Designing an experimental apparatus for auditory functional MRI studies in the zebra finch model

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DESIGNING AN EXPERIMENTAL APPARATUS FOR AUDITORY FUNCTIONAL MRI STUDIES IN THE ZEBRA FINCH MODEL

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Submitted in Partial Fulfillment of the Prerequisite for Honors in Chemistry

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Abstract

The Zebra finch songbird (*Taeniopygia guttata*) shares many developmental, physiological, and genetic characteristics of auditory and vocal function with humans. For this reason, song birds are an ideal model for studying language learning and memory. While many experimental methods exist to study neural development and function in songbirds, few offer the flexibility of longitudinal studies, which is vital for studying time-dependent processes like learning and memory. Functional magnetic resonance imaging (fMRI) is a non-invasive technique that allows for longitudinal studies of neurophysiology.

The focus of this project is to develop and test a functional MRI experimental apparatus to perform future studies on song learning and memory in the zebra finch model. Developing this experimental paradigm has encountered many roadblocks because of the many limitations that zebra finches, and our fMR instrumentation introduce.

Having previously developed the appropriate imaging sequence and technique to acquire fMR images, the next steps in this project were to develop an effective system for sound delivery into the MR instrument. Here, we discuss the process and challenges in developing a sound delivery system that included design and construction of MRI-compatible speakers, an acrylic bird bed to facilitate sound delivery, and a trigger system to synchronize auditory stimuli with the fMRI scans.

In testing this apparatus, it was found that noise and sound distortions associated with MR imaging poses a big problem for effective sound delivery. Further testing is required to confirm the success of the developed apparatus. Other work in this project includes development of data analysis parameters using Statistical Parametric Mapping (SPM) to preprocess and process functional images for experimental analysis.
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Introduction

Background

Understanding the cognitive pathways and mechanisms involved in human speech and auditory recognition calls for the analysis of an overwhelming number of facets of neural plasticity. Anyone who has attempted to study a foreign language can attest to the daunting task of learning, recognizing, and reproducing meaningful speech. Despite this, almost all humans are able to acquire vocal and auditory fluency and flexibility over the course of young childhood. These skills become invaluable in terms of an individual’s ability to communicate with others and integrate his or herself into modern society. As such, complications affecting speech and auditory ability such as learning disabilities, injury and disease can be devastating to a person’s way of life. As with the study of all complex processes, pedagogy and experimental design have shown that the key to understanding complex processes is to model them with similar and simpler situations. In the case of the neural representation of vocal and auditory ability, the study of songbirds has been shown to be a valuable model. In infancy, songbirds exhibit vocal learning through the development of song recognition and vocalizations as first presented by parents (Kuhl, 2003). This shows that an observable behavior change, such as the ability to learn and present vocalizations throughout maturation, can help us better understand certain aspects of neural plasticity and learning.

While there are several differences between both the learning process and vocalization use in songbirds and humans, there are several parallels between these two strata of organisms that rely on higher order auditory and vocal function.
For example, both children and young songbirds exhibit a sensitive period during which they have the greatest propensity for developing vocal language (Doupe, 1999). Additionally, the learning of the two groups during this time heavily depends on their auditory exposure and ability to give learning feedback through vocalizations (Kuhl, 2003). These similarities in the age and quality dependency suggest that the learning mechanisms stem from a similar cognitive process (Wilbrecht & Nottebohm, 2003). On a more anatomical level, there is evidence that sound production and auditory processing in the songbird brain is lateralized, similar to the hemispherical organization of Broca’s and Wernicke’s areas in the human brain (Voss, 2007). Evidence of these specialized neural substrates in humans further speaks to the value in using songbirds as a model for human auditory learning and processing. Most of the information that we have been able to learn about human audition and vocalization has been a result of permanent, lesion or accident based occurrences. Additionally, most techniques used to study and map brain function involve invasive, unethical, and often terminal techniques that would not be suitable for a human sample (Maul, 2009). The use of songbirds for this field of study thus becomes even more ideal because of our ability to manipulate and control the songbird environment, in addition to the ability to sample a large number of birds and use a wider range of techniques.

Thus far, the two methods that have been most extensively implemented in the study of brain function in small animals are electrophysiology and immediate early gene expression, both of which meet a diverging set of specifications for different types of neurological studies. Electrophysiology, for example, has the ability to obtain feedback from the relevant neurons in a matter of milliseconds, which proves to be a very valuable tool for studying rapid neuronal
changes in response to learning. Despite this capability, the technique is limited to measuring feedback from individual cells thus making it difficult to get a holistic view of these changes in response to stimuli (Van Ruijssevelt, 2012). Immediate early gene expression, by contrast, is able to assay a response from as much or as little of an area as necessary; however, data can only be obtained in a matter of minutes to hours after stimulation because of the necessary extraction and treatment of the brain tissue. Additionally, as a terminal technique, it is impossible to perform longitudinal studies on an individual subject (Van der Linden, 2009).

Thus far, these techniques have been able to provide preliminary information on the regional physiology of song learning and vocalizing systems. However, auditory learning studies demand the flexibility of regional specification and longitudinal study due the plasticity and regional organization associated with neural systems. Functional Magnetic Resonance Imaging (fMRI) may prove to be extremely useful in implementing these studies, as it provides whole brain data, with regional specificity through non-invasive means that allow for cross-sectional and/or longitudinal studies.
Figure 1 shows commonly studied regions of the zebra finch brain that are involved in its auditory and song functions. Because applications of techniques like immediate early gene expression, and even initial use of fMRI, have limited research to targeted, region-hypothesized study, much of the published data overlap in regions of interest based on previously known areas of auditory learning in the zebra finch. Studies using immediate early gene expression have suggested that the caudal pallium contains the neural substrate for tutor song memory (Gobes, 2010), and that the HVC in a songbird is linked to learning his own song (Bolhuis et al., 2012). This technique has also been used to demonstrate left-sided dominance in song learning, similar to hemispheric dominance seen in human brains (Moorman et al., 2012).

Functional MRI experiments agree with data from immediate early gene studies, including the hemispherical lateralization of auditory systems (Poirier et al., 2009). Additionally, more regional-specific data have been produced with this imaging technique. For example, Voss et al. (2007) were able to demonstrate a spatial song coding in the songbird forebrain.
Functional MR imaging has also produced data with more detailed spatiotemporal activation. In previous immediate early gene studies the HVC was suggested to be a dominant region in “own song” learning. However, functional MRI studies have shown additional “own song” activity in the dorsal NCM (Poirier, 2011). Since this technique provides whole-brain data, it has also allowed for organizational analysis of the neural auditory system. Boumans et al. (2008) produced data suggesting forebrain organization based on measured functional activity intensity rather than regional selectivity, which appeared to be contradictory to many previous selectivity-based studies.

The ultimate goal of this project is to use fMR imaging to perform longitudinal auditory studies on juvenile zebra finches isolated from song. We expect that these studies will allow us to begin to characterize the regional organization and plasticity in the brains of these birds at their first meaningful auditory experience, and throughout the maturation of song learning. First however, we must develop and validate a fMRI apparatus. Initially, this task may seem to be very simple. When we observe MR imaging in a clinical setting, we lose sight of the intricacy and nuanced nature of the technique: we lie in a large magnet, someone presses a button, and are given an incredibly well-resolved image of the body. The external simplicity and comprehensive result speak to the eloquence and power of this method, which have allowed it to become a widely used diagnostic tool. Beneath the automated protocols we see in hospital settings however, the theory and instrumentation involved in this process are in fact quite complex. Thus, the initial task of method development quickly becomes complicated when assessing the modifications necessary for auditory studies and small animal imaging. In order to
understand this holistically, we must first review the theory and current work in magnetic resonance imaging and functional auditory studies.

