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Using an Internal Auditory Stimulus to Activate the Developing Primary Auditory Cortex: A Fetal fMRI Study

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Abstract

Insight into the rapidly developing brain in utero is scarce. Fetal functional magnetic resonance imaging (fMRI) is a technique used to gain awareness into the developmental process. Previous auditory task-based fMRI studies employed an external sound stimulus directly on the maternal abdomen. However, there has since been recommendation to cease doing so. We sought to investigate a reliable paradigm to study the development of fetal brain networks and postulate that by using an internal stimulus, such as the mother singing, it would result in activation of the fetal primary auditory cortex. Volunteers carrying singleton fetuses with a gestational age of 33-38 weeks underwent two stimulus-based block design BOLD fMRI series. All of the nine fetal subjects analyzed had activation in the right Heschl's gyrus, and seven out of the nine fetal subjects had activation in the left Heschl's gyrus when exposed to the internal acoustic stimulus. Ultimately, this internal auditory stimulus can be used to analyze the developing fetal brain.

Keywords

Functional Magnetic Resonance Imaging, Fetal Brain Development, Auditory Task Stimulus, Fetal Functional Magnetic Resonance Analysis, Fetal Motion

Summary for Lay Audience

Functional MRI (fMRI) is a safe and non-invasive method to investigate the brain. Fetal fMRI provides the ability to investigate the developing brain of a fetus in utero. This thesis investigates areas of the fetal brain that are involved in auditory development such as the primary auditory cortex, putamen, and the middle cingulate cortex. Previous studies investigating fetal response to sound have placed magnetic resonance (MR) safe headphones on the abdomen of the mother. However, there has since been a recommendation to no longer do so. Thus, we proposed that by having the mother sing, representing the auditory stimulus, will activate the fetal primary auditory cortex. Nine pregnant volunteers underwent a stimulus-based fMRI. Our results suggest that out of the nine subjects analyzed, all nine had

activation on the right primary auditory cortex and seven out of nine subjects had activation on the left. It can be concluded that this internal auditory stimulus of having the mother sing, can be used to analyze the developing fetal brain.

Co-Authorship Statement

Estee Goldberg – Subject scans, data collection, data analysis and thesis writing.

Dr. Sandrine de Ribaupierre – Study design, data interpretation and thesis editing.

Dr. Roy Eagleson – Study design, data interpretation and thesis editing.

Dr. Barbra de Vrijer – Study design, and enrollment.

Dr. Charles McKenzie – Study design, and subject scans.

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List of Abbreviations

2D	Two Dimensional
3D	Three Dimensional
4D	Four Dimensional
1.5T	1.5 Tesla
3T	3 Tesla
AFNI	Analysis of Functional NeuroImage
BET	Brain Extraction Tool
BOLD	Blood Oxygen Level Dependent
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
$CMRO_2$	Cerebral Metabolic Rate of Oxygen
CRL	Computational Radiology Laboratory
EPI	Echo Planar Imaging
fMRI	Functional Magnetic Resonance Imaging
FT	Fourier Transform
FOV	Field of View
fMRI	Functional Magnetic Resonance Imaging
fNIRS	Functional Near Infrared Spectroscopy
FSL	FMRIB Software Library

GA	Gestational Age
GE	General Electric
GEM	Geometric Embracing Method
HASTE	Half- Fourier Acquired Single-Shot Turbo-Spin Echo
HG	Heschl's Gyrus
IUGR	Intrauterine Growth Restriction
LHSC	London Health Sciences Centre
MCC	Middle Cingulate Cortex
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
NMR	Nuclear Magnetic Resonance
RF	Radio Frequency
SPM	Statistical Parametric Mapping
SSFSE	Single Shot Fast Spin Echo
TE	Echo Time
TR	Repetition Time
US	Ultrasound Imaging

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1 Introduction

Pregnancy typically lasts 40 weeks and is split into three trimesters which are marked by specific fetal developments marked by a timeline. The first trimester is from week 1-13, the second from week 14-26, and the third from week 27-40 (1).

1.1 Fetal Brain Development

The fetal brain is a rapidly developing organ which grows and thrives in utero. It undergoes substantial structural and functional changes continuously throughout pregnancy with the brain being one of the first structures to form (1). Around five weeks gestational age (GA) the central nervous system begins to form when the notochord tissue infiltrates the embryonic disc and induces overlying embryonic tissue to thicken and fold, fusing to form the neural tube. By the sixth week GA, the neural tube closes and morphs into the forebrain, midbrain, and hindbrain. By the 12^{th} to 15^{th} week GA, most of the structures in the brain are in their final form such as the cerebral hemispheres, basal ganglia, thalamus, hypothalamus, midbrain, pons and medulla. The cerebellar vermis, neuronal migration from the periventricular germinal matrix, the development of sulci and gyri and myelination do not begin to develop until after the 15^{th} week of pregnancy. The corpus callosum develops around the 20^{th} week GA where it induces the formation of the cavum septi pellucidi and the cavum vergae. The cerebellum and vermis are formed around 22 weeks GA and the cortex undergoes complex development at the neuronal level and is mostly finished by 28 weeks GA (1–3).

The third trimester is the most critical period of brain development as myelination, neuronal organization, the development of dendrites and formation of synapses begin. The brain's surface area increases dramatically during this period as sulci and gyri begin to form (4). Brain development itself is not completed by the end of the gestational period and although these processes start in utero, they do not end there. Once born, the neonate

possesses billions of neurons, however, there are few connections or synapses between them as the brain is still developing post birth. Myelination and dendritic growth continue until the age of three with the brain growing three times the size since birth. Thus, the speed of structural and functional brain development does not slow until 3 years post birth (5).

1.1.1 Fetal Auditory Development

Auditory development is critical for cognitive development as it is a pillar for language and speech acquisition (6). The auditory system requires meaningful environmental sounds such as voice, language and music starting from the 28^{th}-30^{th} week GA (7). A study conducted by Webb et al. 2015 investigated exposure of recordings to preterm newborns prior to full term brain maturation and showed that the auditory cortex is more reactive to maternal sounds than environmental sounds after birth (8). A similar study done by Partanen et al. 2013 revealed that term newborns can react differently to familiar versus unfamiliar sounds they were exposed to as fetuses (9).

In utero, auditory development begins structurally around 15 weeks. Around 25 weeks GA the auditory system becomes functional as the ganglion cells of the spiral nucleus in the cochlea connect the inner hair cells to the brainstem and temporal lobe (2). Around the 28^{th}-30^{th} week GA, the neural connections to the temporal lobe become functional. This begins the development of the tonotopic columns within the auditory cortex which are imperative for interpreting, receiving, and reacting to sound (7). By 32 weeks GA the fetus is able to differentiate between male and female voices, phenomes, learn its mother's voice and recognize simple music after birth (3, 6, 7, 10). Thus, by the time of hearing, many neural events have occurred. Neurons from the primary auditory nuclei have developed and migrated to their final and desired destinations, axons have formed and connected to their desired nuclei, and dendrites have formed allowing functioning of synapses between neurons within the auditory network (11). In order to assess these processes, a reliable and non-invasive metric is required to determine if a fetus can hear in utero.

1.2 Fetal Imaging

Two- dimensional (2D) ultrasound (US) is regularly used for obstetric patients throughout the course of their pregnancy. A 2D US can provide the clinician and patient insight into fetal development. An US is used as a baseline metric to assess overall fetal health due to its availability, portability and low cost compared to other imaging modalities (1, 12). However, if an abnormality is suspected or viewed in a fetal US, magnetic resonance imaging (MRI) scan of the fetus is used to provide a more detailed view of the issue of interest due to its soft tissue sensitivities. A fetal MRI can provide clinicians with a more comprehensive analysis as to what may be present in the fetus, leading to a diagnosis and appropriate intervention if necessary (13).

1.2.1 Ultrasound Imaging

US is a prominent tool in obstetric care with an estimation that US was used in 68% of all pregnancies in 2002 (1). US is a safe, noninvasive and easily accessible technique to investigate the developing fetus (12). US is an accurate imaging modality that is conducted multiple times throughout a pregnancy and in real time (14). In the early 1960's US was brought into clinical use for pregnancy and since then, US is typically conducted throughout the pregnancy. US in the first trimester is used to help with pregnancy dating, assessment of bleeding and pain, and nuchal translucency in screening for aneuploidy. Within the second trimester, US is used to assess interval growth and routine survey of fetal anatomy, such as the head and spine of the fetus. During the third trimester, US is predominantly used to assess fetal growth and wellbeing. An emerging system in fetal US is three-dimensional (3D) sonography which can provide a volumetric assessment of the fetal anatomy (1).

1.2.2 Magnetic Resonance Imaging

MRI of the fetus is an invaluable obstetric diagnostic tool due to its soft tissue sensitivity, larger field of view compared to ultrasound, and a multitude of imaging sequences to

provide the most detailed image of the desired area of interest. Fetal MRI is most commonly used to assess the developing fetal brain, but, it can be used to assess any region or pathology in the fetal body (13). The soft tissue sensitivity of MRI allows for the detailed view of the developing brain in order to aid in diagnosis and potential treatment. For example, fetal MRI can aid clinicians in assessing the method of delivery of the fetus, a detailed view of the placenta, or potentially aid in decisions or planning of surgical interventions (13). Previously, pregnancy has been a contraindication of MRI due to potential claustrophobia or the difficulties and potential worry for the mother and fetus; however, MRI is a safe imaging modality to use during pregnancy (13, 15).

1.3 Magnetic Resonance Imaging

MRI is a widely used and powerful imaging modality due to its flexibility and sensitivity to a wide range of tissue properties. MRI is a safe, non-invasive metric to assess different diagnoses for individuals of all ages as it can provide detailed anatomical images without the use of ionizing radiation (16).

The field of MRI began in the 1940's when researchers discovered that hydrogen nuclei rotate at a precise frequency, which depends linearly on the magnitude of the field (17). However, MRI did not take off clinically until the 1980's, and since then, it has become a vital component for diagnostic care. MRI relies on the capability to manipulate the contrast of the region of interest in order to detect the precession of hydrogen spins in water, fat, and tissues. This can be achieved in MRI as the measured signal is dependent on the tissue properties of interest. One can therefore manipulate the image to achieve the correct contrast for the region of interest which is unlike any other imaging modality. With MRI, the image is a map of the local transverse magnetization of the hydrogen nuclei which is dependent on several intrinsic properties of the tissue (16, 17).

1.3.1 Motion Correction for Anatomical Fetal MRI

Fetal motion in MRI is a dubious task and a relevant problem in all fetal MRI studies as this problem does not only exist in fetal MRI but in adult and pediatric studies as well.

Motion correction for MRI has revolutionized the way we understand and visualize our images. Such advancements have even progressed to the domain of fetal MRI, where groups have been able to recover intra-slice motion through the assumption of rigid head motion and through an estimation of the pattern of fetal trajectories (18). Research has been conducted using a two-step process to develop different computational achievements to estimate intra-slice fetal head movement that can be recovered into the 3D positioning of each slice (18–21). Reconstruction of 3D volumes of the fetal brain has been completed by intersecting acquisitions of motion corrupted stacks of 2D slices (22). Bonel et al. 2008 implemented a prospective acquisition correction which was conducted in real-time during the scan using a navigator (23). Without proper localization of the fetal head, the images cannot be acquired as the navigator must be repositioned. This increases the total amount of time the mother and fetus are the scanner which is not desired. Using this navigator-based approach added on average six seconds per slice when acquiring on average 30 slices, resulting in an average time of three minutes per half-Fourier acquired single-shot turbo-spin echo (HASTE) sequence when the usual time is roughly 30 seconds for the number of slices. This approach increases scan time, which can greatly reduce the number of images acquired. Their scan time was planned for 40 minutes but stopped at 50 minutes if there was a delay (23). Ultimately, these groups have tried the methods and approaches outlined in attempts to combat fetal motion in anatomical MRI.

1.4 Functional Magnetic Resonance Imaging

Functional Magnetic Resonance Imaging (fMRI) is a non-invasive imaging modality to determine regional and time-varying changes in the brain (24, 25). fMRI is a powerful tool used to understand functional behavior and to understand how neural activity couples with the Blood Oxygen Level Dependent (BOLD) signal (25).

1.4.1 Echo Planar Imaging

Echo Planar Imaging (EPI) was one of the first imaging methods to be proposed by Sir Peter Mansfield in the 1980's. EPI is now extensively used in neurological imaging through fMRI and diffusion imaging (17). It is a very fast MRI technique capable of acquiring an entire MR image in only a fraction of a second. In single-shot EPI, all the spatial-encoding data of an image can be obtained after a single radio frequency (RF) excitation and the total acquisition time to collect k-space is in a single shot (16, 26). In single shot-EPI, repetition time (TR) is effectively infinite thus one can have a high T_2^* weighted contrast with no T_1 contribution at all. In single shot-EPI, image slices are acquired sequentially, a whole slice at a time from a single RF excitation as shown in Figure 1.4.1. In fMRI the echo time (TE) is the time between the RF pulse and the collection of data encoded to the center of k-space as shown in Figure 1.4.2 (17). For EPI all of the data is encoded into k-space from one single excitation. TE's for EPI typically range between 20 and 60 ms and are an important parameter as a TE is chosen to maximize the BOLD sensitivity. This is what helps to determine its signal to noise ratio (SNR) and contrast in the final image. SNR is dependent on the resolution, as voxel size is directly proportional to SNR (17, 25).

EPI offers major advantages over conventional MR imaging as it reduces imaging time, decreases motion artifact and increases the ability to image rapid physiological processes of the human body. The use of EPI has already resulted in significant advances in clinical diagnosis and scientific investigation, such as in functional imaging of the human brain, heart and abdomen (17).

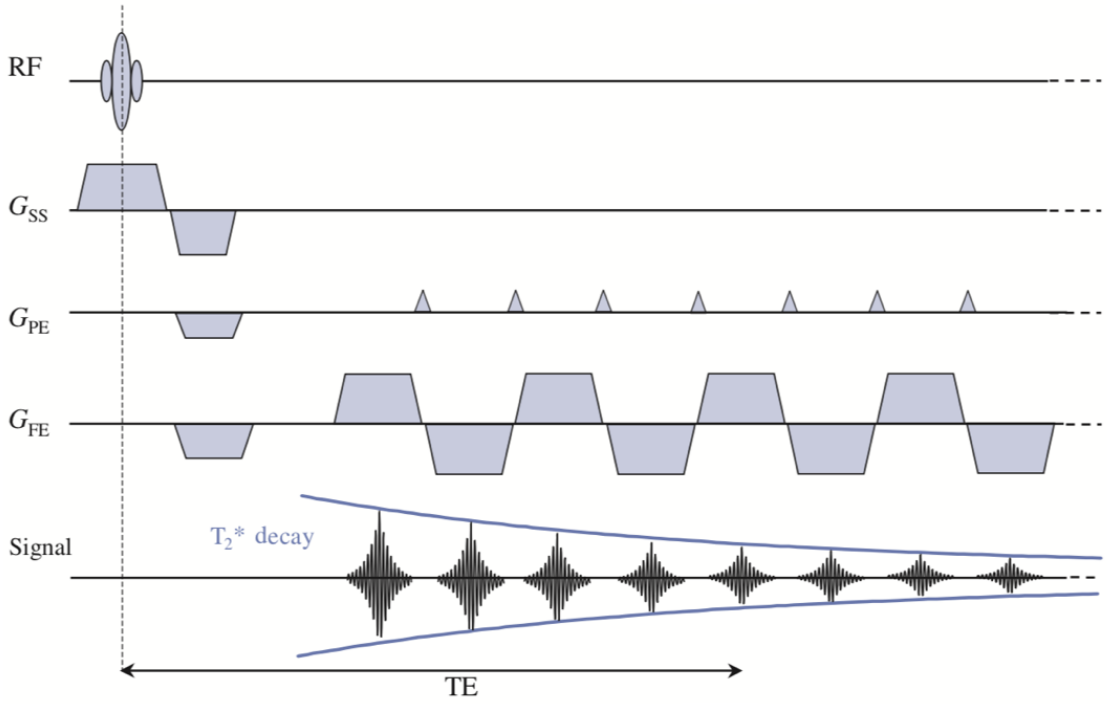


Figure 1.4.1: Example of a Single Shot GE-EPI Sequence from Picture to Proton with permission in Appendix I.

