project cortical output) (reviewed in Logothetis, 2003). The majority of animal electrophysiology reports extracellular single-unit or multi-unit recordings; the ability to use this recording technique in animals is a primary advantage of animal-based experimentation and research.

Multi-Unit Activity and Local Field Potentials

Low-impedance microelectrodes with a slightly wider tip (15-30μm) measure the sum of potentials generated by multiple neurons in a particular area.. The extracellular field potentials (EFPs) detected from populations of neurons can reflect fast spike activity of hundreds of neurons as well as subthreshold, integrative, and slow changes in field potentials from contributions of thousands of neurons. These two types of activity (fast multi-unit spikes and slower regional activity) can be acquired in the same recording and distinguished by band-pass filtering: multiunit spike activity > 300 Hz (preferably > 800-1000 Hz), local field potential activity < 300 Hz (Eggermont and Ponton, 2002).

Multi-unit activity

Multi-unit activity (MUA) involves using high-impedance microelectrodes to record fast-spike activity from single or mutliple neurons. This signal provides good spatial and temporal resolution of neural activity, and primarily reflects synaptic events (i.e. action potentials, activity >800 Hz). Because

of the high spatial resolution of MUA, information in this signal is naturally restricted to the receptive (firing) properties of the single cell or a small group of cells and does not reflect associative activity (or activity from combined receptive fields) that occur at slower time-scales (<300 Hz) (Logothetis, 2003).

Local Field Potentials

As the sensor is moved further from the neural source electrical potentials from the source integrate, and in addition to spikes, much slower and weaker changes in membrane potentials (due to EPSPs and IPSPs) can be observed. These slower changes are often synchronized across neurons and sum to form a signal that spans many frequencies. Very high frequencies of this signal die quickly and relatively locally, but lower frequencies with longer wave lengths can propagate longer distances through extracellular space. Moving further away from the source towards the surface of the brain, LFPs are no longer detectable (from the source) but large scale brain potentials, Electrocorticograms can be detected on the brain surface. Electroencephalogram (EEG) and Event Related Potentials can be recorded by detectors on the skull, at such distances it is often no longer possible to localize the source of the neural activity being recorded (Luck, 2005).

Neural activity or events that occur at frequencies lower than spike activity reflect inetgrative processes and associations between cells, i.e. after-hyperpolarizations which tightly modulate excitability of cells and occur on the order of 10s of milliseconds (Kim and McCormick, 1998). These signals reflect larger populations of neurons; amplitude does not directly reflect amplitude of individual spikes, but rather weighted average of synchronized slow components of synaptic events (i.e. dendrosomatic, rather than axosomatic signals) (Logothetis, 2003). Because the LFP signal is not a direct measure of spike event, spike amplitude does not bias signal. LFP activity reflects density and orientation of cells, current fields in extracellular space are recorded; and each neuron contributes to the field signal; during firing each neuron acts as a simple sink and source, and creates an electric dipole. In LFP recordings dipoles are easily detected; as electrodes are moved further from the source of activity detecting neural generators of activity, or dipole localization, becomes much more difficult.

As noted above, the LFP signal might mirror slow-events that are associated with spike signal, but are not the spike potential itself; for example, slow afterhyperpolarizations, on the order of 10s of milliseconds. The frequency of these signals coincides with EEG frequency bands <100 Hz, and the LFP signal is correlated with aspects of the EEG signal (Logothetis, 2008). EEG band classification (delta 0-4 Hz, theta 4-8 Hz, alpha 8-12 Hz, beta 12-24 Hz, and gamma 24-80 Hz) is based on signal amplitudes (and shape) and specific frequency bands relate directly to behavioral state (Key, Dove, The EEG and LFP signals, with a lack of spatial resolution, provide a wider lens on brain activity, one that can be related to processing and perception at the level of the organism.

The wider spectrum afforded by EEG and LFP signals bring additional problems: measuring neural signal from a distance requires that signal travel from source to sensor. Certain assumptions

are made about extracellular space in local and far-field electrophysiology recordings. The extracellular space acts as a volume conductor with certain properties: 1) signal can travel long-distances through extracellular space, propagation of signal is independent of frequency; 2) extracellular space provides resistance (neuron activity can be viewed as single source); 3) resistitive quality of extracellular space is isotropic (Logothetis, 2003;). These properties simplify detection and quantification of recorded signal (summation signals) but specifics of the extracellular space - resistance of the volume conductor and ultimately features of signal detected – depend on configuration of neurons in the brain tissue stimulated (i.e. open vs. closed field, neuron orientation, and dipole properties), which may vary between species, individuals, and different regions of the same brain.

Long latency evoked potentials (ERPs) and Oscillatory activity (EEG)

Large scale brain potentials are collective contribution of hundreds to thousands for neurons with synchronous activity (Eggermont and Ponton, 2002). These are measured by far-field sensors, on the scalp or dura and the activity recorded at this level has been linked to behavioral state, cognitive processes in humans, and perceptual processing in humans and animals. Two aspects of far-field brain potentials have been explored: signals time-locked to specific sensory events (Event-Related Potentials) and ongoing oscillatory Electroencephalogram brain activity Pfurtscheller and Lopes da Silva, 1999). Both of these signals can provide information about high-level sensory processing and perception.