**MRI Theory**  
*(Hornak, 2011; Sing & Neutze, 2012)*

Magnetic Resonance techniques are dependent on the quantum mechanical property of spin angular momentum. Every electron, proton, and neutron in an atom has a spin quantum number, either expressed as \( \frac{1}{2} \) or \( -\frac{1}{2} \), representing a low or high energy configuration (Figure 2).

![Figure 2. In the presence of a magnetic field, particles exhibit a spin angular momentum, \( m \). Particles can have a low energy spin state, \( + \frac{1}{2} \), or a high energy spin state, \( -\frac{1}{2} \).](http://chemwiki.ucdavis.edu/Physical_Chemistry/Spectroscopy/Magnetic_Resonance_Spectroscopies/Nuclear_Magnetic_Resonance/Nuclear_Magnetic_Resonance_II)

The ratio of spins in the high energy configuration (\( N^+ \)) to the number of spins in the low energy configuration (\( N^- \)) is demonstrated by Equation 1. Boltzmann statistics tell us that the distribution of spins in the high and low energy states depends on the temperature and the difference in energy between the two states. Since the energy difference between two spin states is very small, MR techniques are not as sensitive as other techniques such as infrared spectroscopy.
Opposing spins of like-particles cancel each other, therefore nuclei with an odd number of protons and/or neutrons have a nonzero nuclear spin, and are MR-active. Every element that does not have an odd number of protons, has some isotope with an odd number of neutrons, therefore having some form of MR-activity. However, only elements with a considerable population of these isotopes produce enough signal via energy transitions in a magnetic field, and are therefore commonly studied using magnetic resonance techniques (Table 1.)

\[
\frac{N^-}{N^+} = e^{-\frac{\Delta E}{kT}} \tag{1}
\]

<table>
<thead>
<tr>
<th>Isotope</th>
<th>$^1$H</th>
<th>$^{13}$C</th>
<th>$^{15}$N</th>
<th>$^{19}$F</th>
<th>$^{31}$P</th>
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<tr>
<td>Abundance</td>
<td>100%</td>
<td>1.1%</td>
<td>0.4%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Spin</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{1}{2}$</td>
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**Table 1.** Common MR-active Isotopes  
Data Source: Hornak, 2011

When particles with spin magnetic moments are placed in a magnetic field, the spin magnetic moments align with and against the surrounding magnetic field. In this environment, particles are able to absorb energy of a specific frequency. This frequency, $\nu$, depends on the magnetic field strength, $B$, and the gyromagnetic ratio of the particle, $\gamma$, and typically falls within the radiofrequency range (Equation 2).

\[
\nu = \gamma B \tag{2}
\]

When the energy of frequency $\nu$ is equal to the change in energy between these two configurations (Figure 2), transition will occur from the low energy to the high energy.
configuration within the magnetic field (Equation 3.). This frequency is called the Resonance, or Larmor Frequency.

\[ \Delta E = h\nu_{\text{Larmor}} = h\gamma B \]

When a magnetic resonance technique, such as Nuclear Magnetic Resonance (NMR) or Magnetic Resonance Imaging (MRI), is used, a material with a significant population of MR-active isotopes (such as those in Table 1) is first placed within a magnetic field, \( B_0 \) (Figure 3). Classically, we view the spin magnetic moments of these isotopes as aligning with or against \( B_0 \), precessing about the z-axis (dotted lines in Figure 3A). The z projection of these magnetic moments is called the net magnetization, \( M_z \).

As radiofrequency energy at the Larmor frequency is applied along the y axis, the net magnetization \( (M_0) \) begins to precess about the magnetic field component of the RF energy. If the RF pulse is applied for a particular period of time, then the magnetization will precess to the xy plane (Figure 3). Once the RF pulse is removed, the net magnetization relaxes back to z axis (Figure 3.).
The relaxation of the spin magnetic moments back to the equilibrium magnetization releases energy that is detected by the MR instrument’s receiver coil, and results in a signal that yields the MR data. As the spins’ magnetic moments relax, dissipation of energy into the surrounding alters and reduces the final signal, reflecting variances in each atom’s physical and chemical environment. There are two main types of relaxation that result in this signal reduction: spin-spin and spin-lattice relaxation.

Spin-spin relaxation is the relaxation mechanism though which the transverse magnetization, $M_{xy}$, decays to its equilibrium value of zero. This relaxation occurs as adjacent spins interact with one another. Spin-lattice relaxation, by contrast, reflects the recovery of the longitudinal magnetization component, $M_z$, to its equilibrium value. This type of relaxation occurs as energy dissipates into the surroundings of each active atom. The relaxation times $T_1$ and $T_2$ reflect the rate at which spin-lattice and spin-spin relaxation occur, respectively. More precisely, $T_1$ relaxation refers to the amount of time required for the longitudinal magnetization...
component to relax to approximately 63% of its equilibrium value. Conversely, $T_2$ relaxation refers to the amount of time required for the transverse magnetization component to decay to approximately 37% of its maximum value during excitation. In cases where inhomogeneities in $B_0$ exist, interactions with these inhomogeneous regions result in a second pathway of transverse relaxation. The relaxation time, $T_2^*$, reflects the total transverse relaxation time as a result of spin-spin and spin-inhomogenous-$B_0$ interactions (Equation 3)

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2^{inhomo.}}$$  \hspace{1cm} (3)

While the signal produced through these excitation and relaxation methods encodes for a great deal of information on the chemical and physical environments of the MR-active atoms, there are still a variety of steps involved in processing and interpreting this signal as an image. First, it is important to be able to give the data spatial significance. This is possible through the use of three perpendicular magnetic field gradients to encode the slice, frequency, and phase of the signal. As each gradient coil has a gradient in field strength across its axis, spatial information is organized by small variances in resonance frequency at spatially differentiated atoms.

Spatial resolution is determined by the size of the voxels, discrete three-dimensional parts of the entire MR image. Smaller voxels translate to overall better resolution. Voxel size is determined by the field-of-view (FOV), slice thickness, and matrix size. The number of data points collected in the frequency domain, and the number of phase encoding steps in the other direction dictate matrix size. The greater number of steps in either frequency or phase
encoding results in a larger matrix. Since the matrix will fill the desired field-of-view, a larger matrix will yield smaller voxels and thus better spatial resolution.

After information is spatially encoded through these gradients, it is spatially organized in a map called k-space. Radiofrequency signals are coded by intensity using a grayscale, and then filled in k-space. Once the k-space is filled, the information can be interpreted into an image through a Fourier transform (Figure 4).

![Figure 4. Data Organization & Processing](http://scien.stanford.edu/pages/labsite/2006/psych221/projects/06/cukur/mri.html)

**fMRI Theory**

*(Huettel et al., 2008)*

The basis of functional magnetic resonance imaging lies in determining small, localized changes in the brain that occur during use or stimulation of specific neural pathways. As a region of the brain is used, glucose uptake and cellular respiration increase. In order to fulfill the metabolic demands of the stimulated cells, surrounding oxygenated hemoglobins lose their bound oxygen atoms and become deoxygenated. This shift in oxygen binding is important.
because the hemoglobin’s coordinated iron experiences a shift in electron structure.

Oxygenated hemoglobin is diamagnetic, meaning that it has no unpaired electrons and no magnetic moment. Therefore, it is not magnetically active and will result in no appreciable effect on the magnetic resonance signal. Deoxygenated hemoglobin however, is paramagnetic. It has unpaired electrons, a nonzero magnetic moment, and thus it has an increased magnetic susceptibility. This susceptibility creates small inhomogeneities in the magnetic field, and increases the extent of spin-spin relaxation. As previously discussed, small inhomogeneous regions in the magnetic field contribute to $T_2$ relaxation, and add another component of relaxation from the transverse component of magnetization. Thus, this type of decay is dependent on a $T_2^*$ time constant. By increasing spin-spin relaxation, we expect that the $T_2^*$ time constant would be smaller, resulting in faster decay and reduced signal. This means that signal difference in $T_2^*$ weighted images may reflect compositional changes of oxygenated and deoxygenated hemoglobin in the blood.