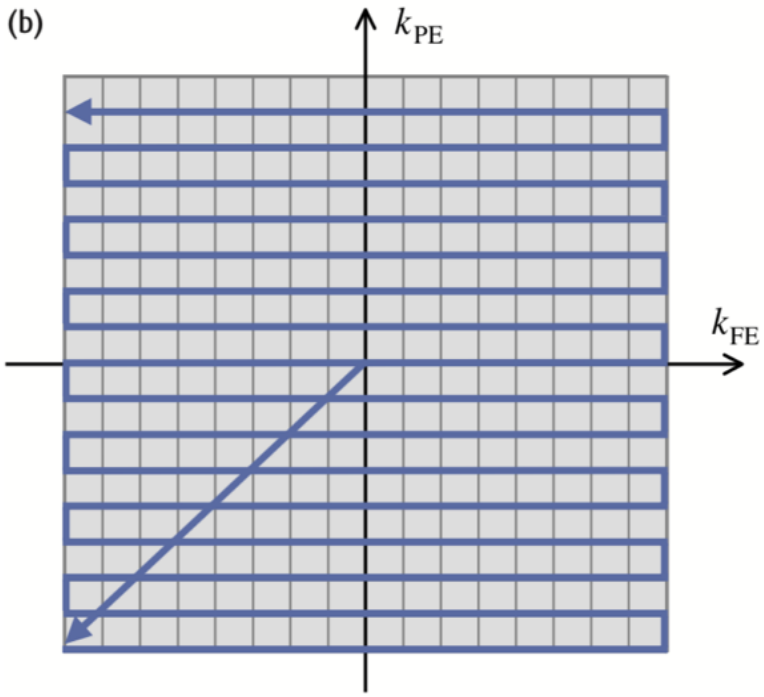


Figure 1.4.2: K- space trajectory of GE-EPI blipped sequence from Picture to Proton with permission in Appendix I.

1.4.2 The BOLD Effect and Hemodynamic Response

Contrast agents can be used to manipulate the susceptibility of the blood to investigate different physiological processes by the researcher or physician. Deoxyhemoglobin, or deoxygenated blood, is used as the contrast agent in fMRI studies. Oxyhemoglobin has the same magnetic susceptibility as brain tissue in comparison to deoxyhemoglobin which is paramagnetic (17). The presence of deoxyhemoglobin changes the magnetic field susceptibility causing the distortions within the magnetic field, affecting the T_2^* in the tissue around the blood vessels (17, 24, 25, 27). When there is an increase in oxygenated blood, there is a decrease in the amount of deoxyhemoglobin present. The change in the ratio of oxygenated and deoxygenated blood is the BOLD response. This effect is the basis of the BOLD contrast, meaning that deoxygenated blood has a shorter T_2^* value and a lower MR signal than fully oxygenated blood. The BOLD effect is widely

used for mapping patterns of activation in the human brain (17, 24, 25, 27). This effect depends not only on the total amount of deoxyhemoglobin within a voxel but on the change of the amount of oxygen within the blood and the changes of overall blood volume itself (24, 25).

It is important to establish the distinction that it is not the BOLD signal and the changes of deoxygenated to oxygenated blood, that are measured during the task phase. The BOLD effect is sensitive to the changes in cerebral blood flow (CBF), the cerebral metabolic rate of oxygen ($CMRO_2$), and the overall cerebral blood volume (CBV). The grouping of CBF, $CMRO_2$, and CBV is known as the hemodynamic response (27). The hemodynamic response represents a rate of change of the BOLD signal in response to a stimulus. Additionally, the interpretation of the BOLD signal is dependent on the accuracy of the localization and can be improved only at the expense of scanner sensitivity. In magnets with a higher field strength, the larger signal changes are usually ignored with the smaller changes resulting in the BOLD signal used for activation mapping. In magnets of weaker field strength, such as 1.5T, the small-signal changes are so impactful to the final outcome that disregarding the larger signal changes would greatly impact the overall activation map (24). Thus, similarly to traditional MRI, BOLD requires trade-offs to ensure proper specificity, sensitivity, and effective localization for a successful acquisition.

The BOLD effect during activation is shown in Figure 1.4.3. Although there is a greater number of deoxygenated red blood cells following neuronal activity, the increase in oxygenated blood delivery results in a reduction of the concentration of deoxyhemoglobin and therefore T_2^* increases along with the MR signal (17).

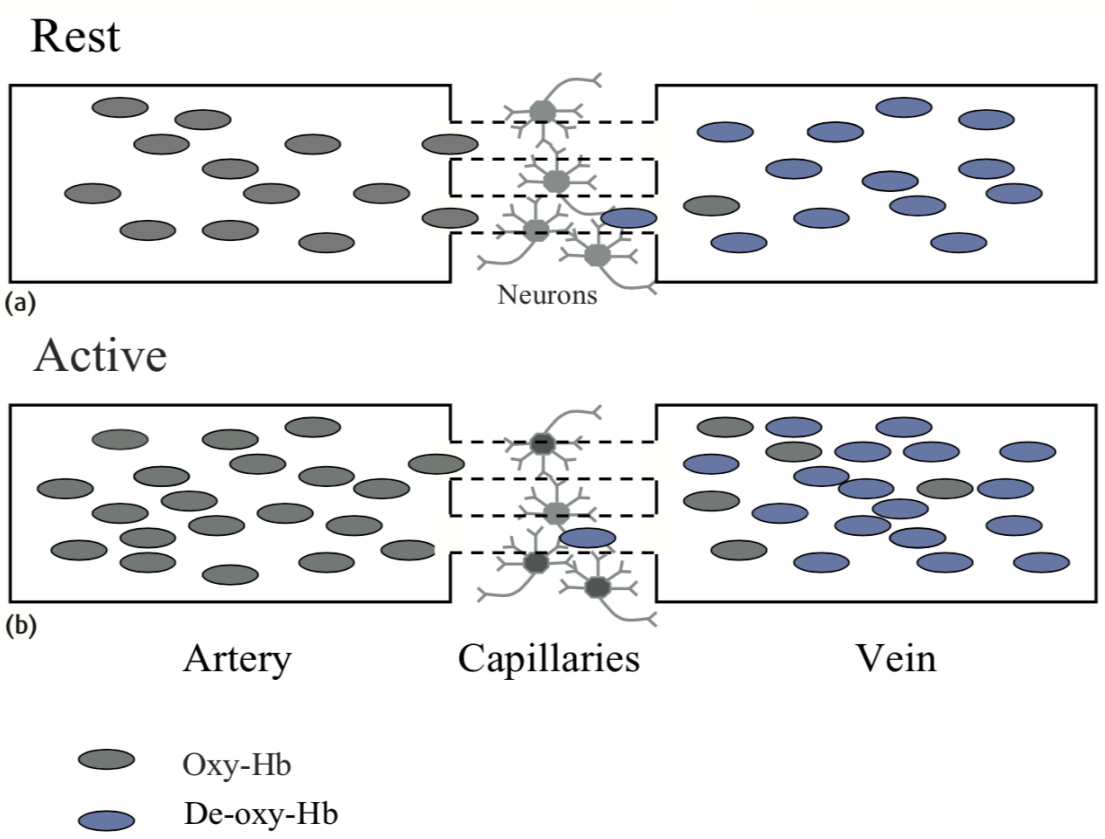


Figure 1.4.3: (a) Shows the origin of the BOLD effect in the rest phase, where there is an increase in deoxygenated blood, decreasing the T_2^* and MR signal. (b) The BOLD effect during activation is shown where following neuronal activity there is an increase in oxygenated blood, which causes a decrease in deoxygenated blood increasing T_2^* and the MR signal. Figure from Picture to Proton with permission in Appendix I.

1.4.2.1 Task and Resting State

The small changes in brain activation that are detected by the MR signal through the BOLD effect have been widely used to study functional connectivity of the brain. An fMRI experiment can be designed as either task based or as resting state. During the task phase, subjects in the scanner are instructed to conduct a task, such as speaking aloud in response to a visual or auditory stimulus or moving a body part (i.e. tapping their fingers). By conducting these tasks at specific time points, investigators are able to

determine the changes through the BOLD effect to determine which areas in the brain are active during the task phase. This produces activation maps which are attained by comparing the difference of signals between resting and the task phases. Task fMRI helps to determine information regarding which areas in the brain are more or less active during specific tasks. During a resting state fMRI, subjects are not instructed to do anything and simply lay there like one would for an anatomical scan (28). During resting state, the fMRI is looking at the fluctuations within the signal that are correlated to one another.

Task fMRI can be designed in two ways to evoke a stimulus, block design and event related design shown in Figure 1.4.4. A block design paradigm is when there are one or more conditions that are alternated to show the differences between the two, such as a rest block which is used as a control and the temporal stimulus pattern is similar to a square wave. Event related designed paradigms are designed in a non-structured way meaning that they are created by evoking randomized stimuli at non-consistent time periods (28, 29).

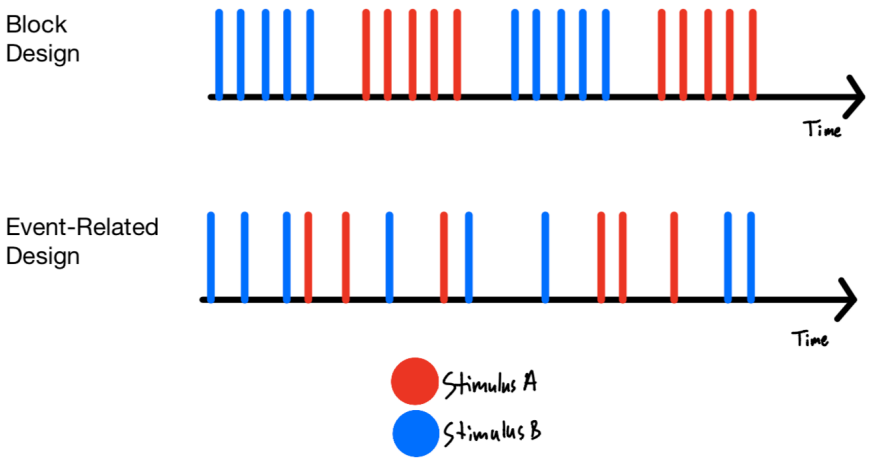


Figure 1.4.4. Top: Block design style of fMRI task paradigm with two blocks of stimulus A and two blocks of stimulus B with four rest blocks in between. Bottom:

Event related design where there are two different stimuli shown in red and blue that are presented in a randomized order, and at varying time intervals with no consistency.

Both task and resting state fMRI can be used when looking at groups of subjects. This can show differences in activation patterns not only on a subject-subject level but within an entire group. Traditionally, task fMRI studies use groups of subjects to compare differences, for example, patient groups compared to control or normal groups. In such, the aim of task fMRI data is to identify small changes that are spatially localized in image intensity due to an experimental task. This is done by collecting a series of images covering the entire brain or the majority of the brain at intervals in seconds and analyzing the results of each voxel that are obtained at that specific time period.

1.5 fMRI Motion Correction

fMRI is highly susceptible to subject motion due to the fast imaging EPI sequence. Subject motion is one of the largest concerns in fMRI acquisitions with rotational and translational head motion being the most common issue to combat. Such head motion results in discrepancies in localization of the anatomical brain, impacting the voxel signal and quality. Motion correction for fMRI was noted by Jiang et al. 1995 as the influence of head motion from the subject during the image acquisition impacted the validity of activation within specific voxels (30). There are many algorithms available for motion correction within fMRI packages such as Statistical Parametric Mapping (SPM), FMRIB Software Library (FSL), Analysis of Functional NeuroImage (AFNI) and BrainVoyager (31–34). Within the programs mentioned there is the basis of image registration, where the fMRI data is aligned to an anatomical template or atlas to ensure comparisons between volumes and subjects are consistent. Additionally, these programs all use some form of a motion correction algorithm with six degrees of freedom (three rotational, three translational). They work by assuming the idealized voxel function based on each image in relation to each other by using interpolation to remove motion and are under the branch of image alignment. Image alignment is imperative for fMRI motion correction

and can be broken down into three steps. First, an algorithm determines the error margin or the differences between the image and the atlas. Second, determination of spatial transformation is done to move the image to adhere to the atlas. The last step of this process is the interpolation of the fMRI data based on the spatial transformation of the previous step. This allows the creation of a new image and is continued throughout the entire dataset. These steps are the basis for all motion correction (image realignment) algorithms for fMRI and can be applied for adult, pediatric, and even fetal data. It is important to note that these algorithms assume that there is motion only once per volume, disregarding the potential intra-slice misalignment within each volume (25). Ultimately, subject motion remains a daunting task for fMRI researchers as there are many avenues and programs available to minimize the effects of motion. At this point in time there is no complete solution to eliminate or avoid motion entirely as subjects do inherently move if conscious, thus employing the need for motion correction in fMRI.

1.6 fMRI in the Fetus

fMRI is a non-invasive method to investigate the neural correlates of brain development and studies have used fMRI to assess fetal brain activity (35, 36). Fetal fMRI is more challenging in comparison to adult fMRI as the fetus cannot be instructed to remain still for the length of the scan and is likely to move between image acquisitions. Images that are longer in duration are more susceptible to larger amounts of motion. This implies that in order to accurately produce effective data using reconstruction and motion correction tools, a fast image acquisition protocol must be in place. Fast MRI sequences allow a snapshot of images within individual slices to be acquired quickly enough to almost freeze the subject while there is motion, such as Single Shot Fast Spin Echo (SSFSE) sequences (37). In an ideal scenario, using virtually motion free data stacks of data from slices of SSFSE's with good image quality can be realigned to reconstruct corrected volumetric data mostly hassle free; yet this is not the case for fetal MRI or fMRI.

Traditional anatomical motion correction pipelines have not been implemented and modified for fetal fMRI nor do they pose the capacity to accommodate the significant amount of motion a fetus may generate during the image acquisition. The reasonable algorithm option for fetal MRI is to utilize a slice-to-volume reconstruction algorithm where manual implementation and intervention of a skilled user are required to accurately select the correct registration template for anatomical MRI (38–40). Motion correction algorithms for fetal fMRI are unchartered territory at this point with in-house programs dominating the field. These programs are challenging to recreate on a different computer as they were designed for specific data on their specific machines.

Open source and widely available programs such as ITK-SNAP are excellent for minor motion of anatomical images, although the program is not designed nor can it accommodate multiple fMRI volumes. This program is a suitable alternative when dealing with anatomical NIFTI files where there is only one volume for the entire dataset (41). As already mentioned, fetal movements cannot be avoided by the researcher or clinician and thus the anatomical data can present with intra-slice movement despite the use of fast MR sequences. The manual registration tool within ITK-SNAP can accommodate registration of the anatomical volume to a fetal brain atlas (41). ITK-SNAP for fetal data is challenging to use in terms of reconstructing and maintaining the integrity of the individual slice and it would therefore have to be reconstructed in a program such as 3D Slicer (42). 3D Slicer is an open-source program that can accommodate DICOM images and was designed for segmentation with the ability to input your own algorithm and program though Python or MATLAB (43, 44). There are a multitude of downloadable extensions that have been previously established for brain motion in MRI such as SkullStripper, Resample Image BRAINS, Crop Volume, Transforms and Landmark Registration (42). Within 3D Slicer, entire DICOM files can be loaded into the program and can convert them into NIFTI files. Other automated programs have been created that provide excellent results for anatomical fetal MRI data, however, these algorithms and programs were built to accommodate structural MRI.