Human auditory global responses assess different auditory processes – frequency selectivity, attention, and orientation. Several middle and long-latency evoked potential components in rats and monkeys parallel human potentials: sensory gating: human P50, rat P15; acoustic change detection: humans N100, rat N50 (Knight, Brailowsky, Scabini, Simpson, 1985). Our aim is to explore response properties of AEP's in the zebra finch – shape, size and consistency of components - and determine the temporal components of the auditory evoked responses (long-latency?) and whether the responses are sensitive to stimulus type and duration.

In sum, advantages and disadvantage exist within both microscopic and macroscopic methods of measuring neural response and neither method provides adequate information for quantifying perception of the stimulus. Microscopic methods of single-unit recordings give high spatial resolution but often cannot capture larger scale patterns, which drive perception and behavior. Macroscopic methods provide information about processing of the stimulus over time but lack spatial resolution. A quantitative analysis of sensory "perception" in the auditory system requires obtaining information across levels of physiological responses to stimuli (Eggermont, 2001; Makeig, 2002).

1.2. Methods of AEP procedures

1.2.1. *Surgical Procedures*

In the following experiments adult birds were implanted with an epidural electrode array forming an electrode cap. Electrode implantation in juveniles required modification of the following procedure. Epidural electrode arrays were constructed several days prior to the implantation, and consisted of copper alloy pin electrodes (0.5 diameter) mounted on a plastic substrate (we used conventional machine interconnect strips, adapted from (Espino, Lewis, Rosenfield, Helekar, 2003). Three electrode pairs (6 electrodes) were implanted in each hemisphere at different positions along the coronal plane (Figure 1). One single pin was implanted above the cerebellum as a reference electrode. Electrode pins were coated with epoxy so that copper alloy was exposed only at the tip. Pins were individually cut to different lengths to conform to the skull curvature.

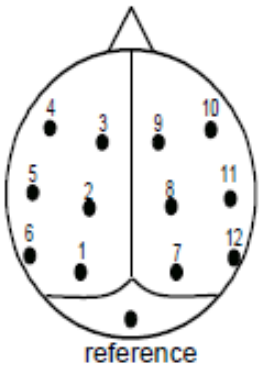
If the bird was not already isolated in a sound recording box, it was isolated twenty-four hours prior to implantation, and food and water were restricted 2 hours prior to implantation. For the electrode implantation surgery the bird was anesthetized with a Xylazine-Ketamine mix (16.25 mg/ml and 8.12 mg/ml respectively in normal saline solution) i.m. and mounted in a stereotaxic device. The bifurcation of the sagittal sinus was used as a reference point (0mm anterior, 0mm lateral) to mark the skull at the following coordinates on the right and left sides (Figure 3-1): Posterior medial: 0.0mm (anterior) 2.0 mm (lateral); Posterior lateral: 0.5mm (anterior), 4.1 (lateral); Medial medial: 2.7mm (anterior), 1.8mm (lateral); Medial lateral: 3.2mm (anterior), 3.9mm (lateral); Anterior medial: 5.0mm , 1.1mm; Anterior lateral 5.5mm, 3.2mm; cerebellum: -2.0mm, 0.0mm. Both layers of skull were perforated at these coordinates using a 27g surgical needle (B-D medical). The bottom pins of the electrode array were inserted into the holes until they touched the dura, and the plastic substrate rested atop the skull. Dental cement (Tylok Plus – Fisher Scientific) was used to secure all electrodes and formed a permanent electrode cap on each bird's skull.

Post-operatively birds were injected with Yohimbine (0.49 mg/ml in normal saline) i.m. to hasten the return to wakeful state. Birds were monitored for several days after the electrode implantation and all birds used in these experiments exhibited normal behavior: singing, perching, eating, and hopping around the cage. The first auditory evoked potentials for each bird were recorded at least seven days after electrode implantation.

Figure 1-1

Electrode Configuration. A. Twelve epidural electrodes were implanted across the skull in pairs along the coronal plane. One electrode was placed over the cerebellum as a reference. **B.** Electrode cap was secured with dental cement. Implanted birds showed normal hopping, singing, and feeding behavior.

A



B

1.2.2. *Recording Procedure and parameters*

Lead wires connect the female side of the six-unit pin connector to the amplifier input. The amplifier-electrode connector fits tightly onto the epidural pin electrodes, but can be removed from the pin electrodes with a gentle pull. The permanent epidural electrodes and removable electrode connector allow us to record auditory evoked potentials at different times in the bird's life without additional invasive procedures. Also, the permanent electrodes and tight connector reduce movements between the bird and the wires and electrode, therefore minimizing movement artifacts in the signal in the unrestrained bird.

Twelve channels of a sixteen channel single-ended DC bioamplifier (Bioelectric Amplifier, SA Instrumentation, Inc.) were used to record evoked activity. Responses were amplified using a x10,000 9-Volt battery-powered Bioelectric amplifier and the analog signals were filtered (0.1 – 1000 Hz) prior to sampling at 5kHz (Data Acquisition Card - PCI-DAS1602/16). All data was acquired using the Matlab data acquisition toolbox and software written in MatLab (S. Helekar and K Maul). The stimulus was sent as a stereo signal to the soundcard (see Figure 3-2). The left channel signal was a short pulse (20 μsec) sent to the speaker and used as the acoustic signal. Due to digitization and downsampling the short duration acoustic stimulus could not be used for trial-alignment. A pulse "marker" with identical onset time to the stimulus, but longer duration (3 ms) was created in the right channel, and was recorded in the PCI as analog data along with the analog signals from electrodes. Recordings were aligned to stimulus onset, via the pulse marker recorded directly into the PCI card.