We refer to this change as Blood-Oxygen-Level-Dependent (BOLD) contrast and use fMRI to detect subtle signal differences between tissue before and after oxygen uptake, as hemoglobin becomes deoxygenated.
To observe the BOLD contrast, images must be acquired very rapidly, within the period of localized hemoglobin deoxygenation, otherwise known as the hemodynamic response. Humans and songbirds have relatively similar hemodynamic response times, of about 7-8 seconds (Van Meir et al., 2005). These images are acquired in sets, or “blocks” which occur within an imaging paradigm of alternating “Stimulus ON” and “Stimulus OFF” blocks. Once the data are obtained, statistical analysis software can be used to compile ON and OFF images, correct for small differences among images, and detect subtle changes in the BOLD response across the whole brain.

Figure 5. ON/OFF Auditory fMRI Paradigm.
Challenges

One of the greatest challenges in this project was the development of the appropriate imaging parameters to produce functional images from our zebra finches. In order for a pulse sequence to be used for functional analysis, it must capture a reasonably resolved image within the subject’s hemodynamic response time to ensure accurate, time-sensitive representation in response to a stimulus. Typically, a gradient echo (GE) pulse sequence is used to acquire functional images. Using techniques like Echo Planar Imaging (EPI), and sometimes, Flash Low Angle Shot (FLASH), a gradient echo image can be acquired rapidly enough to obtain acceptable functional data (Figure 6).

Figure 6. Gradient Echo (GE), EPI-GE, and FLASH Pulse Sequences.
A gradient echo pulse sequence features successive 90 degree radiofrequency pulses (A). The FLASH (B) and EPI (C) sequences shown share a basic GE pulse sequence, but use different slice, frequency, and phase encoding sequences in order to acquire images quickly enough for functional applications.

Birds present a unique problem with these traditional fMR imaging techniques, however, given the small air cavities in their skulls, which aid flight but distort fMR images. These distortions occur because gradient echo pulse sequences are susceptible to artifacts such as signal dropout and distortion at air-tissue interphases, especially in high field strength magnets such as our 9.4 T instrument. Thus, we observed these artifacts and distortions when
trying to produce functional images using gradient echo EPI, and FLASH sequences (Parker, 2013). Previously, fMRI studies in songbirds have reported data using GE sequenced images. However these experiments were limited to studying centrally located regions, such as the primary and secondary auditory regions, which do not contain susceptibility artifacts. Because our experimental goals include whole brain data analysis, it was necessary for us to seek alternate imaging approaches.

Images acquired with a Spin Echo (SE) pulse sequence typically exhibit an increased signal-to-noise ratio in comparison to GE images due to the application of an additional 180 degree refocusing pulse (Figure 7).

Figure 7. Spin Echo (SE) Pulse Sequence. A spin echo pulse sequence features an initial 90 degree radiofrequency pulse followed by a 180 degree refocusing pulse. This is able to eliminate artefacts by....

Because the air-tissue interface of these skull cavities introduce small inhomogeneous regions within the magnetic field, the spin magnetic moments nearby these regions do not dephase at the same rate as those in more homogenous regions of the magnetic field. Ultimately, this results in signal decay in these areas, and the artifacts we observed in our gradient echo images. The refocusing pulse in a spin echo sequence causes the magnetic moments that were returning to the longitudinal component of magnetization more slowly than others, to become
the spins closest to the initial equilibrium magnetization. Eventually, all of the dephasing spins catch up to each other, allowing for a better resolved image despite the small inhomogeneous field regions at the air cavity sites. This technique is able to eliminate air cavity induced artifacts, at the expense of the increased time taken to apply a second radiofrequency pulse. In order to reduce scan time while maximizing signal-to-noise ratio retention, Poirier et al. (2010) were able to zero-fill data acquired at a 64 x 32 matrix size to 64 x 64. In previous experiments, our images exhibited the best results when acquired through a 32 x 32 matrix followed by reconstruction to 64 x 64 rather than zero-filling to a larger matrix (Parker, 2013). Using a Spin Echo (Rapid Acquisition with Refocusing Echoes, RARE) pulse sequence with matrix reconstruction, we were able to produce acceptable images within the zebra finch hemodynamic response time (Figure 7).

![Functional Image of Zebra Finch Brain.](image)

**Figure 7. Functional Image of Zebra Finch Brain.**
Functional images were obtained with satisfactory spatial resolution and acquisition time using a spin echo, RARE imaging sequence with an acquisition matrix of 32 x 32 and reconstruction to 64 x 64.

Having successfully obtained functional images, one of the next challenges in this project is ensuring the delivery of quality auditory stimuli within the magnet. Because we intend to study the physiological response to an auditory stimulus, it is important that the
stimulus is successfully delivered and experienced by the subject zebra finch. Therefore, delivery of the auditory signal must ensure the maintenance of an appropriate amplitude and quality. Unfortunately, any magnetic resonance study restricts use of traditional speakers that rely on internal magnets.

A variety of methods have been used for auditory delivery within the magnet, most of which include passive sound attenuation in the form of ear defenders, and sound delivery through some sort of tubing (Palmer, 2006 & 2001). Alternative methods employ the use of dynamic electromagnetic speakers (Van Ruijssevelt, 2012; Van Meir, 2005; Boumans, 2008). This method allows for the utilization of the bore’s magnetic field to drive movement in the speaker coil (Figure 8), rather than employing an internal speaker magnet that will disrupt the MRI’s magnetic field (Baumgart, 1998). Because of the size restrictions of our imaging probe, we elected to use the last method of sound delivery.

![Figure 8. Speaker Design.](image)

MRI compatible speakers are designed by taking advantage of the bore’s magnetic field, in place of an internal speaker magnet. The forces created by the interaction of this magnetic field with that created by the coil cause diaphragm movements, which produce sound.
An additional auditory challenge we face in this project is overcoming the rather loud noise made by the gradient coils during imaging. This may pose a risk for potential interference with the desired auditory stimulus, as gradient sounds significantly decrease auditory activation due to a desired stimulus (Elliot et al., 1999). Our first attempts using sound piping and, later, dynamic speakers, proved to be ineffective in conjunction with the gradient coil noise. This initially seemed to be due largely to the relatively low audio signal amplitude and power output. We were able to improve the dynamic speaker system in this respect, by choosing to modify speakers with greater power output and lower resistance. As a result, recordings from inside the magnet during scans and auditory delivery displayed clear audio signal in between periodic gradient noise.

While many other studies have employed passive forms of gradient noise reduction such as ear defenders or other physical barriers, we decided against these techniques because of our particular system. The first constraint we faced in this regard was the limited amount of space inside of our radiofrequency probe (Figure 9.).

Figure 9. Bird Setup within the probe.
Before placement into the probe, zebra finches are inserted into an acrylic bed (A). This bed was designed with installments for isofluorane delivery and respiration measurement through a pressure transducer. The small diameter of our bore, and thus our probe limits the potential for the use of physical sound barriers. After insertion, birds are secured within the bed using tissue and surgical tape (B).
The second and perhaps more taxing issue with using physical noise-reducing barriers is the general inflexibility in working with anaesthetized zebra finches. We have found that complete anaesthetization with isofluorane in these birds is not always consistent in dosage and duration. Thus, the bird bed apparatus that we utilize within our probe requires spatial flexibility to allow for quick and simple adjustments in bird placement as well as easily accessible isofluorane and O₂ supply localized to the bird’s nostrils. The third reason why we were unable to use this method is because it would be difficult to selectively filter the gradient coil noise without affecting delivery of the auditory stimulus. Given these constraints, we decided to forgo physical sound barriers and look ahead to other options.
Materials & Methods

Subjects & Subject Maintenance

All experiments were conducted on zebra finch (*Taeniopygia guttata*) or water-based phantom subjects.