Several research groups have investigated both task and resting state fetal fMRI. In regards to fetal task fMRI, Jardri et al. 2008 utilized an auditory stimulus by placing MRI compatible headphones on the maternal abdomen and Fulford et al. 2003 sought to invoke a visual stimulus by using a red LED at the front of the fetal face (35, 45). Both studies had difficulties in analyzing their data due to the severe motion of the fetus (35, 45, 46). Other research groups such as Thomason et al. 2013 forwent a task approach and have conducted many resting state studies on large cohorts of fetal subjects as they are interested in how different areas in the fetal brain are connected to one another (47).

Additionally, it is important to note that fetal imaging is uniquely challenging as the parameters are not strictly defined compared to traditional adult or pediatric fMRI depending on what the target image is. Studies looking at the resting state fetal fMRI on a 3T Siemens scanner by the Thomason group used a TR of 2000 ms and a TE of 30 ms (36, 47, 48). While groups using a 1.5T scanner with similar GA's had a larger spectrum of TR and TE values. Blazejewska et al. 2017 used a TR of 3000 ms and a TE of 43 ms and 100 ms, while You et al. 2016 had a TR of 2000 ms and 3000 ms with a TE of 1000 ms (49, 50). Ferrazzi et al. 2014 implemented a TR of 4000 ms and a TE of 50 ms, and Jaradi et al. 2008 used a TR of 3000 ms and a TE of 80 ms (35, 51). It is important to note that the Blazejewska, Ferrazzi, and Jaradi groups all used a Phillips Achieva scanner except for the You group which used a General Electric (GE) scanner (35, 49–51). From all of the discrepancies, it is evident that there is a lack of uniformity when it comes to TR and TE for fetal fMRI. Groups are still evidently searching for the best possible fetal brain fMRI parameters for their studies.

1.6.1 Task and Resting State

Imaging and assessment of functional norms in utero are challenging due to random fetal and maternal motion, maternal respiration, the small fetal brain, the high water content in the fetal brain compared to adults, and the fact that the head of the fetus is deep within the mother far from the receive coils. Studies using fetal brain fMRI are typically limited to resting state or non-stimulus-based fMRI due to the difficulty to instruct or provide a

stimulus for the fetus. In traditional adult task fMRI, the participant is instructed with a task to complete. Fetal task fMRI can be challenging solely due to the inability for a fetus to conduct or be instructed as to how to do a task. As such, fetal task-based fMRI is understood to be stimulus based, as the fetal brain can react to the stimulus presented. Since there has been a recommendation not to apply sound or visual stimuli on the maternal abdomen due to safety concerns, stimulus based fetal fMRI has been challenging and more researchers have been opting to investigate fetal resting state networks (47). Resting state is typically easier to conduct within fetal fMRI as the scanner is simply acquiring the data. There is no need to coordinate a stimulus with the mother, removing an added complexity to the scenario. However, due to the nature of random fetal motion, both resting and stimulus based fetal fMRI is challenging to acquire and analyze. Ultimately, both study designs require a motion correction phase prior to analysis and some entire data sets, or individual volumes will need to be discarded based on the severity of the motion.

We sought to investigate a reliable stimulus-based paradigm to study normal development of fetal brain networks. Fetal stimulus design fMRI can be successful regardless if the fetus is awake or asleep due to the ability to hear while sleeping (52). A study conducted on sleep-wake cycles for normal fetuses between 30-40 weeks GA showed that within one hour of recording, fetuses spent 74% of their time in an active state and 26% in a quiet state (53). Thus, the fetus can hear both in awake and asleep states resulting in activation of the fetal brain.

Previous fetal fMRI stimulus-based studies have demonstrated temporal lobe activation in response to a direct auditory stimulus; however, since these studies have been published, there has been a recommendation not to apply a direct stimulus to the mother's abdomen due to potential risks to fetal hearing in utero (35, 46, 54–56). A normally occurring alternative to applying a direct auditory stimulus is to have the mother sing. We postulate that this internal auditory stimulus would result in activation in the fetal primary auditory cortex. This pilot study was conducted as a proof of concept to verify that an

internal stimulus would be able to activate the primary auditory cortex of the fetus. This would allow researchers to have a foundation of normal baseline responses from a reliable paradigm to carry further studies and compare healthy versus at risk groups.

1.7 Thesis Objectives and Hypothesis

We hypothesize that an internal auditory stimulus would invoke fetal response within the fetal primary auditory cortex. The objectives of this thesis are to: 1) develop a motion correction pipeline for fetal stimulus-based fMRI, and 2) to verify that an internal auditory stimulus would be able to activate the primary auditory cortex of the fetus.

2 Methods

2.1 Inclusion and Exclusion Criteria

Nine healthy volunteers with uncomplicated, singleton pregnancies and a GA of 33-38 weeks (mean 36.5 GA weeks) were recruited from London Health Sciences Centre (LHSC), Victoria Hospital. Subjects under the age of 18 years, contraindications to MRI, carrying multiple fetuses during the pregnancy and known fetal anomaly or demise were excluded from study participation. MRI occurred during the late third trimester of pregnancy to minimize fetal motion. Subjects were either imaged at Western University's Robarts Research Institute (n=4) or LHSC, Victoria Hospital (n=5).

2.2 Stimulus Design and Fetal fMRI Paradigm on 3T and 1.5T Scanner

Subjects were imaged on a 3T (GE MR750) with a 32 channel GE torso coil and a 60 cm bore at Western University's Robarts Research Institute and a 1.5T (GE MR450w) with a Geometric Embracing Method (GEM) posterior and anterior array coil with a 70 cm bore at LHSC, Victoria Hospital. T_2-weighed SSFSE anatomical images (SSFSE– TR >1200 ms, TE 81.36-93.60 ms, voxel size 0.98*1.96*8 mm^3 and 0.125*0.17*9 mm^3) were acquired prior to the fMRI for the fetus to become familiarized to the sound of the scanner and to localize the position of the fetal brain. Two task-based block design

BOLD fMRI series were conducted with a TR of 2000 ms, a TE of either 45 or 60 ms on the 3T scanner (located at Western University's Robarts Research Institute) and either 60ms or 90 ms on the 1.5T scanner (located at Victoria Hospital). Based on the literature review described in section 1.6 of this thesis, there was little consistency between research groups pertaining to TE values as specific TE of the fetal brain are changing due to the changing GA. The MR physicist assisted in the determination of the best TE for our subjects and scanner. We used two different TE values on both the 3T group and the 1.5T group to determine the differences between the different TE's. The flip angle was 70º, and the voxel size was 3.75*3.75*4 mm^3 with 22 slices per volume on the 3T scanner. The series were acquired while the mother was singing a lullaby ('Twinkle Twinkle Little Star' or 'ABC's') during the task phase. The block design paradigm consisted of 10 seconds of rest followed by 15 seconds of task where the mother was singing the lullaby aloud while listening to the same lullaby through MR safe headphones. Frequent checking and monitoring were conducted during the acquisition to assure that the mother was singing effectively. The sequence was repeated nine times resulting in 112 volumes per dataset for a total scan time of three minutes and 44 seconds for each fMRI acquisition.

2.3 Processing Pipeline

The motion correction pipeline uses SPM 12 (v7219), and FSL, MRIcroGL's dcm2niix, and ClearCanvas Workstation prior standard preprocessing (31, 32, 57, 58). Standard preprocessing involves using tools from SPM 12 (32). Once this was complete, image realignment was conducted through SPM 12 and FSLeyes followed by co-registration within SPM 12 (32, 59). A breakdown of the preprocessing pipeline can be visualized in Figure 2.3.1. After alignment is sufficient, a first level analysis is conducted using SPM 12 (32).

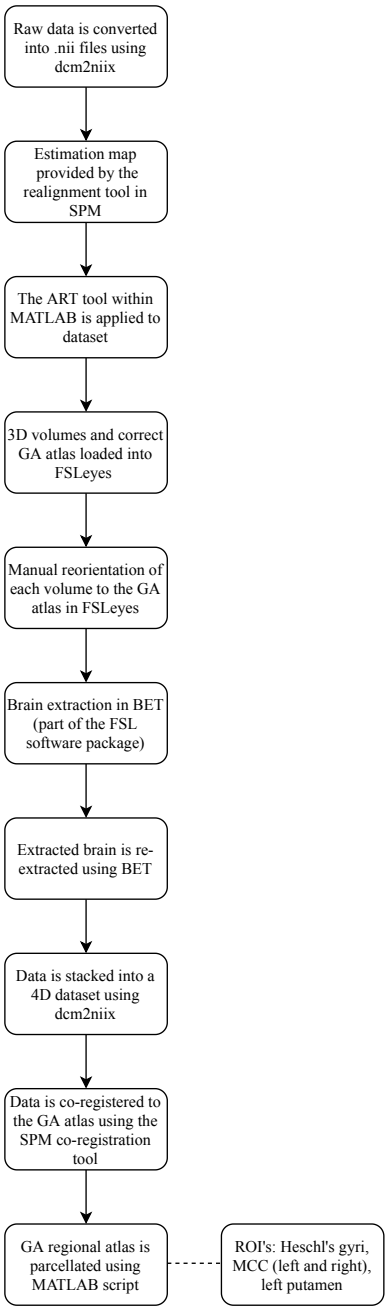


Figure 2.3.1. Preprocessing Pipeline for Fetal fMRI.

2.3.1 Image Conversion

Once the data was acquired, the data was uploaded onto ClearCanvas Workstation which converts the unusable raw files into usable files known as DICOMs (58). Next, all the images in the series were viewed to confirm the correct number of images were within

each given stack as well as to assure integrity of the image, specifically in regard to fetal motion. After visualization, the scans were then filtered by series number and the converted DICOM files were saved into an individualized file for each series acquired during the scan. MRIcroGL's dcm2niix was used to convert the DICOM files into an fMRI friendly format (i.e. NIFTI or .nii files) for programs such as SPM and FSL (57). Standard practice for traditional adult or pediatric fMRI is to convert volumes into 4D.nii volumes through FSL/SPM 8, however due to the challenging nature of fetal imaging, our data was initially manipulated, and motion corrected using each individual 3D.nii volume. The significant motion in fetal imaging and the ability to work with individual volumes and discard them if necessary, instead of discarding the entire data set. To visualize the motion between each volume, the 3D volumes were converted into a 4D stack using dcm2niix FSL/SPM 8 format resulting in 4D.nii volumes. The 4D volumes were converted to see the entire data set for movement between volumes while the 3D is used to detect motion between slices.

2.3.2 Realignment and Manual Reorientation

Each volume was assessed for unpredictable fetal motion by inputting the data into SPM 12's image realignment tool prior to manual reorientation (32). The realignment tool accounts for the changes in signal intensity over time which can arise from motion. The realignment tool estimates six parameters of an affine rigid body transformation that minimizes the differences between each slice by applying the transformation of resampling the data. The tool provides an estimation map indicating the amount of translational and rotational motion for all the volumes in the dataset and provided a coordinate system and position for each volume. To have a greater understanding of which volumes to remove, a program for post-processing of fMRI data called Artifact Detection Tool (ART) was used for each subject after manual reorientation (60). The ART tool provides an assessment of the data and indicates which volumes have too great of a signal intensity and greater than two mm of movement. The tool displays the outliers and volumes that should not be included in the analysis.

Once these motion estimation maps were produced and the ART tool was run, the 4D dataset that was previously mentioned, was loaded into FSLeyes and played in a movie format to visualize all the volumes within the dataset (59). Based on this, the volumes indicated by the ART tool with too great signal intensity and/or motion are recorded and not yet removed to avoid confusion and mistakes as when volumes are removed and stacked, the volume numbers change accordingly. The 4D dataset is removed from FSLeyes and each 3D volume is loaded into the program along with the correct GA fetal brain Computational Radiology Laboratory (CRL) atlases (59, 61). The CRL atlases are already in the correct voxel space and registering our data is a necessary preprocessing step for fMRI analysis (62). Each volume was realigned and reoriented to the atlas using the coordinates provided from the estimation map as well as the coordinates of the atlas itself. It is important to note that these volumes were rotated and not reconstructed eliminating the need for an additional reconstruction algorithm. The 3D volumes that were reoriented to the atlas were stacked into a 4D dataset using dcm2niix for co-registration and a first level analysis in SPM 12 (32, 57). For visualization of reorientation, estimation maps are provided for before and after reorientation in Appendix H for each subject. Based on the results of these estimation maps, initial manual reorientation, and the results of the ART tool, the volumes with too much motion, and too great a range of mean signal intensities were not included in the analysis (60).

2.3.3 Brain Extraction and Co-registration

Once the data was manually reoriented and aligned to the CRL's respective GA atlas, the 4D data was input into FSL's Brain Extraction Tool (BET) (31, 62, 63). Since BET was not able to accommodate the small fetal brain size, each volume underwent a second round of BET where the data was once again input into FSL's BET to obtain tighter margins around the fetal brain. The first round of BET removed the maternal abdomen and surrounding tissues, while the second round of BET provided a reasonable segmentation of the brain. The functional 4D fetal data was then co-registered to the fetal atlas in SPM 12 using the co-registration tool after being reoriented in FSLeyes (32, 59).

2.4 Atlas Parcellation

To identify the regions of interest, each CRL regional fetal brain atlas example shown in Figure 2.4.1. was parcellated to determine which specific regions were active during the stimulus-based phase. Our subjects ranged from 33-38 weeks GA and therefore six separate regional fetal brain atlases were parcellated into 124 regions with the MCC shown as an example in Figure 2.4.1. The 124 regions are listed in Appendix D, but for this study our areas of interest were the right and left Heschl's gyrus (HG), the right and left middle cingulate cortices (MCC) and the left putamen. A script was written using MATLAB to parcellate each region in order to be loaded individually into FSLeyes (Figure 2.4.2.). The script for atlas parcellation is available in Appendix E.

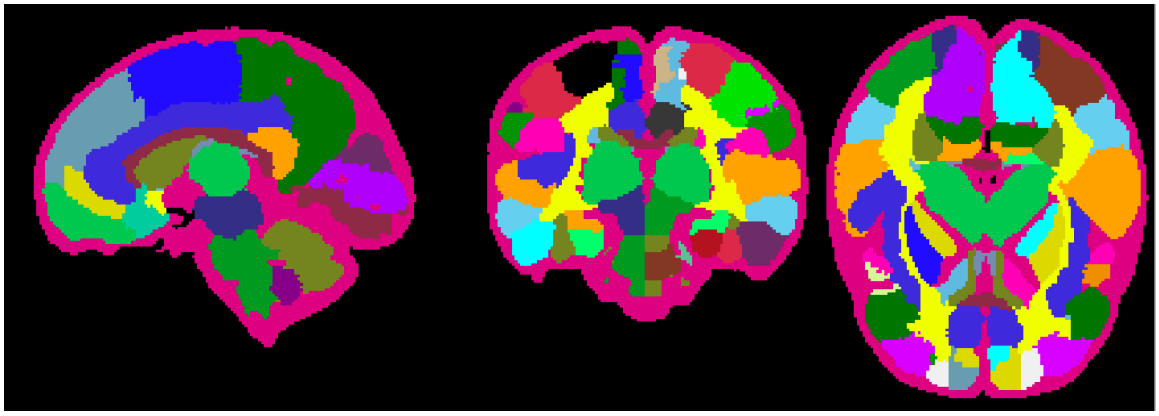


Figure 2.4.1. 37 GA regional fetal brain atlas from the CRL. Each region in the brain is highlighted in a different colour.