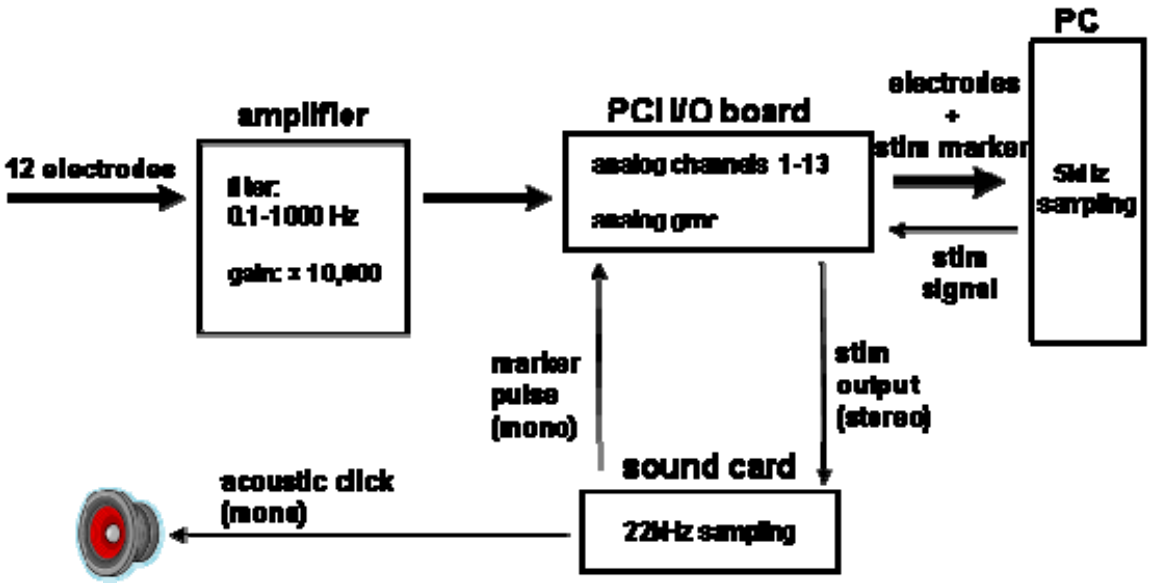
Auditory stimuli were delivered in free-field using a small speaker (KFC-1368S), located approximately 20cm above and perpendicular to the birds head. The sound pressure level of the auditory stimuli at the head position was adjusted to 75 dB. To reduce movement artifacts during recording, birds were placed in a partial restraint (plastic and velcro) and then birds were placed in an electromagnetically shielded and sound-attenuating chamber and electrodes were connected to amplifier leads.

Stimuli were presented at a rate of one click (or one syllable) every three seconds. One block of 100 syllables stimuli were presented each recording session. For each bird, stimulus blocks were

presented consecutively, with 5 minutes of silence before each new stimulus block, and were limited to 5 or fewer blocks per day.

Figure 1-2

Data acquisition setup. An open data acquisition system was used – hardware components were obtained and installed individually (PCI analog/digital input-output card, sound card, bioelectric amplifier) and Matlab software was used to access and program data acquisition components.



1.2.3. *Auditory Stimuli*

Clicks

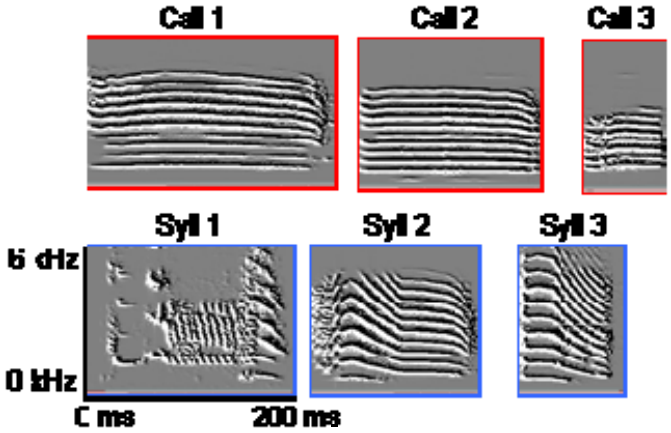
Click pulses were 20 µsec in duration (created in GoldWave sound editing software) and played back at 75 dB at a rate of one click every 3 seconds.

Syllables

Stimuli were naturally produced female calls and male song syllables, produced by birds raised in a colony environment. Calls and syllables ranged in spectral complexity (downsweeps, frequency modulation) and duration (90-250ms), Figure 3-3. These were adjusted to peak amplitude.

Figure 1-3

Natural Stimuli. Female calls and male song syllables vary in duration, spectral complexity and social relevance.