Zebra finches were bred and housed at the Wellesley College animal facility. Subject housing operated on a 12 hour light/dark cycle with a constant supply of food (replenished biweekly), water (replenished as needed), and cage maintenance (weekly cleaning). Prior to each experiment, subjects were isolated from food for approximately 30 minutes to prevent choking during anesthetization.

The experimental group consisted of male adult birds. All birds were identifiable by colored aluminum leg tags that were marked “WC” for Wellesley College followed by a number.

Magnetic Resonance Imaging

All MR images were acquired at 400 MHz in a 9.4-T Bruker Avance vertical wide bore MR spectrometer with microimaging accessory (2.4 G/cm/A gradient strength), using Bruker Paravision 4.0 software.

Animal Preparation

After food isolation, subjects received 2% Isofluorane in O\(_2\) at a flow rate of 0.2 L/min in a plexiglass chamber (Brantree Scientific, Braintree, MA). Once anesthetized, birds were positioned and secured into a custom-made acrylic bed designed and built as a major component of this thesis (see Results and Discussion for details).
Once the bird’s beak is positioned into the beak cone of the bird bed, the bird is secured into the bed with a layer of Kimtech Science Kimwipe® tissue (Kimberly-Clark Professional, Inc.) to prevent feather damage, followed by a layer of 3M Micropore™ surgical tape (3M, St. Paul, MN) (Introduction, Figure 9). Additionally, the bed is outfitted with a pressure transducer to visually monitor respiration using BioTrig Buildier 1.01 software on a Dell Latitude Laptop computer.

Once the bird is successfully positioned and secured into the bed, respiration is monitored to ensure appropriate bird placement and isofluorane levels. If the subject’s respiration rate falls above or below 40-100 breaths per minute, the Isofluorane concentration is adjusted accordingly using a VIP Veterinary Vaporizer (Colonial Med Supply Co. Inc.) to achieve a rate within this range. Once bird installation and desired respiration rate are achieved, the bed is inserted into the radiofrequency (RF) probe (Figure 10), which is then placed into the magnet. The probe is maintained at 35 degrees Celsius during animal imaging.

Figure 10. Instrument Setup. Vertical bore, small animal instrument utilizes a vertical RF probe to receive the MR signal for processing. Once subjects are secured into the bird bed (Figure 3.), the bed is inserted into the probe, which is finally inserted through the bottom of the magnet bore.
Imaging Parameters

Prior to imaging, it was necessary to prepare a shim file suitable for the zebra finch subjects. Previous attempts to manually shim over a zebra finch brain proved to be unsuccessful because of field inhomogeneities caused by air cavities located in the brain. For this reason, a mouse shim file was used.

After this, the RF transmit/receive coil was tuned and matched. This position of this peak was subsequently checked between imaging blocks, to ensure continued signal and image quality. Scout images then were acquired using a “RARE_tripilot” sequence to confirm appropriate brain positioning (See Table 3 below). Each scout image included three orthogonal slices, and was used to set initial geometry parameters and slice orientation (Table 2) to be used for the remainder of each imaging session.

<table>
<thead>
<tr>
<th>Number of Slices</th>
<th>Slice Thickness (mm)</th>
<th>Interslice Thickness (mm)</th>
<th>Field of View (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.75</td>
<td>0.75</td>
<td>2.56</td>
</tr>
</tbody>
</table>

Table 2. Geometry Parameters. Once a scout image has been acquired, the Paravision 4.0 Geometry Editor is used to select the desired imaging region and slice parameters from this initial image. All experimental brain volume in a given subject and imaging period, acquire identical slices to ensure consistency among volumes of different experimental groups.

Anatomical Image Acquisition

At least one anatomical image was acquired for each imaging session and subject. This high resolution image has better-resolved neural structures (Figure 2). In post-processing, processed functional images are superimposed over these anatomical images for a given session to observe and identify activated regions.

All anatomical images were obtained in the coronal plane, using a “RARE_8_basParams” (Table 3), T2 weighted pulse sequence, with the geometry parameters selected from the
“RARE_tripilot” scout image (Table 2). Additionally, each anatomical and functional image acquired on zebra finches following the initial scout image included fat and motion suppression to reduce any fat signal or motion artifacts.

*Functional Image Acquisition*

As previously discussed in the Honors Thesis of Rachel Parker, it is necessary to use a spin echo pulse sequence for functional image acquisition on our imaging system. We followed Rachel Parker’s previously established zebra finch fMRI parameters for the functional images, which are called “RARE_8_bas_recon_64x64” (Table 3) (Parker, 2013). These images use the same initial RARE_8_bas sequence with parameters adjusted to reduce acquisition time enough to fall within the hemodynamic response time of a zebra finch. In order to reduce scan time, images were acquired using a 32 x 32 pixel matrix and subsequently reconstructed to an output matrix of 64 x 64 pixels. In order to compensate for the reduced signal-to-noise ratio associated with decreasing matrix size, imaging parameters were edited to include 3 averages and 2 dummy scans (Figure 11).

<table>
<thead>
<tr>
<th>Scan Name</th>
<th>TR (ms)</th>
<th>TE (ms)</th>
<th>RARE Factor</th>
<th>Number of Averages</th>
<th>Matrix size (pixels)</th>
<th>Field of View (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RARE_tripilot</td>
<td>2000.000</td>
<td>12.5</td>
<td>8</td>
<td>1</td>
<td>128 x 128</td>
<td>2.56 x 2.56</td>
</tr>
<tr>
<td>RARE_8_bas Params</td>
<td>3109.632</td>
<td>15</td>
<td>8</td>
<td>8</td>
<td>256 x 256</td>
<td>2.56 x 2.56</td>
</tr>
<tr>
<td>RARE_64x64_output_recon_8</td>
<td>2000.000</td>
<td>15</td>
<td>8</td>
<td>1</td>
<td>32 x 32</td>
<td>1.40 x 1.40</td>
</tr>
</tbody>
</table>

*Table 3. Imaging Parameters.* The following scans and parameters were used for each scan type: scout image (RARE_tripilot), anatomical image (RARE_8_bas Params), and functional image (RARE_64x64_output_recon_8).
Experimental Paradigm

The experimental paradigm consisted of an ON/OFF block design to measure activation in periods of song exposure in comparison to baseline activation in periods of silence consisted of eight, 40 second alternating blocks of silence and song. Each block consisted of two 8 second dummy scans followed by three 8 second volumes scans.

*Stimulus Design & Delivery*

In order to collect each subject’s own song samples, subjects were isolated for a 24 hour period in sound-proof isolation boxes made from stainless steel-belted coolers (54 Quart Stainless Steel Cooler, Coleman Inc.) which were outfitted with lighting, and water, air, and food supply and kept on sleep and feeding cycles identical to those found in the Wellesley College Animal Facility. Microphones were installed into isolation boxes, and set to record 1 second audio clips every second for the entire 24-hour period (Figure 12). After song collection, audio clips including song were collated using Audacity software to create song samples.
To create experimental rhythmic white noise, stimuli were created in Matlab software (The Mathworks Inc., Natick MA) using a script “whiteMedley” which recognizes rhythmic patterns found in a bird’s song, and reproduces them in a white noise stimulus (Figure 13).