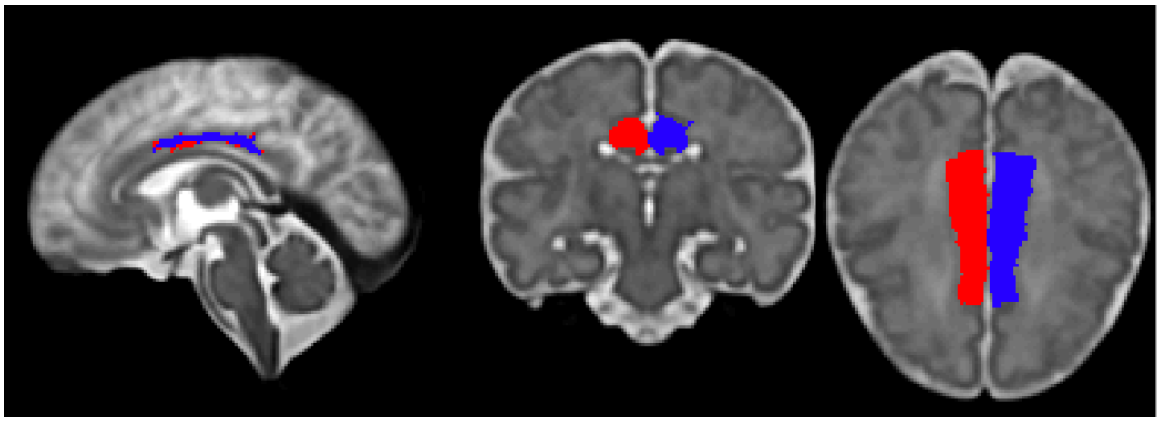


Figure 2.4.2. CRL's 37 GA fetal brain atlas with a parcellated region, with the right MCC shown in red and the left MCC shown in blue.

2.4.1 Analysis

Once the processing pipeline was completed, the segmented functional data was analyzed using SPM 12 as a stimulus fMRI (p uncorrected < 0.05) using a first level single subject analysis (32). The volumes that were not to be included in the analysis were not included in the data selection, and the paradigm was adjusted accordingly (i.e. if volumes 1–3 were removed, the first onset of stimulus was no longer 10 seconds where the TR is 2 seconds as mentioned in section 2.2, the first onset of the stimulus block would be at 4 seconds instead). The First Level Analysis in SPM uses the General Linear Model of $Y = X * \beta + e$. Where Y is defined as the BOLD signal, X is the design matrix (this study used block design), β is the matrix parameters, and e is the error matrix. The activation was found by using the block of activation and subtracting the rest block from the paradigm to acquire the T contrast for all the voxels present in the brain. To identify voxels whose activation increased in response to the stimulus, a T contrast was used for all subjects (32). The respective CRL GA regional atlas was parcellated using a script in MATLAB, available in Appendix E and mentioned in the section above, to assess which regions in the brain were active during the stimulus phase (61). FSLeyes was used to assess the activation for each subject with the correct CRL GA anatomical atlas loaded in primarily, a sagittal view of a 35 GA fetal atlas shown in Figure 2.4.3 as an example.

Each region of interest from the parcellated CRL regional atlas was overlaid onto CRL anatomical atlas shown in Figure 2.4.4. Lastly, the activation map was loaded into FSLeyes overtop of the parcellated regional and anatomical atlases shown in Figure 2.4.5., with the Z score minimum adjusted to 1.96 (this is the equivalent of a p uncorrected < 0.05) (59). The activation was co-registered to the atlas in order to pinpoint which regions had activation during the listening phases. The regions were then assessed with the Z score recorded for regions such as the HG, the MCC and the putamen.

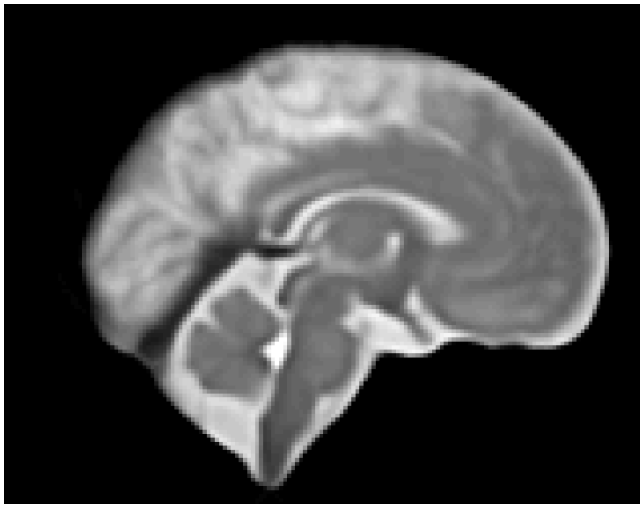


Figure 2.4.3. Sagittal view of the CRL fetal brain atlas at 35 GA.

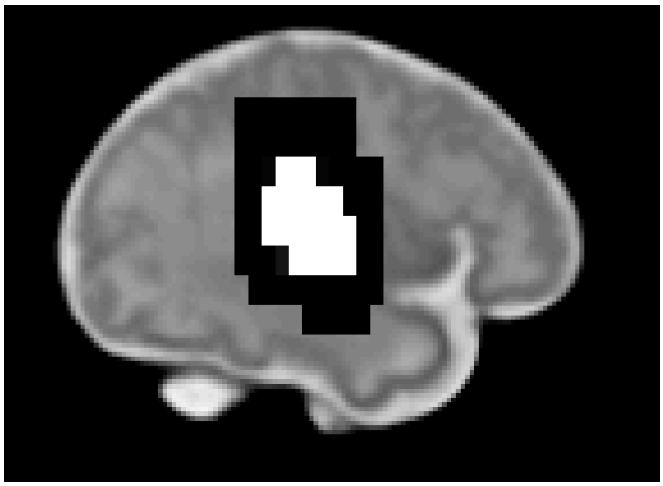


Figure 2.4.4. Sagittal view of a 35 GA fetal brain atlas from the CRL's group with the left HG shown in white, with a black region behind it to highlight the area of interest.

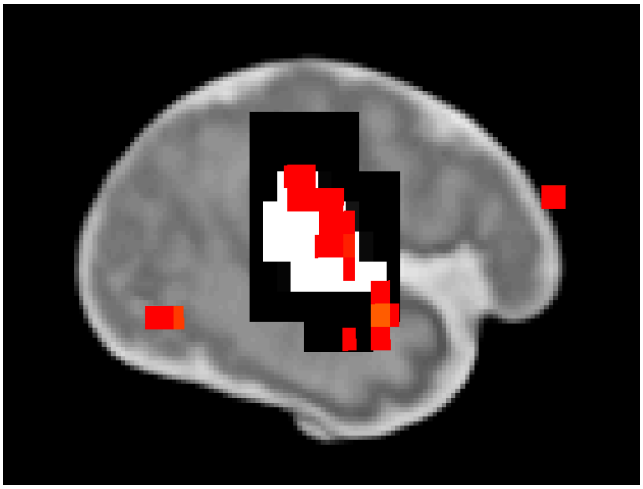


Figure 2.4.5. Sagittal view of a 35 GA fetal brain atlas from the CRL's group with the left HG shown in white, with a black region behind it to highlight the area of interest. The orange/red activation on top of the white HG indicates that there is activation in that region for Subject 6.

3 Results

3.1 Fetal and Maternal Demographics

The maternal demographic characteristics and fetal birth outcomes are detailed in Table 1. This study consisted of nine fetal subjects (mean age of mother 36.33±4.29 years; age range 28-41 years; fetuses imaged mean GA 36.14±1.40 weeks; GA range 33-38 weeks). Two (1 male, 1 female) out of the 9 (5 male, 4 female) fetuses were born preterm (36.4 and 36.9 weeks GA), while the remaining fetuses were delivered at term (mean GA 38.57±1.50). One subject was carried by a mother who had a Body Mass Index (BMI) > 30 km/m^2 and gestational diabetes (Subject 3), while another subject measured small for gestational age (Subject 1) with known intrauterine growth restriction (IUGR) and had a scheduled caesarean section.

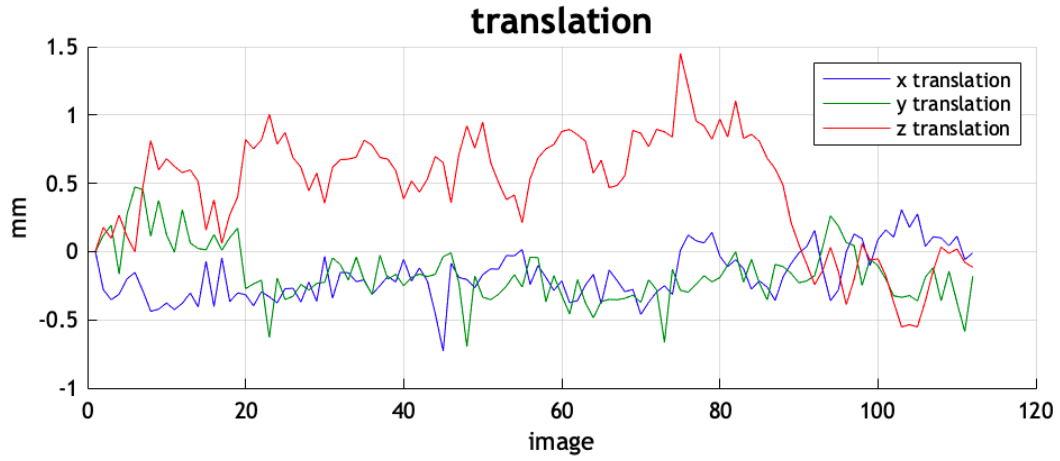
Table 1: Fetal and maternal characteristics including fetal MRI and birth GA, sex of subject, birth weight, percentile, parity, maternal age, and maternal medical conditions.

Subject	**Maternal Age**	**Maternal Medical Conditions**	**GA (weeks) at MRI**	**GA (weeks) at Birth**	**Birth Weight (g)**	**Birth Weight Percentile** (20)	**Sex (F/M)**
1	41	Crohn's, asthma, pernicious anemia, IUGR	35.6	36.4	1860	< 1	F
2	41	None	36.4	40.7	3300	42.10	F
3	28	Hypothyroidism, gestational diabetes, obesity	36.1	38.3	3360	37.80	M
4	40	None	36.9	37.4	3380	39.40	M
5	34	Overweight	37.3	41.1	3900	85.80	F
6	31	Overweight	35	38.3	2950	18.40	F
7	36	Chronic fatigue, depression	36.6	36.9	3750	66.30	M
8	38	None	38.1	39.1	4180	90.30	M
9	38	Hypothyroidism	33.3	38.9	3630	71.60	M

3.2 Volume Degradation

Fetal motion was visualized through the realignment tool in SPM which provided an estimation map displayed in Figure 3.2.1. and through the ART tool functional within MATLAB displayed in Figure 3.2.3. (32). The estimation maps provided a reasonable assessment of how large the range of translational and rotational movement were present within each dataset with an example shown in Figure 3.2.1. The ART tool was instrumental as it indicated which volumes were outliers and should be removed prior to analysis as they either had greater than two mm of motion and too great signal intensity shown in Figure 3.2.3. By viewing the images as a movie in FSLeyes, a software part of the FSL package, it was clear that the estimation maps and the ART tool provided an accurate assessment of fetal movement and which volumes should not be included in the final analysis. While the ART tool did provide information on which volumes to remove, it was ultimately decided which volumes should be discarded based on the data quality through visualization in FSLeyes. Thus, volumes that were not suggested by ART were excluded at times and in some circumstances, volumes that were suggested by ART were not excluded from the dataset. The results of ART for each subject are present in Appendix G. Satisfactory artifact-free data were acquired for eight out of nine subjects, and only 273 out of the total 1008 volumes were discarded (27.08%) with an entire subject, Subject 3 having the whole dataset removed from the final cohort. Figure 3.2.2. shows the same subject shown in Figure 3.2.1. only after the volumes indicated by the ART tool and visualization in FSLeyes were removed. It is important to note that not all artifact is due to motion and can occur during the acquisition of the data. In our case, spin history artifact was presenting in Subject 3 and it was therefore decided that the entire dataset would be discarded due to the debatable activation quality thus questioning the accuracy, despite the bilateral activation found which is indicated in Table 2.

(A)



(B)

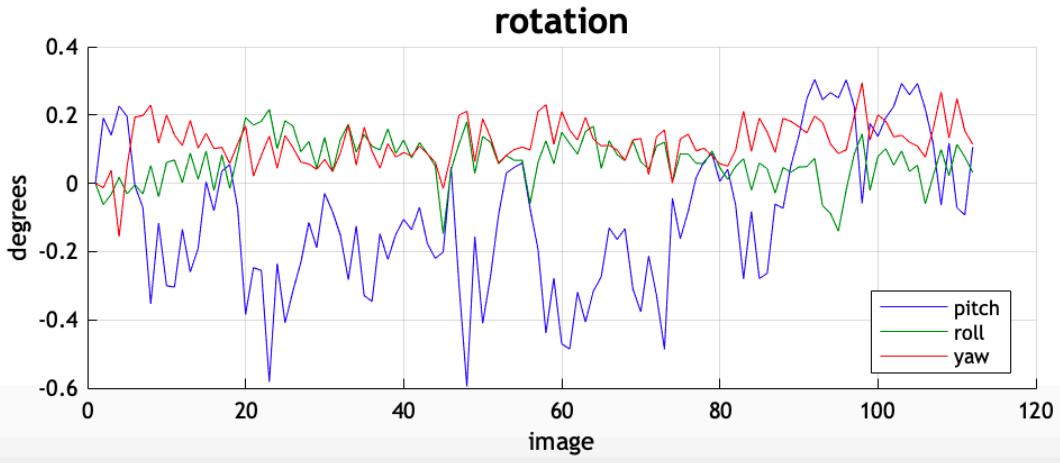
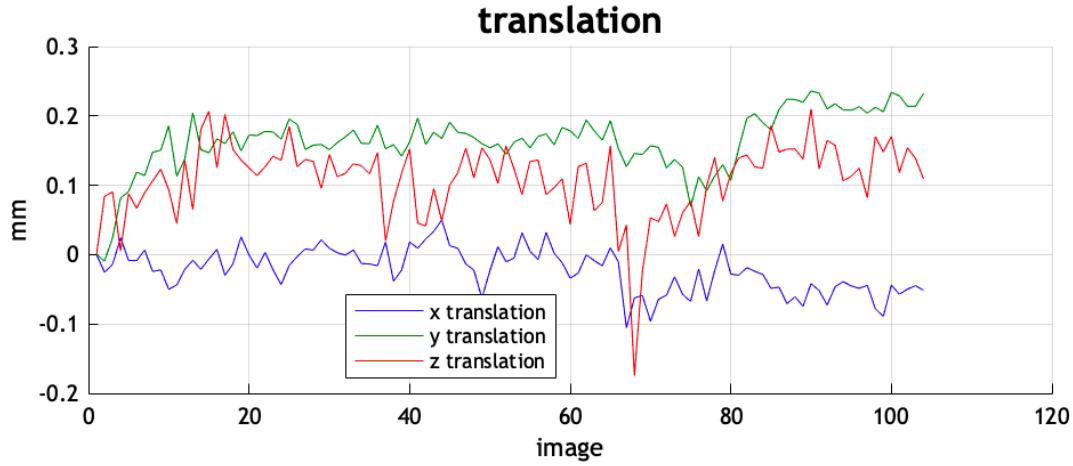


Figure 3.2.1. Estimation Map of Subject 1 prior to ART and reorientation. The y axis indicates mm for (A) and degrees for (B) motion over time at an image rate of 1/11 milliseconds. As shown, the scale for (A) is -1 to 1.5 mm and the scale of (B) is -0.6 to 0.4 degrees.