1.2.4. *Birds*

All birds hatched in the City College aviary. For click stimuli normally trained male birds were used. The experimental protocol for training birds is as follows: between day 4-7 post-hatch the father is removed and the cage is taken, together with the nest and the mother, to a colony room occupied by females and chicks only. This procedure prevents early exposure to songs, as zebra finches have elevated hearing thresholds prior to day 10 post hatch while their auditory system is still immature (Amin, et al., 2007). Chicks are raised by their mother, who does not sing, until day 29 post hatch. From day 30 and on males are kept individually in sound-attenuating chambers. Each bird used in the click auditory evoked potential experiments was trained with one of three song models, from day 43 post hatch until day 90 as described in Tchernichovski, et al. 1999. Briefly, we placed two keys in each box, and the birds learned to peck the keys to trigger a brief (about 1.5 sec) song playback. Each bird is exposed to only one song model, repeated without variation throughout development. Such song playbacks are sufficient to reinforce key pecking, and induce significant imitation of the playbacks, in all birds. We limited playbacks to 20 per day, divided to morning and afternoon sessions. On day 90 post hatch, we removed the keys and stopped the training. Birds were kept isolated in the sound chambers without training for at least 3 weeks (and usually longer) before we measured auditory responses. All auditory responses were measured in adulthood between ages 4-11 months post hatch.

In natural stimuli experiment (calls and song syllables) trained birds and three additional groups were tested: Colony males, isolated male birds, and isolated female birds. Trained birds were raised as described above. Colony males were randomly selected from the City College zebra finch colony, where birds are kept in family cages of one reproductive pair and their offspring (of up to 10 birds per cage). These birds are exposed to a rich auditory and social environment. Isolated female and male birds were raised as in the training design (described above) but were *not* given the

opportunity to peck on keys for song playback, and were not exposed to any song during development.

1.2.5. *Data Analysis:*

ERP Post-processing:

Data was acquired trial by trial, i.e. separate file for each stimulus presentation; each data file contained 12 channels of biological signal, plus one channel for stimulus marker. Each trial was partitioned into segments (epoched) of 2.4 s, with ample pre-stimulus baseline, and processed as follows: median subtraction, notch-filtering (2^{nd} order Butterworth filter, 58-62 Hz), artifact-rejection (1.5 standard deviations from noise floor), and down-sampling to 500 Hz. This initial processing resulted in a single data file that contained all accepted trials, all channels, for the specific bird and stimulus. The 3 ms pulse marker was used to align recordings to stimulus onset. Event related potentials (ERP) were then obtained by averaging across trials for each bird and each stimulus.

Features of evoked response to natural stimuli:

ERP time course: To determine the overall time course of the evoked activity we computed ERP *power* as the square sum across electrodes for each sample.

ERP spatial distribution: Visible amplitude and polarity differences were noticed in anterior-posterior electrodes. This motivated us to use the potential difference between rostral electrodes and caudal electrodes as a measure of ERP amplitude. This captures the average activity over caudal areas (coinciding with NCM/Field L) with the most distant (rostral) electrodes as reference. To quantify the strength and predominant distribution of the evoked activity principal component analysis was performed. The first (spatial) principal component of the evoked potentials computed using all time samples, birds, and syllables indicated a rostral-caudal response gradient (explaining 74% of the variance in the ERP) with left-right symmetry.

Event-related de-synchronization: To quantify potential effects on oscillatory activity we computed trial-averaged spectrograms of the EEG in the mean across electrodes. This is the conventional approach used in EEG studies to asses the strength of oscillatory activity. We observed in the average over syllables a prolonged post-stimulus decrease in power (0.2 – 0.8 Sec.) in the 4-16 Hz frequency band. In humans this is commonly referred to as event-related de-synchronization.

1.3. Results

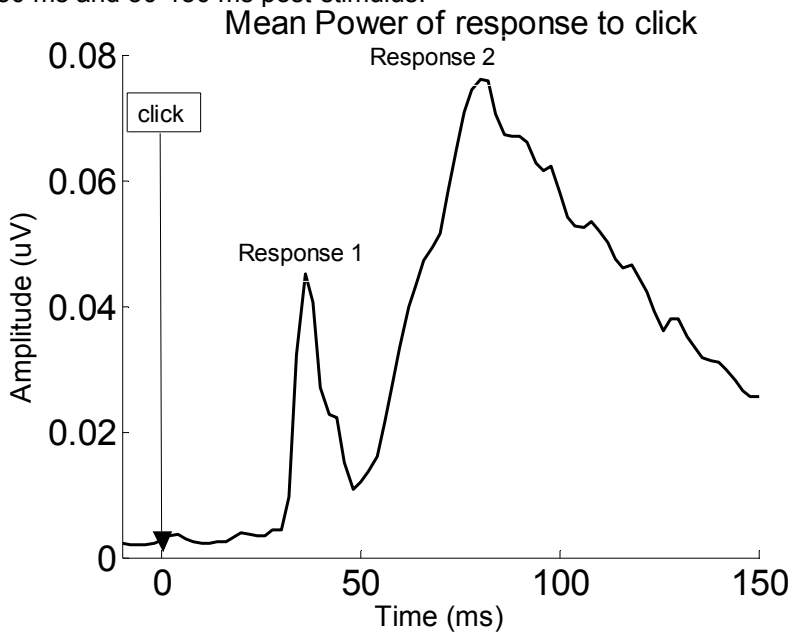
1.3.1. *Responses to clicks*

Single click pulses elicit long-latency auditory evoked potential responses that have multiple temporal components which are relatively stable across birds. Evoked response power averaged over electrodes and birds has two distinct response components: a low amplitude response prior to 50 ms post-stim, and broad, high-amplitude peak between 50-120 ms (Figure 3-4). These two temporal components were observed in every bird; the first response was more consistent across

birds with less variation in amplitude and absolute latency. The second response component was variable in amplitude across birds,