**Figure 12. Sound-proof Isolation Boxes.** Sound-proof isolation boxes were made from stainless steel-belted coolers, outfitted with a light and air source, as well as a microphone and speaker. Subjects would be placed in these boxes inside a cage with ample food and water, to collect song samples for experimental stimuli, or to expose them to sound before experiments. Closed/outside view (left), open/inside view (right).
function whiteMedley = whiteNoiseEditsTwo(inSong, newFileName)
%whiteMedley = whiteNoiseEdits(insong, newFileName)
%with the inputs of a song or medley and a name for the new file, this
%function replaces every portion of a song which is more than slightly
%above the baseline in amplitude with white noise. The output is the
%amplitude vector of the resulting white noise medley.
%KSB

if nargin == 1
    newFileName = 'test';
end

[medley fs] = wavread(inSong);
medley = medley(100:end);
medley = medley/max(medley);

[white fs] = wavread('WhiteNoise');
white(abs(white) > mean(abs(white)+(2*std(abs(white)))) = mean(white);
white = white/max(white);

ywhite = white;
%duplicate white noise file until it is at least the length of medley
while length(ywhite) < length(medley)
    ywhite = [ywhite; white];
end
ywhite = ywhite(1:length(medley));
%create white noise medley file
thresh = mean(abs(medley))+.1*(std(abs(medley)));
whiteMedley = zeros(length(medley),1);
whiteMedley(medley > thresh) = ywhite(medley > thresh);
output = specgram(medley);
rouput = real(sum(output));
normout = routput/max(abs(rouput));
vals = floor(linspace(1, length(medley), length(normout)));
for i = 1:(length(vals) - 1)
    scale = max(abs(medley(vals(i):vals(i+1)-1)));
    whiteMedley(vals(i):vals(i+1)-1) =...
    whiteMedley(vals(i):vals(i+1)-1)*scale;
end
whiteMedley = whiteMedley/max(whiteMedley);
wavwrite(whiteMedley, fs, newFileName); %will need to change to audiowrite in future

%make amplitude plot
figure
hold on
plot(medley)
plot(whiteMedley, 'r')
set(gcf, 'Color', 'w')
box off
ylabel('Amplitude', 'FontSize', 15)
axis([0 .8E5 -1 1])

disp(['Done. White Noise Medley saved as ', newFileName])
end

Figure 13. Script for function whiteMedley.

Script was used to recreate rhythmic song patterns within white noise to produce experimental stimuli.
The auditory stimulus consisted of a random zebra finch tutor song medley and periods of silence. The speech and phonetics analysis software, Praat (Version 5.3.49, Phoenetic Sciences, University of Amsterdam), was used to create the auditory stimuli. The program queued recorded zebra finch song samples (generated in Audacity 2.0.3) to create 16 second ON/OFF blocks.

A trigger system was setup in order to synchronize the auditory stimuli with the imaging sequences. The scanner was connected to a relay switch, which was switched every 15 slices (between ON and OFF blocks) using Paravision's trigger out function. The switch was additionally connected to an alternate computer which delivered randomized stimuli using Presentation software (Neurobehavioral Systems Inc.)

Data Analysis

Statistical Parametric Mapping (SPM8, University College London) was used to perform voxel based statistical analyses. The image origin was assumed to be the origin set by the scanner (32, 24.6, 5.33), and the data were normalized based on the previously described high resolution anatomical image (FOV=2.56 cm, TR =2000ms, TE=60ms, Acquisition Matrix = 256 x 256, Slice Thickness=0.75mm). Statistical analyses were set to assume a voxel size of 0.4 x 0.8 x 0.75 mm, and image dimensions of 64mm x 32mm x 15mm. Given the paradigm, used in these experiments, imaging conditions were set with an interscan interval of 7 seconds and 40 second block durations for the two conditions: 1. Sound, 2. Silence. Data were then analyzed for activation displaying Sound > Silence.
Results & Discussion

Accurate auditory delivery is a key feature of the desired experimental apparatus. In order to ensure experimental efficacy, all auditory stimuli must be delivered to zebra finch subjects in synchronization with the fMRI scans, within an audible range and free of significant distortion and noise. As previously discussed, many characteristics of the MRI instrument introduced challenges in designing these aspects of the auditory fMRI apparatus.

Speaker Construction & Testing

Delivering auditory stimuli into the magnet proved to be a difficult challenge largely because of incompatibility of magnetic speaker components with the instrument magnet. In order to tackle this problem, we first looked to previous auditory fMRI studies in humans, which piped sound into the magnet (Palmer, 2001).

We created two simple piping systems to deliver sound. The first apparatus consisted of a polypropylene powder funnel (CAT # 414004-270, VWR International LLC, Radnor, PA) sealed to Tygon tubing, with a rigid tubular end made of card stock (Figure 14). The second model consisted of soft latex laboratory tubing (CAT # 62996-473, VWR Scientific, West Chester, PA) without any rigid ends (Figure 14). Both prototypes were inserted through the top of the instrument bore, with the funnel end secured approximately five feet away from the magnet to collect sound emitted from Gateway G-MAX2000 speakers (Max Power Output = 3 watt, Frequency Range = 90-20000 Hz).
In order to test sound delivery with sound piping systems, audio signals were played through the sound piping systems and recorded using a MicroOptics Technologies fiberoptic microphone to analyze signal changes and validity. Two signals were played through the magnet: a sine wave (440 Hz) and a sample of zebra finch song. Each of these signals was played and recorded through the magnet during periods of scanner inactivation, and during the acquisition of a RARE 8 bas image in order to test sound delivery in the presence of gradient noise. MATLAB was used to create frequency spectra and map amplitude over time to observe signal differences.

Figure 14. Sound Piping Systems. Two piping systems were produced to test sound transmission into the magnet, both including tubing connected to a plastic funnel for sound collection. One system included tygon tubing and a rigid card stock end, the other latex tubing. Each system is installed into the top of the bore, with the funnel secured away from the magnet to collect sound from a traditional magnetic speakers.
Figure 15. Amplitude vs Frequency (Hz) Spectra of Sound Piping System-delivered sine 440 Hz signal in Time Domain. A 440 Hz sine signal was produced using Audacity software (C), and played into the magnet using the sound piping system during a RARE 8 bas scan (A), and scanner inactivation (B). A fiber optic microphone was used to record the signal within the magnet, and the amplitude of each recording was mapped in the time domain using MATLAB.

Figure 16. Amplitude vs Time (s) Spectra of Sound Piping System-delivered sine 440 Hz signal in Time Domain. A 440 Hz sine signal was produced using Audacity software (C), and played into the magnet using the sound piping system during a RARE 8 bas scan (A), and scanner inactivation (B). A fiber optic microphone was used to record the signal within the magnet, and the amplitude of each recording was mapped in the time domain using MATLAB.
Overall, the amplitude vs time spectra for each test show the fundamental signal at 440 Hz (Figure 15), demonstrating that the sound piping system does indeed deliver the auditory signals into the magnet. The frequency spectrum of the sine 440 Hz signal during scanner inactivation (Figure 16B) shows that there is some frequency distortion resulting from the piping and magnet bore, even in the absence of gradient noise. Subtle differences in amplitude are also shown. While the pure sine wave shows a constant amplitude with a range of ±0.8 (Figure 16), the recorded sample has a much lower amplitude falling between ±0.015 (Figure 16C).

The addition of gradient noise makes the signal analysis more complicated. Figure 3A shows the recording of the amplitude of the gradient noise and the sine wave over time. It can be observed that the amplitude of the gradient noise (seen as the repetitive pulses) reaches a maximum range of ±1, while the sine wave feedback (as shown as the thick blue line across the horizontal) has an amplitude that falls under the ±0.2 range. Listening to this recording, it can be observed qualitatively that the sine wave is generally inaudible in comparison to the gradient noise.