(A)



(B)

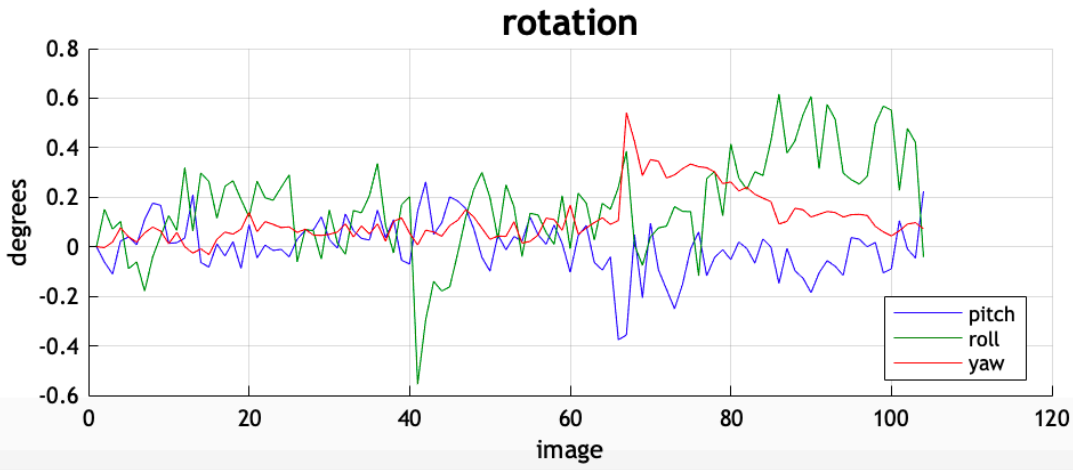


Figure 3.2.2. Estimation Map of Subject 1 after ART, with the suggested volumes removed and reorientation. The y axis indicates mm for (A) and degrees for (B) motion over time at an image rate of 1/11 milliseconds. As shown, the scale for (A) is -0.2 to 0.3 mm and the scale of (B) is -0.6 to 0.8 degrees.

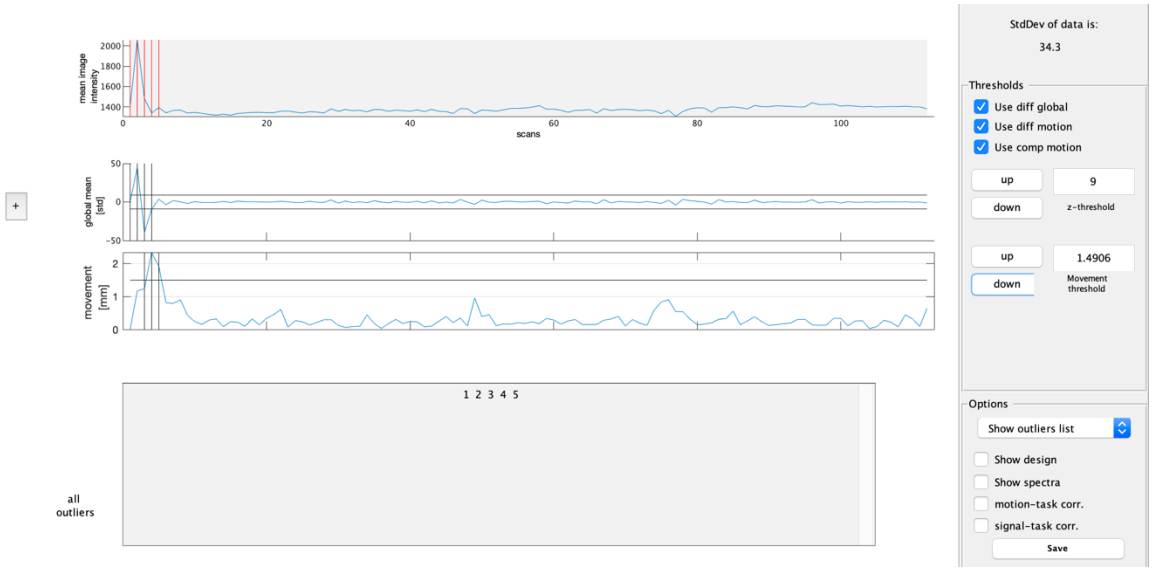


Figure 3.2.3. Result of ART tool for Subject 1 indicating which volumes need to be discarded due to too great of a range of mean signal intensities.

3.3 fMRI Results

Based on the results of the First Level Analysis, each of the eight fetal subjects that were included in the final cohort showed consistent statistically significant activation in the right HG, when exposed to the internal acoustic stimulus, while six out of the eight had significant activation in the left HG (p uncorrected < 0.05). Table 2 shows the average for all the voxels present within the region for each subject (including Subject 3). Activation maps for Subjects 1-2, 4-9 are displayed in Figures 3.3.1-3.3.8 with a legend indicated in each figure. Figure 3.3.9 and 3.3.10 show the respective activation maps overlaid on the functional data for further visualization of the activation in the right and left HG with a colour legend indicated for each image. Other notable regions of activation are the right (mean: 2.44) and left (mean: 2.41) MCC, and the left putamen (mean: 1.32). The right HG on average had a higher Z score compared to the left side, with the right side averaging 2.45 and the left side averaging 2.20. Five subjects underwent the fMRI at a 1.5T GE MR450w scanner with a TE of 60 ms and three of those subjects also had a TE of 90 ms. Four subjects underwent the fMRI at a 3T GE MR750 scanner with a TE of 45 ms and two subjects also had a TE of 60 ms. For the

1.5T cohort, the 90 ms TE scans were not used in the analysis for any subject due to signal loss within those scans and thus the 60 ms TE was superior. For the 3T cohort, the 45 ms provided useable data for all subjects with one out of the two subjects imaged with a TE of 60 ms (Subject 6) had useable data from both 45 ms and 60 ms. The other subject's signal loss deemed the data unusable.

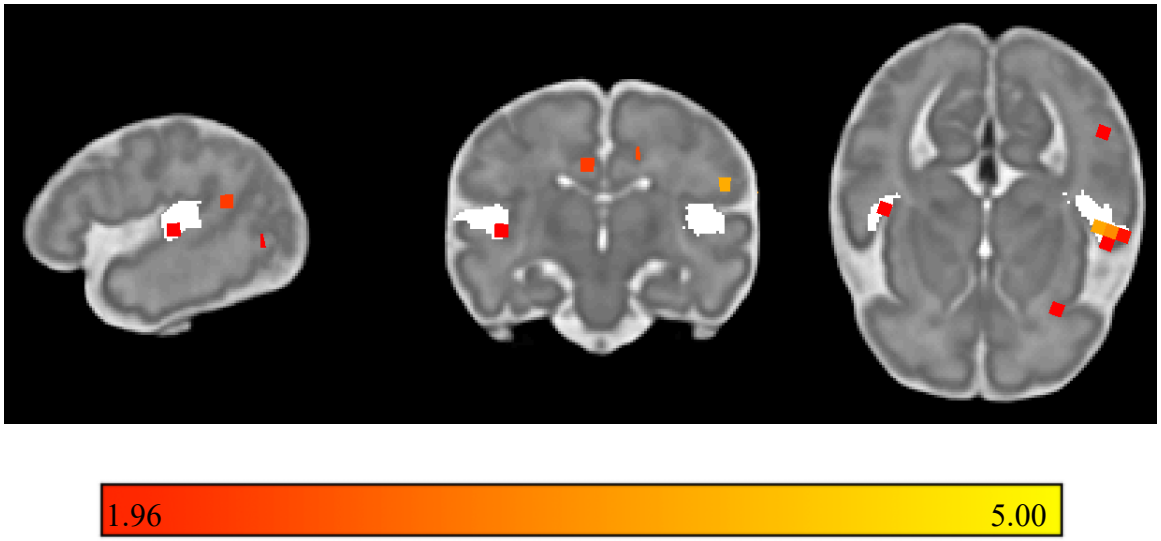


Figure 3.3.1. Activation Map of Subject 1. Activation shown in red/orange overlaid onto the Heschl's gyri shown in white onto the 35 GA CRL anatomical atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

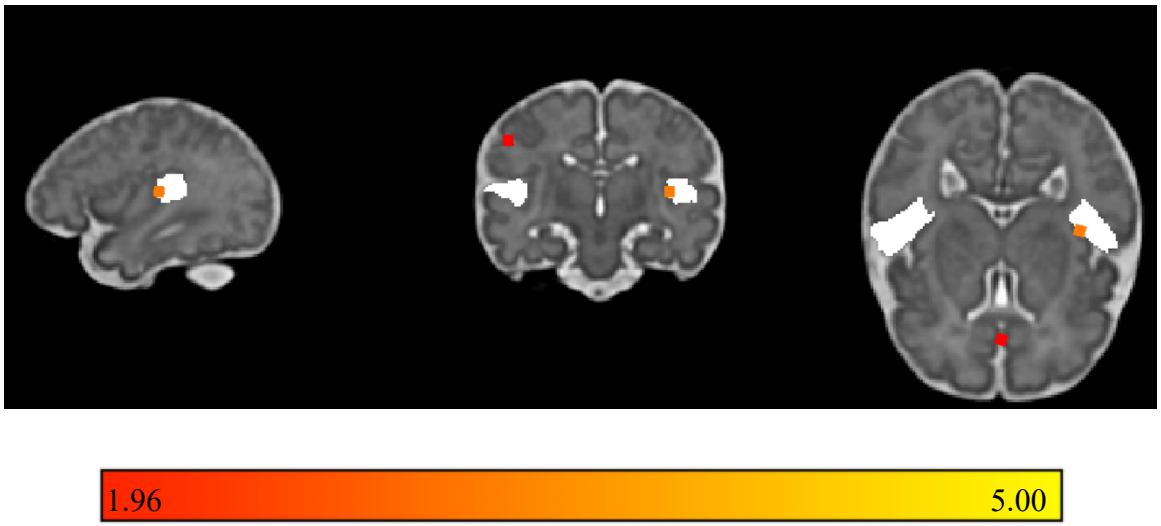


Figure 3.3.2. Top: Activation Map of Subject 2. Activation shown in red/orange overlaid onto the Heschl's gyri shown in white onto the 36 GA CRL anatomical atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

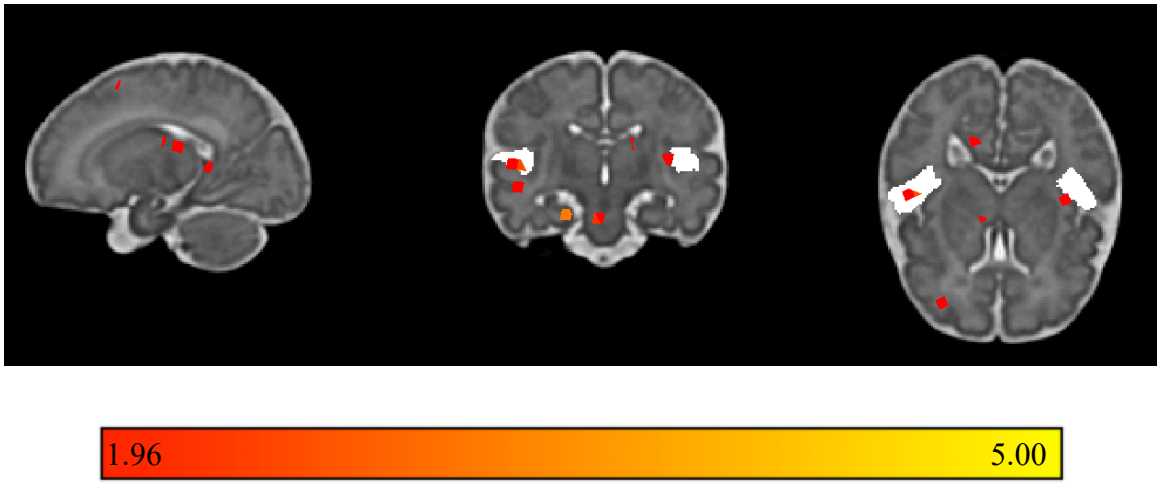


Figure 3.3.3. Top: Activation Map of Subject 4. Activation shown in red/orange overlaid onto the Heschl's gyri shown in white onto the 37 GA CRL anatomical atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

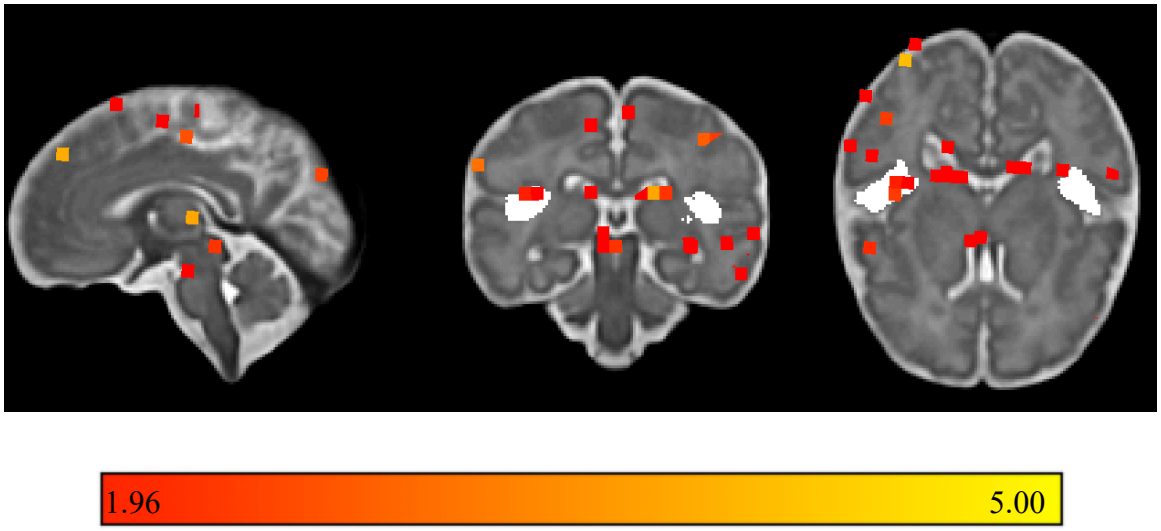


Figure 3.3.4. Top: Activation Map of Subject 5. Activation shown in red/orange overlaid onto the Heschl's gyri shown in white onto the 37 GA CRL anatomical atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

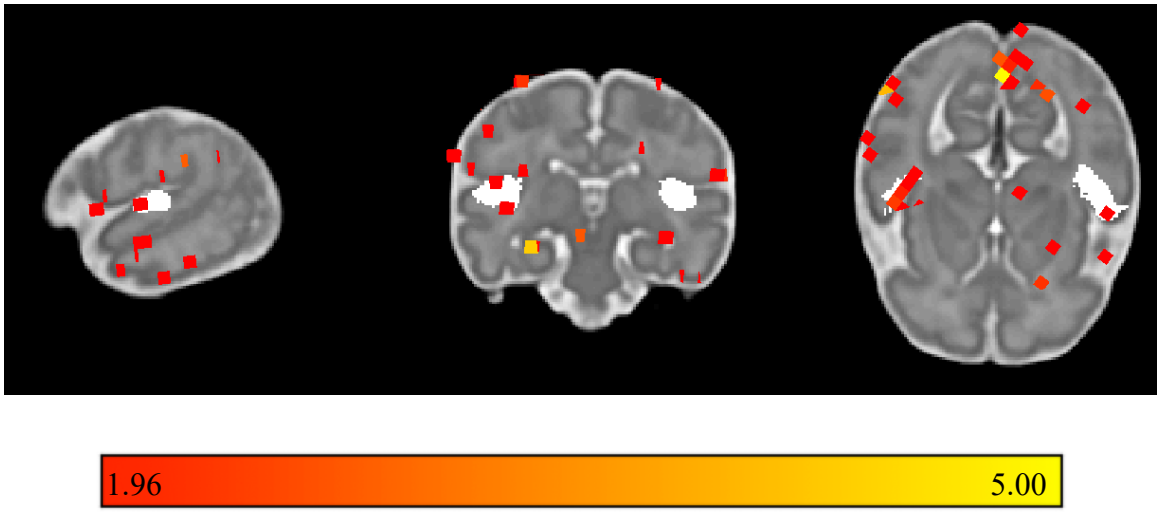


Figure 3.3.5. Top: Activation Map of Subject 6. Activation shown in red/orange overlaid onto the Heschl's gyri shown in white onto the 35 GA CRL anatomical atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