Figure 1-4

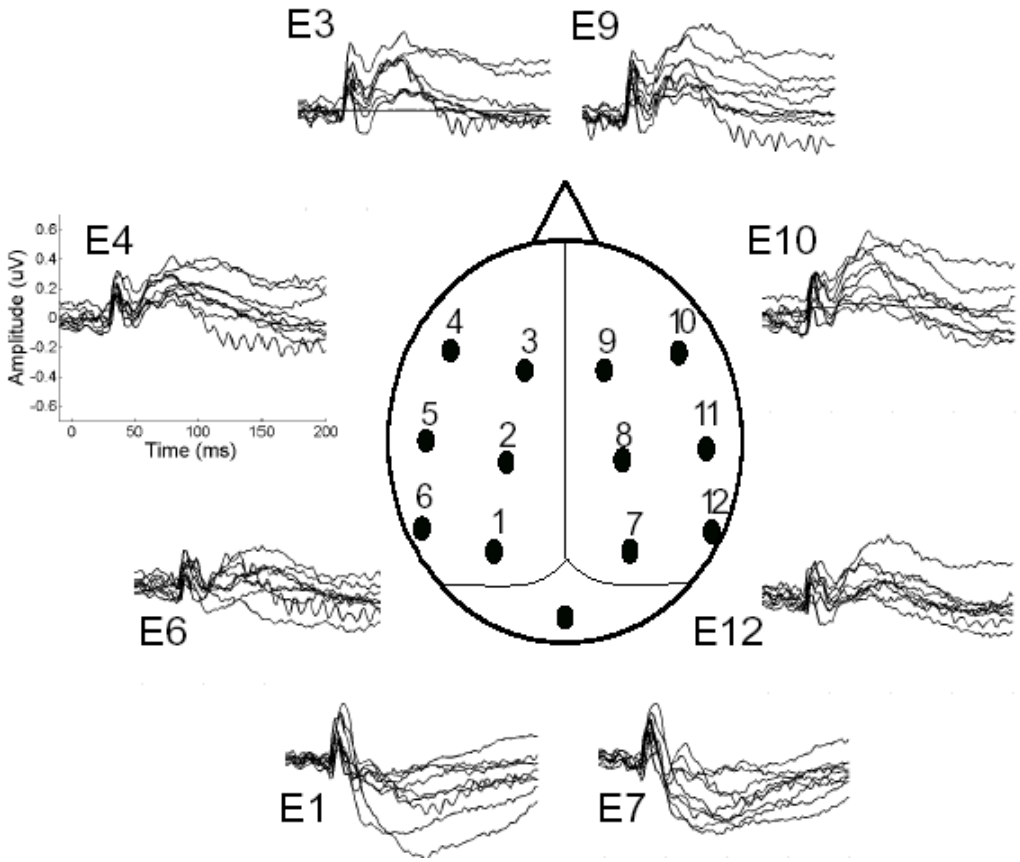
Temporal components to click response: Average Mean Power. Mean power for each bird across electrodes (n=11) and averaged across birds (n=9). Two response components are seen: 25-50 ms and 50-150 ms post-stimulus.



Two response components were seen consistently in electrodes across the dura (Figure 3-5), but initial component had a more stable distribution, a consistent, small positive peak. The second component varied in amplitude and polarity, with the posterior-medial electrodes showing the largest amplitude deflection negative, rather than positive. These two temporal components (1st peak and 2nd peak) were consistently seen in all birds and in all electrodes but one (in one bird anterior-lateral electrode 10 did not provide signal). Based on visual inspection, absolute peak amplitudes and latencies of the two components varied across birds and electrodes; between birds there was more variation in amplitudes than latencies (i.e. temporal conserved across birds), and generally greater amplitude variation was seen across birds than electrodes.

Figure 1-5

AEP response topography. Overlaid responses of 9 birds: Peak 1 is preserved in direction (positive) and amplitude across electrode locations. In posterior-medial electrodes Peak 2 is inverted and amplitude is greatest; peak inversion is detected in all birds.



1.3.2. *Response components to natural stimuli*

Syllables

ERP responses to syllables and calls are similar in initial components to the ERP responses to clicks, with an additional later component. The ERP power, when averaged over birds and syllables, showed multiple response components in time (Figure 3-6 shows the root mean square in μV): onset response at 0-40 ms; a main peak between 40-120 ms; offset responses between 120-350 ms; and a weak prolonged response from 350-1000 ms. Offset components correspond to the duration of the stimulus.

Figure 1-6

Power of evoked response to natural stimuli. Power averaged over stimuli and bird groups for four groups of birds (root-mean-square of the ERP for each group with mean over electrodes and syllables). Four distinct response components were observed: (**A**) onset response: 10-40 ms post-stimulus; (**B**) main peak of response, 40-120 ms; (**C**) offset response: 120-350 ms; and (**D**) weak prolonged response: 350-~1000ms. The onset component of the response provided stimulus specific information; additional components confirmed group differences.