To try to achieve better sound delivery, MRI compatible speakers were made by removing internal magnets from commercially purchased speakers. Based on size, quality, cost effectiveness, and speculated ease of magnet removal, three models of speakers were chosen as candidates for this prototypes: A. 20 mm, 50 Ω Hoisden HDR960 (All Electronics, Van Nuys, CA, CAT #SK-215); B. 40 mm, 300 Ω (All Electronics, CAT #SK-300), and C. 50 mm, 4 Ω (All Electronics, CAT# SK-504) (Figure 17). Each speaker model was outfitted with leads and 3.5 mm Mono, Metal audio plugs (All Electronics, CAT# SMPM) (Figure 17). Connections were systematically tested with the magnet in place, before removal was attempted.
Once the magnets were successfully removed, each speaker was tested perpendicular to the magnetic field of the instrument bore to observe each model’s initial working capacity. The initial three models were unsuccessful for a variety of reasons. Models A and C were housed in magnetically active metal casing which could not be removed. Model B was able to produce sound inside the instrument’s magnetic field, but exhibited minimal output at maximum power and thus was essentially inaudible. It was speculated that this model’s relatively high impedance may have restricted current flow, and limited the speaker coil’s induced magnetic field as a result. Ultimately this reduced magnetic field could result in a reduced repulsive force against the instrument magnetic field and thus limited interaction with the speaker diaphragm, reducing sound output. Therefore, a speaker with lower impedance, Model D. 20 mm, 8 Ω (Digikey, #102-1542-ND) was purchased and tested (Figure 17D). This model seemed to produce a qualitatively more audible signal, and was subsequently tested inside the magnet by taping it inside the probe, perpendicular to the instrument’s magnetic field vector (Figure 18).
Audio signals were recorded from inside the probe using a fiberoptic microphone (MicroOptics Technologies, Inc., Microphone Model FDMI-MR, Driver Model FDMI-DRI) mounted inside the probe, 4-5 inches below the receiver coil (Figure 18). Signal from this source was collected on a Sony Vaio computer using Audacity 2.0.2 Digital Audio Editor.

In order to test the signal delivered into the magnet, audio signals were played through each sound apparatus and recorded to analyze signal changes and validity. A sample of zebra finch song was played and recorded through the magnet during periods of scanner inactivation, and during the acquisition of a RARE_tripilot, and a RARE 8_64x64_output_recon_8 image (Table 3) in order to measure the effect of gradient noise on the signal.
Figure 19. Zebra finch song played and recorded inside the MRI instrument. Zebra finch song was played and recorded inside the magnet probe using the MRI compatible speaker prototype, and a MicroOptics Technologies fiberoptic microphone. Audio spectra (Amplitude vs Time (s)) of both the recorded song (bottom) and the original zebra finch song file (top) were mapped and compared using Audacity software. In order to improve visibility of the signal pattern, the recorded song was amplified by 25dB, to a peak amplitude of 7.7dB. Matching periods of signal in the original and recorded samples are outlined in green (Song Period 1) and yellow (Song Period 2). Baseline noise is outlined in red.

Figure 20. Zebra finch song played and recorded inside the MRI instrument during a RARE_64x64_output_recon_8 functional MRI scan. Zebra finch song was played and recorded inside the magnet probe during functional image acquisition (RARE_64x64_output_recon_8, Table 2.) using the MRI compatible speaker prototype, and a MicroOptics Technologies fiberoptic microphone. Audio spectra (Amplitude vs Time(s)) of both the recorded song (top) and the original zebra finch song file were mapped and compared using Audacity software. Scanner gradient noise is outlined in orange.
Figure 21. Zebra finch song played and recorded inside the MRI instrument during a late-onset RARE_64x64_output_recon_8 functional MRI scan. Zebra finch song was played and recorded inside the magnet probe using the MRI compatible speaker prototype, and a MicroOptics Technologies fiberoptic microphone. A functional image (RARE_64x64_output_recon_8) was acquired 2.3 seconds after the song onset. Audio spectra (Amplitude vs Time(s)) of both the recorded song (top) and the original zebra finch song file were mapped and compared using Audacity software. In order to improve visibility of the signal pattern, the recorded song was amplified by 15 dB, to a peak amplitude of 11.5 dB. Matching periods of signal in the original and recorded samples are outlined in green (Song Period 1) and yellow (Song Period 2). Baseline noise is outlined in red, scanner gradient noise is outlined in orange.

Figure 22. Zebra finch song played and recorded inside the MRI instrument during a RARE_Tripilot MRI scan. Zebra finch song was played and recorded inside the magnet probe during a scout image acquisition (RARE_Tripilot) using the MRI compatible speaker prototype, and a MicroOptics Technologies fiberoptic microphone. Audio spectra (Amplitude vs Time (s)) of both the recorded song (top) and the original zebra finch song file were mapped and compared using Audacity software. In order to improve visibility of the signal pattern, the recorded song was amplified by 10 dB, to a peak amplitude of 5.2 dB. Matching periods of signal in the original and recorded samples are outlined in green (Song Period 1) and yellow (Song Period 2). Scanner gradient noise is outlined in orange.
The sound efficacy tests were able to prove successful function of our MRI-compatible speakers, showing audible and recognizable periods of song in comparison to the original song sample (Figure 19). However, even in the absence of image acquisition, it appears that the recorded signal shows some appreciable noise. This may be a result of speaker and/or microphone limitations given the frequency and power of the sound signal, as well as distortions and echoes produced within the enclosed probe environment.

While there seems to be relatively successful sound delivery into the magnet, recordings of sound played during image acquisition introduced new problems. As expected, gradient coil noise was present in the recordings, and interfered with the desired audio signal. This noise was most apparent during functional image acquisition (RARE_64x64_output_recon_8), which exhibited constant, high frequency noise and completely overpowered our delivered signal (Figures 20). The signal was audible and recognizable between scans, and during scans with intermittent gradient noise such as the RARE_Tripilot scout image and RARE_8_bas anatomical image (Figures 21 & 22).

**Gradient Noise Reduction**

Having confirmed audio delivery within the magnet, the next efforts focused on reducing gradient noise interference with the desired auditory stimuli. One approach that showed promising results in the literature is active noise cancelling, to reduce gradient noise through deconstructive interference (Goldman et al., 1989). To try this method, a trial recording of RARE 8 bas gradient noise was collected in the magnet to study potential manipulations of this signal. A digital filter was produced in MATLAB to actively cancel gradient noise recorded inside the magnet. This was accomplished by first performing a Fast Fourier Transform on the
RARE 8 bas gradient noise recording to convert the signal from the time domain, into the frequency domain. Then, the signal was converted back to the time domain using an inverse Fast Fourier Transform. Finally, the signal was inverted to produce a signal that would destructively interfere with the original gradient noise signal. When played aloud, the cancellation signal did seem to deconstructively interfere with the original gradient recording signal to effectively reduce the noise.

While this approach offers an effective mechanism for gradient noise reduction, it was ultimately not pursued further for a variety of reasons. First, the cancellation by interference model relies on the cancellation signal to be perfectly in phase with the gradient noise. This is difficult in practice, because scanning paradigms may change slightly from experiment to experiment due to human error in scan triggering or due to adjustments within the instrument that may result in time delays. It would eventually be possible to use a scanner-auditory stimulus trigger system (discussed below) to synchronize the cancellation signal with the images, although this may not necessarily ensure that the gradient noise pulses are in phase with the cancellation signal. Since the cancellation signal is produced from a single gradient noise sample which may not reflect all gradient noises to the desired accuracy. Eventually, these problems could be circumvented and a better filter could be designed using an adaptive filter method. This type of signal filter would periodically assess the gradient noise, and produce new cancellation signals in real time using new cycles of recorded noise. In theory this approach works well (Chambers et al., 2007; Li et al., 2007); however the multi-step, periodic process has an inevitable time lag between gradient noise sampling and cancellation signal delivery which poses further complications. For this reason, although this type of adaptive filter has been used
for gradient noise reduction in MRI before (Moelker & Pattynama, 2003; Chambers et al, 2007), applications to functional MRI would be especially difficult given the time sensitive nature of fMRI paradigms. Another reason why this method would be especially difficult to adapt for our apparatus is that a lot of electronic hardware would need to be introduced into the magnet. Even a minimal design would include additional speakers and microphones, for which there is minimal free space within the instrument, and other electronic components which may require further considerations to ensure MRI-compatibility.