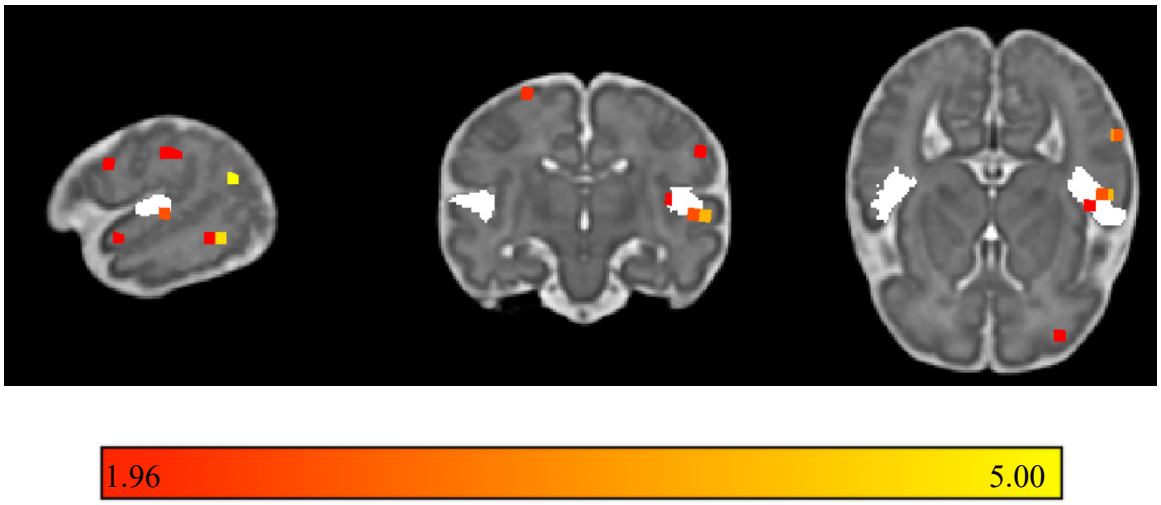


Figure 3.3.6. Top: Activation Map of Subject 7. Activation shown in red/orange overlaid onto the Heschl's gyri shown in white onto the 36 GA CRL anatomical atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

(A)

(B)

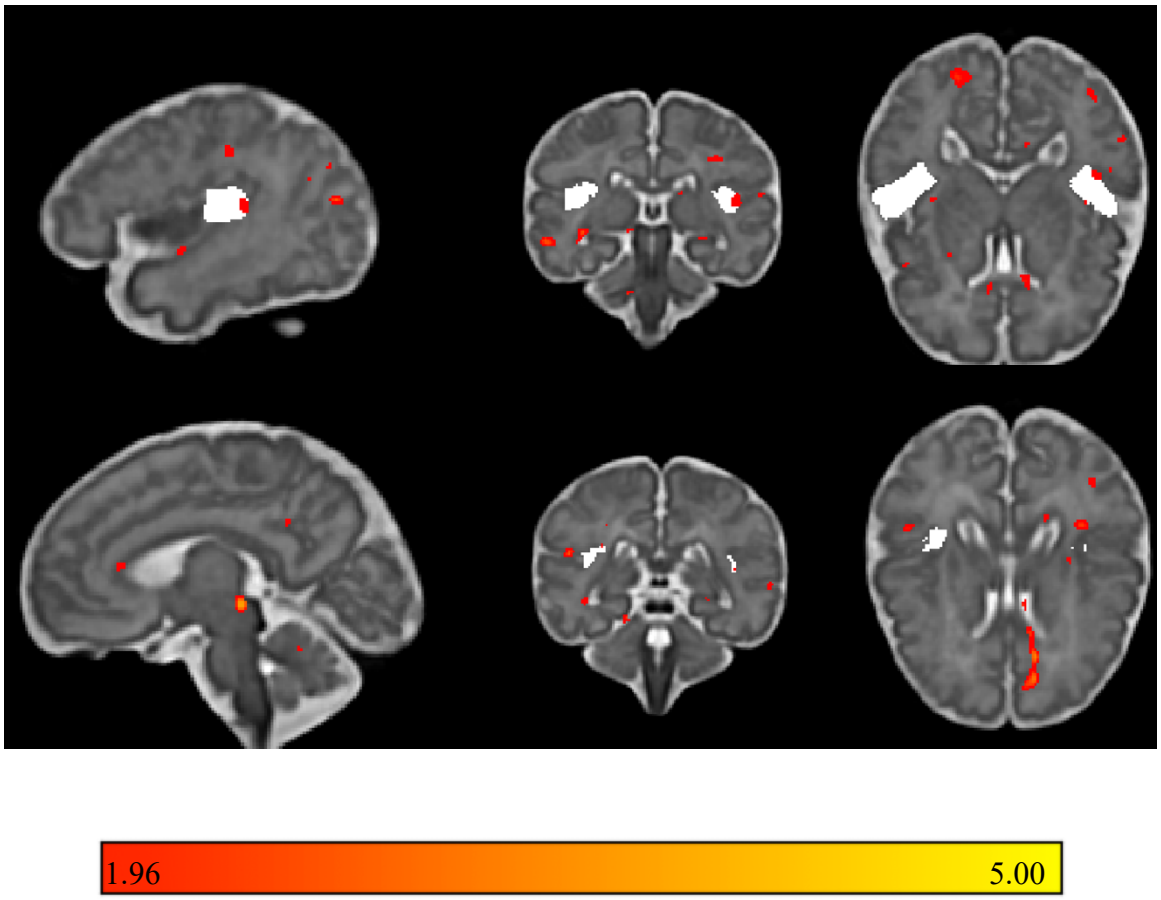


Figure 3.3.7. Top: Activation Map of Subject 8. (A) indicates activation on the right Heschl's gyrus, while (B) indicates activation on the left. Activation shown in red/orange overlaid onto the Heschl's gyri shown in white onto the 38 GA CRL anatomical atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

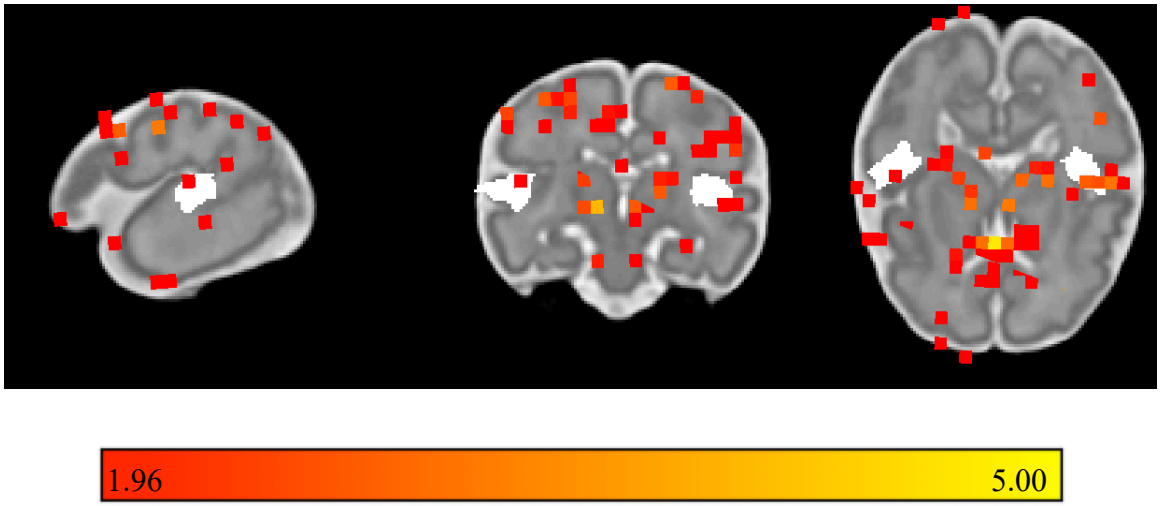


Figure 3.3.8. Top: Activation Map of Subject 9. Activation shown in red/orange overlaid onto the Heschl's gyri shown in white onto the 33 GA CRL anatomical atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

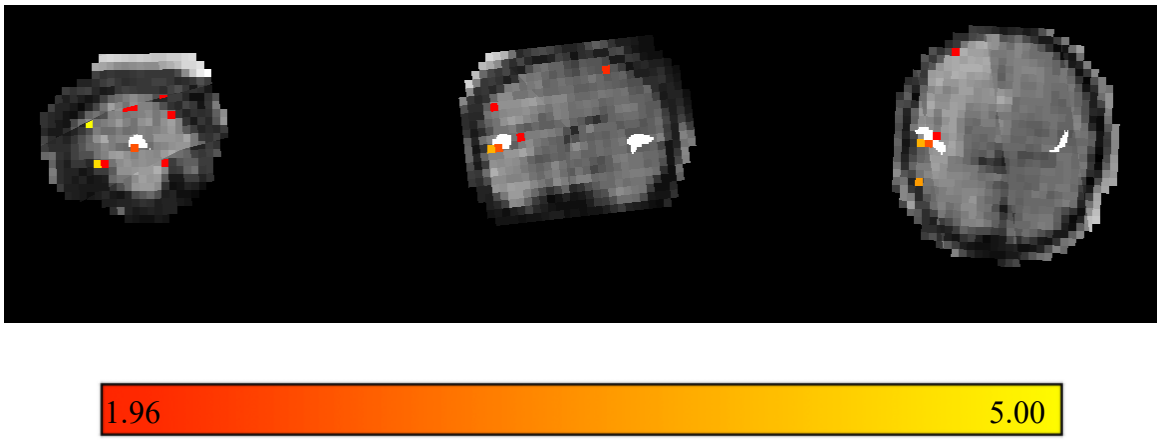


Figure 3.3.9. Top: Activation map of Subject 7 overlaid onto the functional data instead of the CRL atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

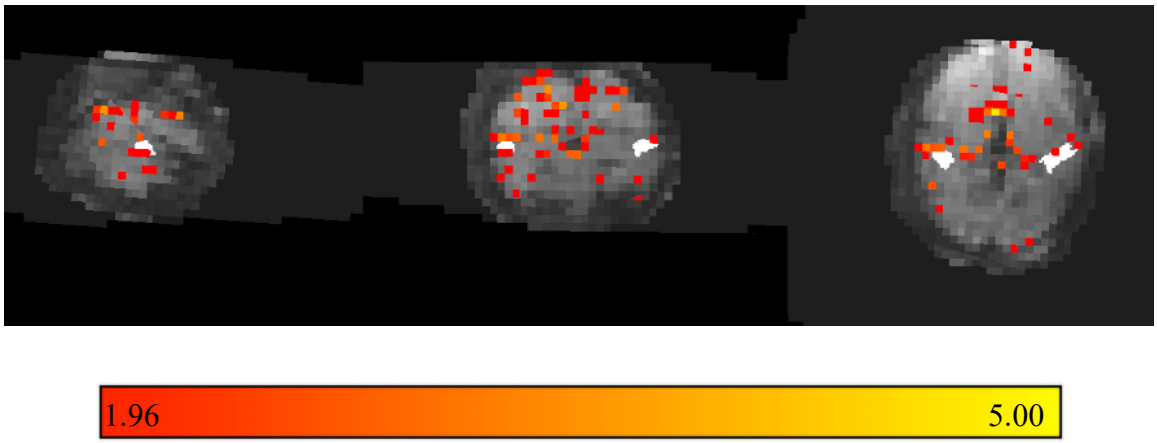


Figure 3.3.10. Top: Activation map of Subject 9 overlaid onto the functional data instead of CRL atlas. Bottom: Legend of the colour map indicating the Z score thresholds for the activation maps.

Table 2: The Z score average of the right and left HG for each subject with Subject 3 italicized to indicate the bilateral activation despite the removal from the final cohort.

Subject	Z Score Average for Right Heschl's Gyrus	Z Score Average for Left Heschl's Gyrus
1	2.30	2.13
2	2.59	No Activation
3	*2.25*	*1.88*
4	1.86	2.44
5	2.21	2.45
6	2.27	2.38
7	2.54	No Activation
8	2.28	2.09
9	2.78	2.15

3.4 Discussion

This thesis demonstrates that when a fetus between 33-38 weeks GA is exposed to an internal acoustic stimulus generated by maternal singing, the auditory network of the fetus becomes activated. Additionally, this thesis demonstrates that there is activation in the right and left MCC along with the left putamen, which is consistent with neonatal studies of both pre-term and term infants in response to an auditory stimulus with activation (64).

This thesis is the first to our knowledge to localize activation in response to an auditory task using an internal stimulus to specific regions of the fetal brain. Previous investigations by the Jardri group have utilized a stimulus applied directly onto the maternal abdomen to localize findings to the temporal lobe (35, 65). Due to safety concerns, it is now considered inappropriate to apply direct stimulation to the mothers' abdomen. Naturally occurring sound exposure in utero generated by the mother's singing is a reasonable alternative to an external stimulus due to the prosodic characteristics emphasized in utero and the internal vibrations of the maternal larynx and diaphragm (66).

The primary auditory cortex, HG, is the first cortical area of the brain to process sound. A study using light and electron microscopy of the fetal auditory cortex stated that the left cochlear nerve of a fetus develops earlier than the right (67). However, our study is looking at fetuses in a GA window that has surpassed this stage in development and therefore can account for the bilateral activation in six out of the eight subjects, excluding the one subject's dataset that was discarded due to artifact. Additionally, a Functional Near Infrared Spectroscopy (fNIRS) study has shown that the auditory network is already able to differentiate between male and female voices by the 32nd week GA demonstrating that at the 33rd-38th weeks GA studied, the cochlear system has developed sufficiently bilaterally to result in higher level auditory cortex development that enables processing of more complex auditory signals (6, 10).

Subjects were imaged either on a 1.5T or a 3T magnet to assess the functionality of the paradigms at those respective field strengths. The use of different strength MRIs in this study was the consequence of unavailability of a large bore 3T at Western University or affiliated hospitals. For participant comfort we elected to scan the more advanced pregnancies in the large-bore 1.5T at LHSC, Victoria Hospital, with the exception of petite women. This provided us the ability to assess the paradigm, internal acoustic stimulus and parameters for two different field strengths. A successful response was

measured in the brain areas associated with hearing in all of the fetuses scanned just by having the mother sing while undergoing an fMRI.

A limitation to this study is the small subject size and limited GA window used. However, we were successful in measuring a response in brain areas associated with hearing in all of the fetuses scanned, just by having the mother sing while undergoing an fMRI. Additionally, all fetuses who underwent a hearing test at birth passed. This GA was chosen due to the natural restriction of fetal motion in the late stages of pregnancy. To truly understand auditory development and be able to aid clinicians in the assessment of brain function in premature infants, investigations need to span the complete viable GA range (23 weeks onwards) and additional studies will need to be conducted to assess the functionality of our internal acoustic stimulus at different GAs.

Another limitation to this study is the amount of time in each of the blocks and the number of blocks in this study; as we wanted to keep the mother for a minimum of time in the magnet. With 10 seconds for each rest block and 15 seconds for each task block that is a very short amount of time to track the activation. Additionally, there are only 9 rest blocks and 9 task blocks, resulting in a short block design study. The length of paradigm was 3 minutes and 44 seconds, and this was decided as if there was too much fetal movement, the scan could be repeated. For our study, our subjects were subject to no more than 45 minutes within the scanner. Due to fetal movement, localization scans were required to take place between each scan due to the increased movement a fetus makes compared to adults. Thus, the anatomical scans took longer than usual for an adult study. With the anatomical scans taking on approximately 20 minutes, had the paradigm been longer, the mother might not have been able to tolerate it.

Fetal motion is unpredictable and cannot be controlled, thus we sought a pipeline to correct for motion in our scenario. Jardri et al. 2008 tried to combat fetal motion by sedating the mothers prior to the task-based fMRI and used a whole dataset analysis resulting in only two out of the six fetal datasets being analyzed (35). Our approach of single volume rejection preserves the maximum number of datasets while still providing

enough volumes for each fetus to assess fetal response to an internal auditory stimulus. There are specific cases where certain volumes have distortion that data were deemed unsuitable for analysis and unfortunately, we have yet to find a way to preserve these volumes effectively. Aside from the significant motion due to the nature of the scan, and the data being deep within the mothers' tissues, fetal fMRI data cannot be treated the same way as traditional adult data. Adults do not have a large amount of tissue surrounding their brain and can maintain their positions during image acquisitions and as a result, adult fMRI has the assumption of negligible motion within intra-stack volumes, large clusters of activation and excellent quality data (24, 25, 68). When engaged in the pelvis of the mother with the fetal head faced down, the fetus still has the ability to rotate and translate in all directions without having large displacement. These movements are similar to how a neonate would be when swaddled in a vacuum blanket (69). Both rotational and translational motion of the fetal brain must be corrected in order to assess accurate localization of activation. These volumes cannot have traditional adult motion correction techniques applied to them as these programs do not accommodate fetal data as both the fetus and the mother are moving.