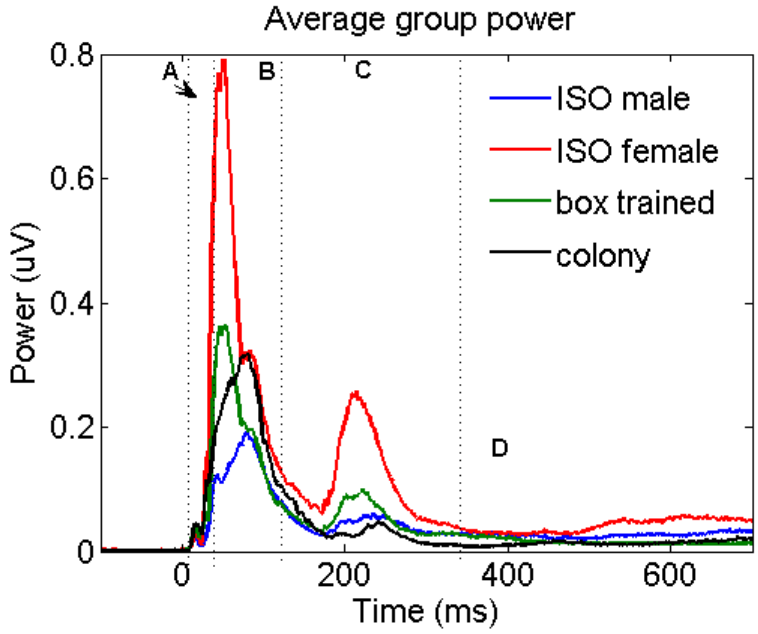
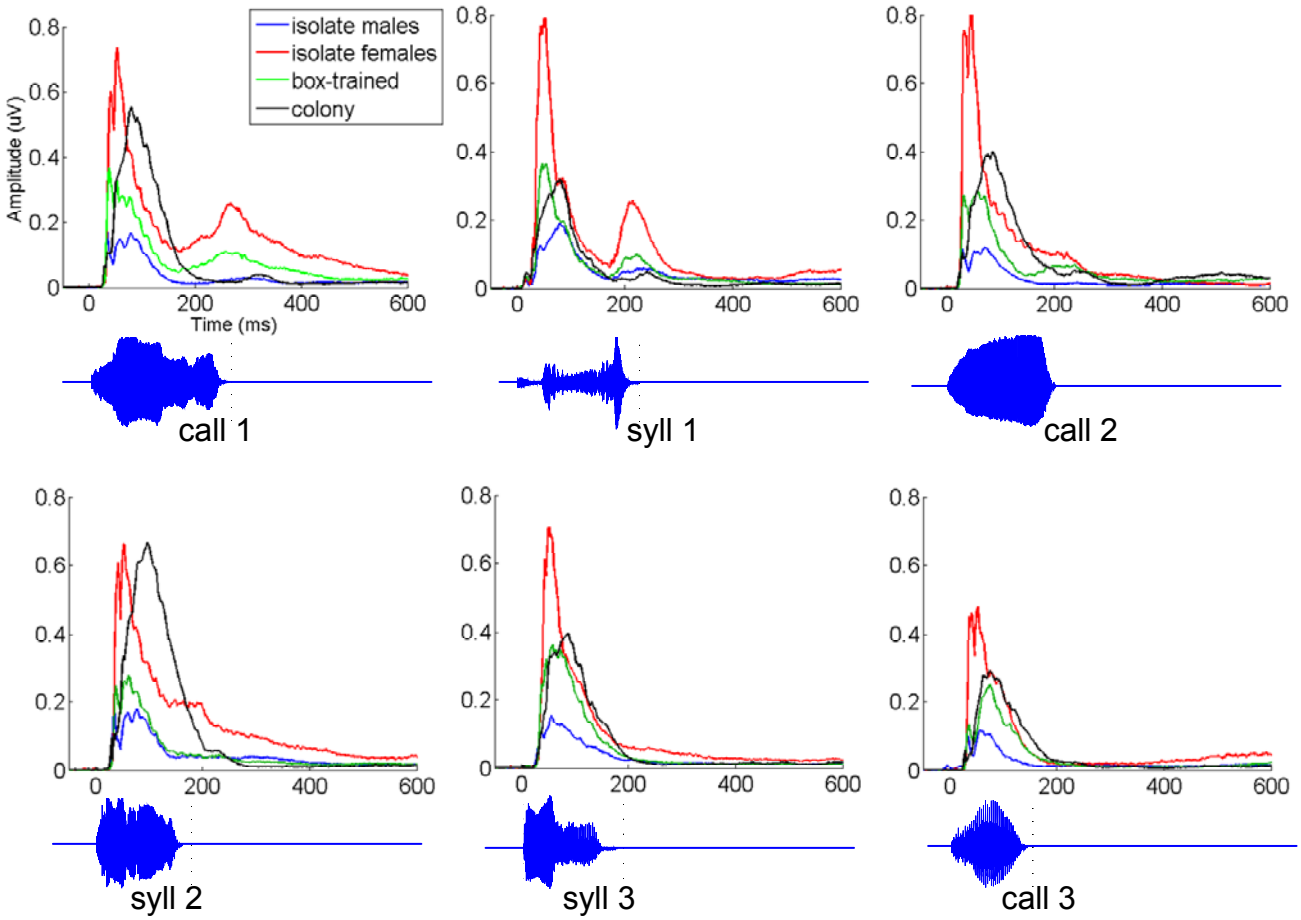


Figure 1-7

Evoked potential offset responses. Natural stimuli: female calls and male song syllables, sorted by length. Offset response is visible for longer stimuli (>150 ms), but is hidden in main peak for shorter stimuli.