Instead, simpler routes to gradient noise reduction were sought to meet the needs of the experiments. Other routes to gradient noise reduction include pre-experimental subject habituation to gradient noise. To do this, isolation boxes (see Materials & Methods) were outfitted with speakers to expose bird subjects to gradient noise samples periodically, 1-3 weeks before experimental imaging. While this method may not prevent interference between sound stimuli and gradient noise, it could potentially prevent false positive activation from gradient noise rather than from the desired experimental stimuli.

Because sound tests exhibited audible stimuli between scans, another method that could aid in reducing gradient noise-related experimental problems is changing paradigm design. Typically, there is no lag time between ON and OFF blocks in a fMRI paradigm. However, it may be possible to design a paradigm with short non-scanning intervals before each scan in an ON block. Ultimately, this method might have different implications for the data but could be viable if designed and analyzed with care.
Another major apparatus adjustment that was made to improve sound delivery was redesign of the bird bed. The first bird bed created for this project was an acrylic, cylindrical structure that successfully allowed for anesthesia delivery, respiration monitoring, and bird placement within the magnet during imaging. Once the MRI-compatible speakers were developed, however, it became clear that the bed required design adjustments to optimize sound delivery. The previous design included a closed system around the bird head, which restricted sound flow to the ears. In order to facilitate sound delivery, a new bed was designed with an above-head compartment to contain the speaker. This compartment features tunnels through the bed to deliver sound directly to each ear. Additionally, the anesthesia delivery was altered to direct incoming anesthesia above the bird head to allow for gravitational flow of the gas down past the bird beak, and out through the exhaust tubing. Other components of the bed remained similar to those of the previous bed (Figure 23).

Figure 23. Bird bed Redesign. A new bird bed (bottom) was designed based on the original cylindrical, acrylic design (top), with additions to improve sound and anesthesia delivery.
The bed contains two chambers, one each to deliver anesthesia and auditory stimuli over the duration of each experiment (Figure 24).

Figure 24. Bird Bed Schematic. The bird bed consists of two major systems: auditory delivery (green) and anesthesia delivery (blue). The auditory delivery components allow sound flow from the speaker directly to holes located at each ear. The anesthesia delivery is housed below the auditory delivery system, and directs incoming anesthesia to the top of the anesthesia chamber. From this point anesthesia flows down to the bottom of the chamber to the inhalation tube for subject inhalation, and excess gas flows through the exhaust line.

The anesthesia chamber directs incoming isofluorane/O₂ into a beak cone, and excess gas out through an exhaust line into a Vapor Guard Activated Charcoal Adsorption Filter #1931401, Vet Equip) (Figure 11).

The original prototype of the new bed was designed to provide speaker housing above the bird head to offer more localized sound, rather than taping the speaker several inches
below the bird as done in previous work (Figure 18). We wanted to design a bed that would maximize flexibility for future experimental modifications, and to prevent the need to redesign or rebuild the bed in future years. It was necessary that the speaker compartment include a removable cap, to allow for speaker removal and replacement over time in the case of future damage or wear. Additionally, considerations were taken for future experiments that might image un-anesthetized birds, which would require substantial restraint. Existing zebra finch stereotaxis tools rely on the insertion of small metal bars into each ear as a restraint mechanism. An idea for potential restraint within the bed would include future installments of flexible ear bars, which would ideally pipe sound directly to birds’ ears as well. Given this vision, the original prototype was modified. Initially, this design delivered sound through holes in the speaker compartment directly to holes around the beak cone.

**Figure 25. Bird Bed Design Changes.**
The original acrylic design (A) was first rebuilt with modifications to include speaker installment above the head, with sound flow to the head compartment around the beak cone (C). This prototype was further modified to deliver sound directly to the zebra finch ears (B).
Given the location of zebra finch ears at each cheek, we decided to modify the design to direct sound from the speaker compartment directly to holes at each ear. This made sense because sound delivered in closer proximity to each ear would maximize perceived signal intensity with respect to the external gradient noise, because of proximity and insulation produced from the acrylic holes surrounding each ear. Additionally these holes could be outfitted with hollow, flexible ear bars in the future for facilitated sound delivery and effective subject restraint without anesthesia.

Once a second prototype was made to reflect this design, initial tests showed two major required adjustments. By introducing more localized sound delivery, the depth of the head encasement increased, which complicated bird installment (Figure 26).

![Figure 26. Bird Installation in Old (left) and New (right) Bird Beds.](image)

Because zebra finch stability under isofluorane can be unpredictable, it was necessary to practice and adjust bird installation techniques in order to place the bird’s beak in the anesthesia flow quickly enough.
Another issue that appeared was electromagnetic interference between the speaker leads and the RF receiver coil. All prototypes directed the speaker leads through the bed and receiver coil, however this dramatically interfered with the MR signal exhibited major image artifacts (Figure 27).

Leads were wrapped in latex tubing for insulation; however this was not successful in preventing electromagnetic induction of the speaker leads and the receiver coil. Finally, two holes were drilled into the speaker cavity cap and the speaker leads were directed out of the top of the bed to prevent this problem. (Figure 28)
As a result, the bed must be placed in the bore from the top of the magnet bore. The speaker leads exit the magnet from the top of the bore (Figure 29). After this, the bird can be positioned into the bed and probe which, can finally be inserted into the bore from the bottom.

**Figure 28. Bird Bed.** An acrylic bird bed was custom designed to house zebra finch subjects within the magnet probe. The bed is outfitted with two compartments: one for auditory delivery, and another for anesthesia deliver. The bed also includes a pressure transducer to monitor subject respiration rate.

As a result, the bed must be placed in the bore from the top of the magnet bore. The speaker leads exit the magnet from the top of the bore (Figure 29). After this, the bird can be positioned into the bed and probe which, can finally be inserted into the bore from the bottom.

**Figure 29. Probe Installation.** Because all speaker components must stay outside of the receiver coil, the bed and speaker must first be inserted through the top of the bore before bird insertion. Once the bird is successfully secured into the bed, it can be inserted into the instrument probe and finally into the bore through the bottom entrance.
Once the technical aspects of the bed were finalized, the bed’s sound delivery system was tested. To do this, the bed was setup to image an NMR tube water phantom to allow for sound flow through the bed during testing (Figure 30).

Samples of looped zebra finch song and white noise were played through the speaker (Figure 31) inside the bed, and subsequently recorded by a MicroOptics Technologies fiberoptic microphone taped directly below the bed (Figure 30). Zebra finch song and white noise were played and recorded in the magnet during scanner inactivation and during the acquisition of RARE_Tripilot, RARE_64x64_output_recon_8, and RARE_8_bas images.
Ultimately, the sound tests were unable to prove successful sound delivery in the new bird bed. In the absence of image acquisition, recorded samples exhibited extensive noise and distortion, and unrecognizable signal. The white noise recording specifically, seemed to transmit well initially, but experienced increased noise over time. Figure 32 shows noise patterns outlined in green, which seem to increase in amplitude over time. It is possible that this may be due to constructive interference of noise or any frequency in the audible range which could occur very quickly in a confined place such as the probe. The recorded zebra finch song shows some repeated signal patterns, which may suggest some transmission of the original signal. However when observed aurally, the recorded zebra finch song was inaudible (Figure 32)

Figure 31. Looped Zebra finch song and White Noise samples. Audacity software was used Zebra finch song looped to create a long audio sample to test the new bird bed’s auditory delivery. Audacity was also used to generate white noise for additional testing.
As seen in previous sound studies, gradient noise was still present in the recorded signals. This noise was most apparent during functional image acquisition (RARE_64x64_output_recon_8), just as in previous speaker tests (Figure 34). Some signal is observed between scans, and during scans with intermittent gradient noise such as the RARE_Tripilot and RARE_8_bas images (Figures 33 & 35). Any signal between these scans however, is inaudible or aurally unrecognizable.