An additional limitation to our study was the mapping of our fetal data onto the CRL's atlases. Due to human error, and confounding error from each step within the pipeline mentioned, the alignment may not be exact. Hence why the average of all the voxels present within the HG, MCC, and left putamen were used instead of a single voxel analysis. The average of the Z score for each voxel was provided in SPM with the same value corresponding in FSLeyes. These values for the voxels were the ones used to determine and calculate the average of the voxels present within that region for each subject. Additionally, as we could not remove all of the motion within the data, there was residual motion artifact present for some activation voxels. This can be due to the misalignment error during the pipeline and the residual fetal motion within that slice. Since our cohort consisted of 9 fetal subjects, there is not enough evidence to track the residual motion artifact as it was not consistent for any of our subjects however, it is important to note.

Lastly, the amount of activation differs between an adult and a fetus (70). There is less activation in a fetus due to the natural immaturity of the fetal brain, however, the focus of this study was to localize activation in the primary auditory cortex and the amount of fetal activation remained sufficient to be measurable in utero. Despite limitations to image quality due to the nature and consequence of fetal imaging, the scan itself is being taken of the maternal abdomen, and the hemodynamic response signal may be interfered due to blood flow of other organs, such as the placenta (71). fMRI of the maternal abdomen poses challenges as there can be obstructions, such as maternal bowel gas, that can present near the fetal brain generating susceptibility of artifacts that can disrupt detection of regional activation. Maternal breathing and uterine contractions can cause additional motion that must be corrected for prior to analysis. We were able to achieve sufficient fetal brain activation for eight out of the nine subjects through modification of the scanning parameters such as TE. Unfortunately, the data for Subject 3 was not included in the final analysis due to artifact from the scanner. The artifact was too intrusive of the data and compromised the activation quality and thus the accuracy was in question despite the bilateral activation found resulting in removal of the dataset. Additionally, it is worth noting that both Subject 2 and Subject 7 did not have activation present in the left HG.

A TE of 45 ms and 60 ms were evaluated to determine the best fetal brain activation for the 3T scanner at Western University's Robarts Research Institute. It was deemed that a TE of 45 ms provided sufficiently better activation than a TE of 60 ms for our specific parameters as a greater TE resulted in more signal loss. Additionally, this was also conducted for the 1.5T scanner, where the two TE's measured were 60 ms and 90 ms. However, for all subjects imaged on the 1.5T scanner a TE of 60 ms was analyzed as there was signal loss at a TE of 90 ms. Ultimately, a TE of 60 ms was selected for this scanner as it provided fetal brain activation based on our specific parameters mentioned in the methods section of this thesis. Many studies typically do not use a 3T scanner for fetal data, possibly due of the hesitation of maternal size and claustrophobia due to the smaller bore. Studies on preterm neonates with similar age ranges to our subjects do use a

3T Philips Achieva scanner and use a TE of 45 ms (72, 73). For 1.5T (GE Signa Excite) scanner, Lee et al. 2012 found that a TE of 60 ms provided better signal than a TE of 130 ms. Despite the notion that an optimal TE provides more signal than a shortened one as they mentioned that 20% of the data collected using a TE of 130 ms was unusable due to signal loss (74).

This thesis provides clinicians with a reliable paradigm to begin assessing preterm brain development and compare differences between premature infant brain development outside the womb versus physiological brain development in utero.

An internal auditory task can consequently be a tool to analyze the developing auditory cortex in the fetal brain to help guide clinicians and provide previously unknown answers regarding fetal auditory development. This supports the evidence of fetal response to a maternal voice and that an internal auditory stimulus can be used to assess fetal brain responses.

4 Conclusion

4.1 Overview of Objectives

This thesis assessed the functionality of a motion correction pipeline for preprocessing fetal fMRI images and the reliability of a stimulus-based fMRI to invoke fetal response in the primary auditory cortex. The increasing research emerging in non-stimulus or resting state fetal fMRI is allowing researchers to cover new ground to assess fetal brain functionality at a variety of GAs. The specific objectives of this study were to develop a motion correction pipeline along with assessing the fetal response to an internal auditory stimulus-based fMRI paradigm.

4.2 Summary of Results

A motion correction pipeline was developed using widely available tools with the flexibility to discard individual volumes if necessary; in addition to being able to realign

them. It was concluded that this manual motion correction pipeline is a method that can be utilized by groups that do not have large data sets and have time to manually correct the data. Overall, by using manual reorientation and the ART tool, allowed us to have sufficiently good results despite scenarios with problematic motion that may have originally resulted in degradation of the entire dataset. In summary, we sought to investigate a method to assess fetal response to a stimulus in utero and observed activation in the primary auditory cortex in response to an internal auditory stimulus. Out of all the fetuses that were able to be analyzed, there was activation present in the primary auditory cortex on both sides with the exception of two fetuses showing no activation on the left side (Subject 2 and Subject 7 indicated in Table 2).

4.3 Future Directions

For this master's thesis, the development of a motion correction workflow and fetal response to an internal auditory stimulus were assessed. The motion correction workflow on fetuses late in GA, from 33-38 weeks, aims to allow future scan of younger fetuses (potentially as early as the second trimester). However, it is still in question whether this technique will yield similar high-quality results in pregnancies at earlier GAs when fetal movement is more extreme and remains uncertain. In order to accurately assess a wider range of GAs and expand the scope of this pilot project, validation of the workflow must be conducted.

One subject needed to be removed entirely due to spin history artifact present within the data. In the future, the slicing should be an additional factor to paradigm design. Such as one paradigm using interleaved slices as was done in this study, with another using continuous slices. This would provide the opportunity to explore the potential differences between continuous and interleaved slices for fetal fMRI while also potentially providing a reduction of this artifact that was present within our data.

To truly understand auditory development and be able to aid clinicians in the assessment of brain function in premature infants, investigations need to span the complete viable

GA range and additional studies will need to be conducted to assess the functionality of our internal acoustic stimulus at different GAs.

Lastly, in the future, with a larger data set, a group level analysis of the non-stimulus (resting state) fetal data will be conducted to assess the functional connectivity of normal fetal brains in utero. Using the baseline responses from our control subjects outlined in this thesis, a new study will be conducted to assess the fetal brain in response to maternal cannabis ingestion throughout the pregnancy. Alternatively, this pilot study lays the foundation of baseline responses to be applied to a study investigating a spectrum of fetal abnormalities.

4.4 Conclusions

This thesis set out to establish a full pipeline for fetal stimulus fMRI from the acquisition, preprocessing and image analysis. Fetal motion correction pipelines vary between groups and the need to establish a user-friendly motion correction pipeline can allow many researchers to preprocess their data without the need of developing an automated algorithm. Shifting the focus of research groups who want to focus on the data and results instead of the development side. This workflow also allows investigators who want to teach their students how to manipulate and determine a basic understanding of fetal motion within fMRI without the investment. This workflow is a first step in the attempts to minimize fetal fMRI motion and in the future could be automated as an additional research project. Specifically, within the scope of fetal fMRI, it is vital to work around existing algorithms that assume motion is only present once per volume while assuming negligible intra-slice motion. Thus, the importance for this pilot project to manually work with each volume in attempts to minimize was a vital step in having a relatively low volume rejection rate. Once the motion correction was a normal part of the regular workflow similar to adult preprocessing, analyzing the fetal fMRI data was similar to that of any other subject. The fetal response to a maternal internal auditory stimulus can open many avenues for clinicians to answer previously unknown questions using a reliable and

reproduceable study design and apply it to a multitude of study ideas. Ultimately, by incorporating our acquisition parameters, preprocessing motion correction workflow and analysis steps we were able to analyze fetal response to an internal auditory stimulus.

References

1. Rumack CM, Wilson SR, Charboneau JW, Levine D: *Diagnostic Ultrasound Obstetrics & Gynecology.* W B Saunders Co; 2014.

2. Graven SN, Browne J V: Auditory Development in the Fetus and Infant. *Newborn Infant Nurs Rev* 2008; 8:187–193.

3. Wedenberg E: Auditory Tests on New-Born Infants. *Acta Otolaryngol* 1956; 46:446–461.

4. Bouyssi-Kobar M, Du Plessis AJ, McCarter R, et al.: Third trimester brain growth in preterm infants compared with in utero healthy fetuses. *Pediatrics* 2016; 138.

5. DiPietro JA: Baby and The Brain: Advances in Child Development. *Annu Rev Public Health* 2000; 21:455–471.

6. Ream MA, Lehwald L: Neurologic Consequences of Preterm Birth. *Curr Neurol Neurosci Rep* 2018; 18:1–10.

7. Hepper PG, Shahidullah S: Development of fetal hearing. *Arch Dis Child* 1994; 71:8–9.

8. Webb AR, Heller HT, Benson CB, Lahav A: Mother's voice and heartbeat sounds elicit auditory plasticity in the human brain before full gestation. *PNAS* 2015; 112:3152–3157.

9. Partanen E, Kujala T, Tervaniemi M, Huotilainen M: Prenatal Music Exposure Induces Long-Term Neural Effects. *PLoS One* 2013; 8:1–6.

10. Mahmoudzadeh M, Dehaene-Lambertz G, Fournier M, et al.: Syllabic discrimination in premature human infants prior to complete formation of cortical layers. *Source* 2013; 110:4846–4851.

11. Jones CT (Ed): *Fetal and Neonatal Development*. Ithaca, New York: Perinatology Press; 1988.

12. Stratmeyer ME, Greenleaf JF, Dalecki D, Salvesen KA: Fetal Ultrasound. *J Ultrasound Med* 2008; 27:597–605.

13. Prayer D, Brugger PC, Asenbaum U: Indications for Fetal MRI. 2010:1–17.

14. Campbell S: A short history of sonography in obstetrics and gynaecology. *Facts, views Vis ObGyn* 2013; 5:213–29.

15. Ray JG, Vermeulen MJ, Bharatha A, Montanera WJ, Park AL: Association Between MRI Exposure During Pregnancy and Fetal and Childhood Outcomes. *JAMA* 2016; 316:952.

16. Brown RW, Cheng YCN, Haacke EM, Thompson MR, Venkatesan R: *Magnetic Resonance Imaging: Physical Principles and Sequence Design: Second Edition*. 2014.

17. McRobbie DW, Moore EA, Graves MJ: *MRI from Picture to Proton*. 2017.

18. Studholme C, Rousseau F: Quantifying and modelling tissue maturation in the living human fetal brain. *Int J Dev Neurosci* 2014.

19. Shuzhou Jiang S, Hui Xue H, Glover A, Rutherford M, Rueckert D, Hajnal JV: MRI of Moving Subjects Using Multislice Snapshot Images With Volume Reconstruction (SVR): Application to Fetal, Neonatal, and Adult Brain Studies. *IEEE Trans Med Imaging* 2007; 26:967–980.

20. Salehi SSM, Hashemi SR, Velasco-Annis C, et al.: Real-time automatic fetal brain extraction in fetal MRI by deep learning. *Proc - Int Symp Biomed Imaging* 2018; 2018-April(Isbi):720–724.

21. Clouchoux C: Advancing Fetal Brain MRI: Targets for the Future. *Semin Perinatol* 2009; 33:289–298.

22. Rutherford M, Jiang S, Allsop J, et al.: MR imaging methods for assessing fetal brain development. *Dev Neurobiol* 2008.

23. Bonel H, Frei KA, Raio L, Meyer-Wittkopf M, Remonda L, Wiest R: Prospective navigator-echo-based real-time triggering of fetal head movement for the reduction of artifacts. *Eur Radiol* 2008.

24. Buxton RB: *Introduction to Functional Magnetic Resonance Imaging: Principles and Techniques*. 2009.

25. Jezzard P, Matthews PM, Smith SM: *Functional MRI : An Introduction to Methods*.

Oxford University Press; 2001.

26. Moore JK, Linthicum FH: The human auditory system: A timeline of development. *Int J Audiol* 2007; 46:460–478.

27. Buxton RB, Uludağ K, Dubowitz DJ, Liu TT: Modeling the hemodynamic response to brain activation. *Neuroimage* 2004; 23:S220–S233.

28. Petersen SE, Dubis JW: The mixed block/event-related design. *Neuroimage* 2012; 62:1177–84.

29. D. Seixas, G. Ebinger, J. Mifsud JS, von Koch SA: *Functional Magnetic Resonance Imaging Understanding the Technique and Addressing Its Ethical Concerns with a Future Perspective*. 2013.

30. Jiang A, Kennedy DN, Baker JR, et al.: Motion detection and correction in functional MR imaging. *Hum Brain Mapp* 1995.

31. Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM: FSL. *Neuroimage* 2012; 62:782–790.

32. Friston KJ: Statistical Parametric Mapping. In *Neurosci Databases*. Boston, MA: Springer US; 2003:237–250.

33. Goebel R, Esposito F, Formisano E: Analysis of functional image analysis contest (FIAC) data with brainvoyager QX: From single-subject to cortically aligned group general linear model analysis and self-organizing group independent component analysis. *Hum Brain Mapp* 2006; 27:392–401.

34. Cox RW: AFNI: Software for Analysis and Visualization of Functional Magnetic Resonance Neuroimages. *Comput Biomed Res* 1996; 29:162–173.

35. Jardri R, Pins D, Houfflin-Debarge V, et al.: Fetal cortical activation to sound at 33 weeks of gestation: A functional MRI study. *Neuroimage* 2008; 42:10–18.

36. Thomason ME, Scheinost D, Manning JH, et al.: Weak functional connectivity in the human fetal brain prior to preterm birth. *Sci Rep* 2017; 7:39286.

37. Levine D, Barnes PD, Sher S, et al.: Fetal fast MR imaging: Reproducibility, technical quality, and conspicuity of anatomy. *Radiology* 1998.

38. Kuklisova-Murgasova M, Quaghebeur G, Rutherford MA, Hajnal J V, Schnabel JA: Reconstruction of fetal brain MRI with intensity matching and complete outlier removal. *Med Image Anal* 2012; 16:1550–64.

39. Rousseau F, Glenn OA, Iordanova B, et al.: Registration-Based Approach for Reconstruction of High-Resolution In Utero Fetal MR Brain Images. *Acad Radiol* 2006; 13:1072–1081.

40. Rousseau F, Oubel E, Pontabry J, et al.: BTK: an open-source toolkit for fetal brain MR image processing. *Comput Methods Programs Biomed* 2013; 109:65–73.

41. Yushkevich PA, Piven J, Hazlett HC, et al.: User-guided 3D active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. *Neuroimage* 2006.

42. Fedorov A, Beichel R, Kalpathy-Cramer J, et al.: 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magn Reson Imaging* 2012.

43. Python: A dynamic, open source programming language

44. Mathworks: Statistics and Machine Learning Toolbox ™ User's Guide R2017a. *MatLab* 2017.

45. Fulford J, Vadeyar SH, Dodampahala SH, et al.: Fetal brain activity in response to a visual stimulus. *Hum Brain Mapp* 2003; 20:239–245.

46. Krueger C, Horesh E, Crossland BA: Safe Sound Exposure in the Fetus and Preterm Infant. *J Obstet Gynecol Neonatal Nurs* 2012; 41:166–170.

47. Thomason ME, Dassanayake MT, Shen S, et al.: Cross-hemispheric functional connectivity in the human fetal brain. *Sci Transl Med* 2013; 5:173ra24.

48. Thomason ME, Brown JA, Dassanayake MT, et al.: Intrinsic functional brain architecture derived from graph theoretical analysis in the human fetus. *PLoS One* 2014.

49. Blazejewska AI, Seshamani S, McKown SK, et al.: 3D in utero quantification of T2* relaxation times in human fetal brain tissues for age optimized structural and functional MRI. *Magn Reson Med* 2017; 78:909–916.