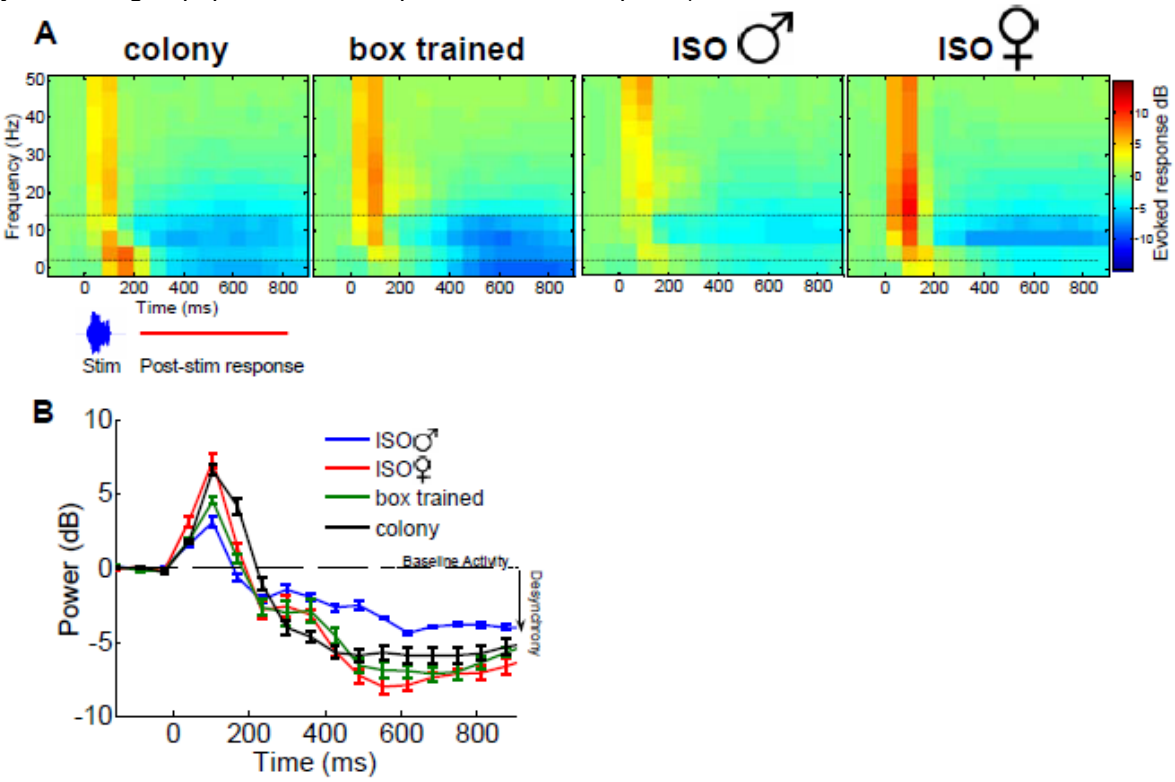
Temporal components of the response were seen within the duration of the response: onset, middle peak and offset response (10-250 ms after stimulus onset). However, the evoked response continues 100-200 ms after stimulus onset and a more subtle response may be seen hundreds of milliseconds after stimulus presentation (Espino, et al., 2003). In this longer post-stimulus period we found a decrease in oscillatory activity as compared to the pre-stimulus activity. Figure 3-8 shows trial averaged spectrograms of the ERP in the mean across electrodes. The initial peak reflects the broadband evoked response to the stimulus (0-200 ms). Following stimulus presentation, oscillatory power decreases in the 4-16 Hz frequency band (300-800 ms post-stimulus). A similar effect has been documented in humans: alpha activity (10 Hz) is reduced during and after engagement in perceptual and motor tasks (Stam, 2000). This is often referred to as “event-related desynchronization” (Pfurtscheller and

Lopes da Silva, 1999): attending to a stimulus and engaging in a perceptual task is thought to reduce large scale synchrony which is reflected in decreased oscillatory electrical potentials.

Although the decreased power in the 4-16 Hz frequency band was not sensitive to different stimuli (no significant stimulus-specific response patterns), when averaged over stimuli the desynchrony effect was markedly weaker in male isolates as compared to all other groups (Figure 3-8). This group difference was confirmed by a 2-way ANOVA: group, $p < 0.01$; stimuli, $p = 0.7$; interaction, $p = 0.9$.

Figure 1-8

Post-stimulus de-synchrony in EEG alpha activity . (**A**) Trial-averaged spectrograms of the EEG across electrodes. Spectrograms, averaged across stimuli, show decreased power in the 4-16 Hz frequency band (between dotted lines) approximately 200-800 ms after stimulus onset. This effect is weak in isolated males compared to all other groups. (**B**) Mean power in the 4-16Hz band: there is significantly less decrease in power (desynchrony) in the isolate males compared to all other groups (2 way ANOVA: group, $p<0.01$; stimuli, $p=0.7$; interaction, $p=0.9$).