Figure 32. Zebra finch song and White Noise played and recorded inside the MR instrument using the new bird bed. Zebra finch song and White Noise (Figure 31.) were played and recorded inside the magnet probe during a period of scanner inactivation using the MRI compatible speaker inside the new bird bed, and a MicroOptics Technologies fiberoptic microphone. Audio spectra of both the recorded song (top) and white noise (bottom) were mapped and compared using Audacity software. Repeating signal patterns in the zebra finch song recording are outlined in yellow. Noise patterns in the white noise are outlined green.
Figure 33. Zebra finch song and White Noise played and recorded using the new bird bed during acquisition of a RARE_Triplot image. Zebra finch song and White Noise (Figure 31.) were played and recorded inside the magnet probe during a RARE_Triplot scan using the MRI compatible speaker inside the new bird bed, and a MicroOptics Technologies fiberoptic microphone. Audio spectra of both the recorded song (top) and white noise (bottom) were mapped and compared using Audacity software. Periods of signal are outlined in green. Noise patterns are outlined in red, scanner gradient noise is outlined in orange.

Figure 34. Zebra finch song and White Noise played and recorded using the new bird bed during acquisition of a functional MR image. Zebra finch song and White Noise (Figure 31.) were played and recorded inside the magnet probe during a RARE_64x64_output_recon_8 scan using the MRI compatible speaker inside the new bird bed, and a MicroOptics Technologies fiberoptic microphone. Audio spectra of both the recorded song (top) and white noise (bottom) were mapped and compared using Audacity software. Periods of signal are outlined in green. Noise is outlined in red, scanner gradient noise is outlined in orange.
Gradient noise is expected, as the placement of the microphone did not change appreciably between the speaker and bird bed sound delivery tests. The bed’s design was only expected to have some gradient noise reducing qualities as a result of localized sound delivery. Because the microphone was not set up to record the sound in the same, bilateral fashion that a bird’s ears would receive the auditory signal, any gradient noise reduction due to this design would not be expected. Microphone placement during these tests was a major problem, because the microphone did not fit into the bed and therefore results did not accurately reflect sound delivery. The bed is not designed to deliver sound throughout or outside of the

Figure 35. Zebra finch song and White Noise played and recorded using the new bird bed during acquisition of a RARE_8_bas image. Zebra finch song and White Noise (Figure 31.) were played and recorded inside the magnet probe during a RARE_8_bas scan using the MRI compatible speaker inside the new bird bed, and a MicroOptics Technologies fiberoptic microphone. Audio spectra of both the recorded song (top) and white noise (bottom) were mapped and compared using Audacity software. Periods of signal are outlined in green. Gradient noise is outlined in orange.
bed. Had the speaker been set up in an open space within the probe, we would expect to observe relatively high signal in our recordings simply because sound flow would be largely uninterrupted and distributed more equally throughout the probe space. By designing a localized sound delivery system, free signal flow throughout the probe was sacrificed in order to concentrate sound at each zebra finch ear. This was evident in previous speaker tests, where the speaker was mounted freely in the probe and within close proximity to the microphone (Figure 18). Had it been possible to place the microphone inside of the bed, better signal recording might be expected.

Overall, these last tests are inconclusive. Because of the major experimental flaw in microphone placement, it is possible that the bed successfully delivers sound; however there is no proof that this is necessarily the case. In the future, it would be important to retest the bed using a smaller microphone, preferably two small microphones placed bilaterally at each ear hole. Another error that may have caused inconclusive results is the possibility of speaker malfunction. The bird bed design process required various adjustments of speaker placement, which called for multiple instances of unsoldering and resoldering leads, in addition to heavy handling. It is possible that any of these actions may have damaged connections in the speaker and thus made it unable to function properly. In order to avoid doubt over speaker function, installation of solderless 3.5mm audio jacks would ease speaker movement for regular testing.

Auditory Stimuli Triggering

The last component of sound delivery needed for future auditory fMRI studies is a trigger system to synchronize auditory stimuli and functional images within a paradigm. Other
songbird fMRI studies have used Presentation Software (Nerobehavioral Systems, Inc.) to interface between an imaging computer and an auditory stimulus computer and track stimulus and scan initiation (Van Ruijssevelt, 2013). Because our scanner computer runs on a Linux OS, and our scanner’s “trigger out” function operates using an analog signal, interfacing with this software posed many challenges initially.

Previous work in our lab utilized a relay switch, which was developed initially to receive and deliver triggering signals (to a bubbler) from Paravision, on our scanner computer (Figure 36). This circuit consists of a bilogic switch. Paravision can be programmed to send a 5V pulse to the relay switch, and switch the circuit logic after every phase, slice, or volume acquired.

In order to interface the Presentation Software with this relay switch, some sort of analog to digital converter is required to convert the analog trigger signal into a digital signal to be inputted into a computer running Presentation (Figure 38). Because Presentation may not recognize the converted signal, further hardware may be needed to interface between the two media.
Two simpler systems could be designed in order to avoid complications with Presentation Software interfacing. The first system relies on only one circuit within the relay switch, which would be connected to the ground lead of the speaker. If the speaker is connected to a device, such as a computer, which plays the desired stimulus in a running loop the speaker can be turned on and off as the relay switch connects and disconnects the speaker circuit (Figure 37). A sample model of this setup was built using a spare speaker (20 mm, 50 Ω Hoisden HDR960, All Electronics, Van Nuys, CA, CAT #SK-215, All Electronics, Van Nuys, CA) to test triggering of an audio signal from the scanner. This model works well, but is not ideal for auditory fMRI studies in the long term since it limits the use of many stimuli to one per paradigm.

Figure 37. Direct Trigger Schematic. In order to avoid interfacing problems between the relay switch and the stimulus releasing device a direct trigger system can be built. This system would connect the speaker directly to the relay switch, as well as with the stimulus releasing device. If the stimulus device plays the desired stimulus on a loop, the speaker will turn on or off depending on the relay switch to complete its circuit.
Another method would be to use the bilogic nature of the relay switch, to create two circuits that interface into the stimulus releasing device (Figure 38). If an interface were created that released two different signals (one for each circuit on the relay switch), a program could be written in Matlab to play a different stimulus file for each switch. This would involve some work in designing and building the appropriate hardware to correctly interface with Matlab; however it would allow for the opportunity to use randomized stimuli in a paradigm by indexing the ON block stimuli to play a variety of different stimuli at a given block.
Conclusions & Future work

Designing and developing an effective sound delivery system for this project proved to be a major challenge because of the hardware restrictions for many small animal MR imaging instruments. Two major installments within the experimental apparatus: MRI compatible speakers, and a new bird bed to facilitate sound delivery.

Initial testing with the speakers showed successful sound delivery into the magnet; however further tests need to be performed to test sound delivery within the new bird bed design. This will require smaller microphones, to more accurately measure sound delivery at the bird’s ear. Additionally, the speaker should be adjusted to be more flexible for regular tests to ensure consistent function.

Once ideal sound delivery function in the speaker and bed apparatus can be determined, the next major step in sound delivery is trigger set up to synchronize scan and stimulus onset. Currently, the speakers can be easily modified to create the trigger paradigm as shown in Figure 14 (Results & Discussion); however a more flexible paradigm will be useful for future experiments. This will most likely be possible with the implementation of some analog to digital converter. Ideally, a trigger system will utilize the Presentation Software (Neurobehavioral Systems, Inc.) since this program tracks scans and outputs data points that will be useful in preprocessing steps in the Statistical Parametric Mapping software. If this software does not interface well with the given system however, a script in Matlab may be able to achieve the desired synchronization.
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