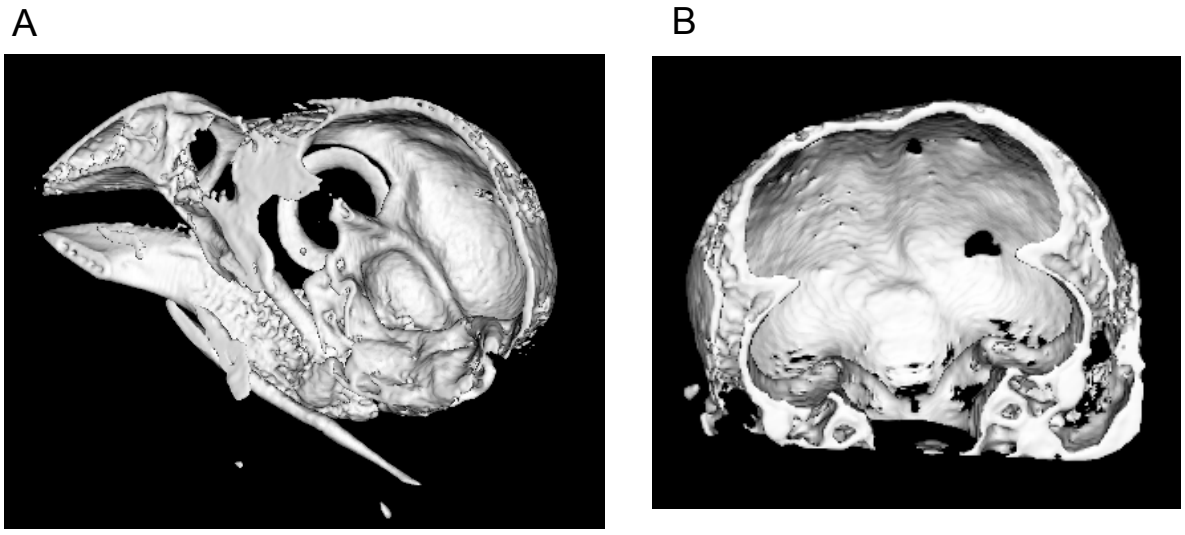
1.3.3. *Source localization*

Ideally we would look at global neural activity in response to auditory stimuli and simultaneously observe the local neural generators of such activity. Source localization of evoked potential signals is a difficult problem even in the human literature, where parameters of electrode type and location are standardized and shape and size of the skull, volume conduction in the brain and anatomical landmarks have been established. Major efforts for dipole fitting of electrical sources in the human brain have made source localization tools relatively accessible to researchers using event-related potentials on human

subjects (e.g. open-source EEGLAB, BESA, LORETA). Such tools (in the present form) do not accommodate physical parameters and characteristics of the zebra finch brain. The geometry of the zebra finch skull and brain and the electrode configuration that we used present challenges for accurate dipole localization: 1) zebra finch skull is elongated (elliptical) and half-open (Figure 3-9); and 2) electrodes are located on top of the head. Dipoles are easier to fit on a spherical model with detectors that pick up signal in multiple planes; using a spherical approximation of the zebra finch brain (ignoring elliptical shape and open bottom) would provide a rough estimate of source location, but low-accuracy dipole fitting may not be useful given the small size of the brain (diameter: 1-2 cm).

Figure 1-9

CT scan illustrating complex geometry of zebra finch skull. **A.** The zebra finch skull is elliptical with an open-bottom and two large frontal indentations with open centers (orb for eyes). **B.** View of the internal posterior skull; electrodes were located on in the axial plane on the superior part of the brain (epidural) in a 2X3 configuration seen in indentations and holes. CT scan images created by Luis Cardozo, CCNY, BioMedical Engineering, used with permission.



1.4. Discussion – AEP in zebra finches

Click stimuli elicit long-latency auditory evoked responses, 30ms-300 ms. Two main components were observed in the averaged power: peak between 10-50ms and 2nd peak centered on 100 ms. In the evoked potential responses the first peak (15-30 ms) shows uniform polarity across all electrodes and birds. This uniformity suggests a spatially symmetric deep central source, which may be the same for all electrode locations. The brainstem could be the source of these uniform response components within the first 30 ms post-stimulus. The 2nd peak is relatively uniform in latency and variably negative or positive depending on electrode site. In the posterior medial electrodes of both the left and right hemispheres the broad 2nd response (50-150 ms) is a deep negativity between 30-130 ms. Differences in polarity of the

2nd peak detected on the dura suggest a superficial or asymmetric source or multiple sources for this component and reflect an anterior-posterior potential gradient. The posterior medial electrodes are in closest proximity to Field L, but it is not presumed that the signal recorded in these electrodes is a direct measurement of the auditory response from Field L.

Results indicate that zebra finch auditory evoked responses to song syllables reflect acoustic information in the syllable; stimulus type and stimulus duration. Further studies could investigate the relationship between specific acoustic features, such as mean pitch and frequency modulation, and temporal response patterns. We are also interested in determining whether the evoked response is sensitive to subtle changes in these acoustic features. Stimulus-specific temporal response patterns may be a global representation of the auditory signal.