50. You W, Evangelou IE, Zun Z, Andescavage N, Limperopoulos C: Robust

preprocessing for stimulus-based functional MRI of the moving fetus. *J Med Imaging* 2016.

51. Ferrazzi G, Kuklisova Murgasova M, Arichi T, et al.: Resting State fMRI in the moving fetus: A robust framework for motion, bias field and spin history correction. *Neuroimage* 2014; 101:555–568.

52. Suzuki T;, Kobayashi K;, Umegaki Y: Effect of natural sleep on auditory steady state responses in adult subjects with normal hearing. *Audiology* 1994; 33:274–279.

53. Suwanrath C, Suntharasaj T: Sleep-wake cycles in normal fetuses. *Arch Gynecol Obs* 2010; 281:449–454.

54. Hykin J, Moore R, Duncan K, et al.: Fetal brain activity demonstrated by functional magnetic resonance imaging. *Lancet (London, England)* 1999; 354:645–646.

55. Fulford J, Vadeyar SH, Dodampahala SH, et al.: Fetal brain activity and hemodynamic response to a vibroacoustic stimulus. *Hum Brain Mapp* 2004; 22:116–121.

56. Moore RJ, Vadeyar S, Fulford J, et al.: Antenatal determination of fetal brain activity in response to an acoustic stimulus using functional magnetic resonance imaging. *Hum Brain Mapp* 2001; 12:94–99.

57. Li X, Morgan PS, Ashburner J, Smith J, Rorden C: The first step for neuroimaging data analysis: DICOM to NIfTI conversion. *J Neurosci Methods* 2016; 264:47–56.

58. Synaptive Medical: ClearCanvas – ClearCanvas. .

59. McCarthy P: FSLeyes. 2018.

60. Whitfield-Gabrieli S., Ghosh S., Nieto-Castanon A. GRL: *Artifact Detection, Rejection and Quality Assurance of FMRI Data Increase Accuracy in Task Activation and Functional Connectivity Studies*. .

61. Gholipour A, Rollins CK, Velasco-Annis C, et al.: A normative spatiotemporal MRI atlas of the fetal brain for automatic segmentation and analysis of early brain growth. *Sci Rep* 2017; 7:1–13.

62. Gholipour A, Rollins CK, Velasco-Annis C, et al.: A normative spatiotemporal MRI atlas of the fetal brain for automatic segmentation and analysis of early brain growth. *Sci*

Rep 2017; 7:476.

63. Smith SM: Fast robust automated brain extraction. *Hum Brain Mapp* 2002; 17:143–155.

64. Lordier L, Loukas S, Grouiller F, et al.: Music processing in preterm and full-term newborns: A psychophysiological interaction (PPI) approach in neonatal fMRI. *Neuroimage* 2019; 185:857–864.

65. Jardri R, Houfflin-Debarge V, Delion P, Pruvo J-P, Thomas P, Pins D: Assessing fetal response to maternal speech using a noninvasive functional brain imaging technique. *Int J Dev Neurosci* 2011; 30:159–161.

66. Voegtline KM, Costigan KA, Pater HA, Dipietro JA: Near-term fetal response to maternal spoken voice. *Infant Behav Dev* 2013; 36:526–533.

67. Ray B, Roy TS, Wadhwa S, Roy KK: Development of the human fetal cochlear nerve: a morphometric study. *Hear Res* 2005; 202:74–86.

68. Satterthwaite TD, Wolf DH, Loughead J, et al.: Impact of in-scanner head motion on multiple measures of functional connectivity: Relevance for studies of neurodevelopment in youth. *Neuroimage* 2012; 60:623–632.

69. Cusack R, Wild C, Linke AC, Arichi T, Lee DSC, Han VK: Optimizing Stimulation and Analysis Protocols for Neonatal fMRI. *PLoS One* 2015; 10:e0120202.

70. Rivkin MJ, Wolraich D, Als H, et al.: Prolonged T2* values in newborn versus adult brain: Implications for fMRI studies of newborns. *Magn Reson Med* 2004; 51:1287–1291.

71. Fulford J, Gowland PA: The Emerging Role of Functional MRI for Evaluating Fetal Brain Activity. 2009; 33:281–288.

72. Doria V, Beckmann CF, Arichi T, et al.: Emergence of resting state networks in the preterm human brain. *Proc Natl Acad Sci U S A* 2010.

73. Arichi T, Moraux A, Melendez A, et al.: Somatosensory cortical activation identified by functional MRI in preterm and term infants. *Neuroimage* 2010.

74. Lee W, Donner EJ, Nossin-Manor R, Whyte HEA, Sled JG, Taylor MJ: Visual

functional magnetic resonance imaging of preterm infants. *Dev Med Child Neurol* 2012.

Appendices

Appendix A: Ethics Approval

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Sandrine de Ribaupierre
Department & Institution: Schulich School of Medicine and Dentistry\Clinical Neurological Sciences,Western University

Review Type: Full Board
HSREB File Number: 109515
Study Title: Monitoring of early brain development with fetal and neonatal brain Magnetic Resonance Imaging

HSREB Initial Approval Date: February 09, 2018
HSREB Expiry Date: February 09, 2019

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Western University Protocol		2018/02/07
Recruitment Items	Telephone script for neonatal	2018/02/01
Letter of Information & Consent	Neonatal	2018/01/25
Letter of Information & Consent	Main - Maternal	2018/02/07
Advertisement	Poster	2017/10/25
Data Collection Form/Case Report Form		2017/10/25
Instruments	Example of ASQ questions	2018/01/15
Instruments	Example of Bayley-III questions	2018/01/16

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB

Appendix B: Letter of Information and Consent

London Health Sciences Centre

LETTER OF INFORMATION & CONSENT

Study Title: Monitoring of early brain development with fetal and neonatal brain Magnetic Resonance Imaging

Principal Investigator: Dr. Sandrine de Ribaupierre, LHSC-Victoria Hospital, [redacted]

Co-Investigators:
Dr. Barbra de Vrijer, Obstetrician/Gynaecologist, LHSC-Victoria Hospital, Associate Professor, Western University
Dr. Charles McKenzie, Professor, Department of Medical Biophysics, Western University
Dr. R. Eagleson, Professor, Faculty of Engineering

Funding: BrainSCAN

INVITATION TO PARTICIPATE
You are being invited to participate in this research study to understand better how the brain develops in a fetus because you have been seen in the Obstetrics Department at LHSC-Victoria Hospital.

PURPOSE OF THIS LETTER
The purpose of this letter is to provide you with information required for you to make an informed decision about whether you would like to participate in this study.

BACKGROUND
The in utero (inside the womb) environment can impact childhood development. This study aims to develop new methods to monitor the development of the baby's brain with Magnetic Resonance Imaging (MRI) and detect abnormal fetal brain development, improve diagnosis, and possibly provide earlier intervention. An MRI is the use of magnetic waves to take pictures of the inside of your body.

PURPOSE
To develop MRI tools that can be used during pregnancy to detect abnormal pattern in the fetal brain.

STUDY PROCEDURE
The MRI may take place at LHSC-Victoria Hospital **OR** at the Robarts Institute at Western University.

London Health Sciences Centre

Maternal/Fetal MRI

If you agree to participate in the MRI scan, we will ask you to lie on your left side on a table. A special coil will be placed around your torso and chest during the MRI scan. This coil receives a signal from the magnet and helps to create the image. You will be asked to lie still during the MRI. The bed that you will be lying on will slide you feet first into the MRI scanner. Pictures of your abdomen and unborn baby will be taken. While some of these pictures are taken, you may be asked to hold your breath for about 20 seconds. This will stop blurring of the pictures that would be caused by your abdomen moving as you breathe. Also, during part of the scan, children songs and lullabies will be played through your headphones and we will ask you to sing or talk along. You will be observed by a technologist during the entire procedure. An intercom in the scanner allows you and the technologist to communicate. The MRI will take about 40 minutes.

The research team will collect information such as your: weight; pregnancy outcome; whether you had any complications such as preeclampsia, gestational diabetes, preterm birth; as well as recording your baby's weight, height, Apgar scores and whether your baby had complications that required admission to the neonatal unit. We will access infant's routine hearing assessment data.

40 patients pregnant with one baby who are 18 years of age or older and plan to deliver at LHSC will be recruited.

OPTIONAL: After your baby is born you may be presented with the option to participate in a 5 year follow-up of your baby that includes responding to questionnaires about your baby and an MRI of your baby. (Study 2)

WHAT ARE THE RISKS AND HARMS OF PARTICIPATING IN THIS STUDY?

There are no known biological risks associated with MR imaging. Some people cannot have an MRI because they have some type of metal in their body. For instance, if you have a heart pacemaker, artificial heart valves, metal implants such as metal ear implants, bullet pieces, chemotherapy or insulin pumps or any other metal such as metal clips or rings, they cannot have an MRI. During this test, you will lie in a small closed area inside a large magnetic tube. Some people may get scared or anxious in small places (claustrophobic). An MRI may also cause possible anxiety for people due to the loud banging made by the machine and the confined space of the testing area. You will be given either ear plugs or specially designed headphones to help reduce the noise.

BENEFITS

There are no known benefits to you associated with participating in this research study. Information learned from this study may help enhance diagnostic methods to detect abnormal fetal brain development, improve diagnosis, and possibly provide earlier intervention.

POSSIBLE DISCOVERY OF UNEXPECTED FINDINGS

While the MRI images obtained in this study are for research only and may not be of sufficient quality to diagnose, there is a slight chance that they may reveal a previously unsuspected abnormality in you and/or your unborn baby. A trained radiologist will look at the images. If

he/she determines that there may be an abnormality, your primary doctor and Dr. de Vrijer, the high-risk obstetrician associated with the study, will be notified. They will contact you to discuss what was found, the implications, the potential need for a clinical MRI scan, and information about options for clinical care.

COMPENSATION
Parking costs for each study visit will be reimbursed.

VOLUNTARY PARTICIPATION
Your participation in this study is voluntary. You may leave the study at any time without affecting your care.

WITHDRAWAL FROM STUDY
If you request to be withdrawn from the study, you have the right to request withdrawal of your information. Data collected up to the point of your withdrawal will be retained for analysis in order to protect the integrity of the research. Let your study doctor know. If you do not deliver at LHSC-Victoria Hospital your data will be withdrawn.

CONFLICT OF INTEREST
The doctor treating you also may be a collaborator in the study.

CONFIDENTIALITY
All information collected from you and your electronic/paper hospital chart will remain confidential and accessible only to the investigators of this study. Upon entry into the study, you will be assigned a study number, and your name will not be used in connection with the study data. All information will be coded and kept in a password-protected computer and accessed only by the research team members of this study. If the results are published, your name will not be used. If you choose to withdraw from this study, your information will be removed and destroyed from our database. Your research records will be stored in the following manner: paper records will be kept in a locked filing cabinet; electronic files will be stored on the hospital secure network drive. Representatives of the Western University Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the way the research is being conducted. The Quality Assurance and Education Officers from Lawson Health Research Institute (Lawson) may audit this research study for quality assurance purposes

WHOM DO PARTICIPANTS CONTACT FOR QUESTIONS
If you require any further information regarding this research project or your participation in the study you may contact Dr. Sandrine de Ribaupierre [redacted]

If you have any questions about your rights as a research participant or the conduct of this study, you may contact the Patient Experience Office at [redacted] or access the online form at: https://apps.lhsc.on.ca/?q=forms/patient-experience-contact-form.

A copy of this letter is yours to keep for future reference once it has been signed.

London Health Sciences Centre

CONSENT – Maternal (Study 1)

Study Title: Monitoring of early brain development with fetal and neonatal brain Magnetic Resonance Imaging

Principal Investigator: Dr. Sandrine de Ribaupierre, LHSC-Victoria Hospital, [redacted]

This study has been explained to me and any questions I had have been answered. I know that I may leave the study at any time. I agree to take part in this study.

You do not waive your legal rights by signing the Consent Form.

OPTIONAL Study 2 – Infant

I am willing to be approached about the optional MRI and 5-year follow up study on babies after my baby is born? ☐ Yes ☐ No

_____	_____	_____
Print Study Participant's Name	Signature	Date (DD-MMM-YYYY)

My signature means that I have explained the study to the participant named above. I have answered all questions.

_____	_____	_____
Print Name of Person Obtaining Consent	Signature	Date (DD-MMM-YYYY)

Appendix C: Recruitment Poster

Monitoring of Early Brain Development with Fetal and Neonatal Brain Magnetic Resonance Imaging (MRI)

Principal Investigator: Dr. Sandrine de Ribaupierre, LHSC-Victoria Hospital, [redacted]

We are seeking volunteers for a study to better understand how the brain develops in a baby.

The study involves having an MRI during pregnancy.
There is also the option for your baby to have an MRI.

Inclusion Criteria

- Pregnant with one baby
- 18 years of age or older
- Plan to deliver at LHSC

If you are interested in hearing more about this research please contact our **research coordinator** at:

[redacted]

Funding: BrainSCAN

Version: August 20, 2018

Appendix D: Label Key for CRL regional brain atlases for parcellation.

1	171	42	78	1	1	1	"Precentral_L"
2	171	42	78	1	1	1	"Precentral_R"
3	180	96	0	1	1	1	"Frontal_Sup_L"
4	180	114	0	1	1	1	"Frontal_Sup_R"
5	193	83	59	1	1	1	"Frontal_Sup_Orb_L"
6	193	83	59	1	1	1	"Frontal_Sup_Orb_R"
7	50	168	101	1	1	1	"Frontal_Mid_L"
8	50	168	123	1	1	1	"Frontal_Mid_R"
9	140	82	0	1	1	1	"Frontal_Mid_Orb_L"
10	140	82	0	1	1	1	"Frontal_Mid_Orb_R"
11	160	111	0	1	1	1	"Frontal_Inf_Oper_L"
12	160	111	0	1	1	1	"Frontal_Inf_Oper_R"
13	218	115	62	1	1	1	"Frontal_Inf_Tri_L"
14	218	115	62	1	1	1	"Frontal_Inf_Tri_R"
15	202	128	0	1	1	1	"Frontal_Inf_Orb_L"
16	202	128	0	1	1	1	"Frontal_Inf_Orb_R"
17	118	94	0	1	1	1	"Rolandic_Oper_L"
18	118	94	0	1	1	1	"Rolandic_Oper_R"
19	110	97	0	1	1	1	"Supp_Motor_Area_L"
20	110	97	0	1	1	1	"Supp_Motor_Area_R"
21	100	100	0	1	1	1	"Olfactory_L"

```
22   100  100    0        1  1  1    "Olfactory_R"

23   156   68    0        1  1  1    "Frontal_Sup_Medial_L"

24   156   68    0        1  1  1    "Frontal_Sup_Medial_R"

25   179   98    0        1  1  1    "Frontal_Med_Orb_L"

26   179   98    0        1  1  1    "Frontal_Med_Orb_R"

27   114  128    0        1  1  1    "Rectus_L"

28   114  128    0        1  1  1    "Rectus_R"

29    77  106    0        1  1  1    "Insula_L"

30    77  106    0        1  1  1    "Insula_R"

31    62  109    0        1  1  1    "Cingulum_Ant_L"

32    62  109    0        1  1  1    "Cingulum_Ant_R"

33    74  165    0        1  1  1    "Cingulum_Mid_L"

34    74  165    0        1  1  1    "Cingulum_Mid_R"

35    68  137    0        1  1  1    "Cingulum_Post_L"

36    68  137    0        1  1  1    "Cingulum_Post_R"

37   255  147  230        1  1  1    "Hippocampus_L"

38     0   29  255        1  1  1    "Hippocampus_R"

39     0   95  117        1  1  1    "ParaHippocampal_L"

40     0   80  117        1  1  1    "ParaHippocampal_R"

41   104  255   34        1  1  1    "Amygdala_L"

42   255  247    0        1  1  1    "Amygdala_R"

43   120    0   48        1  1  1    "Calcarine_L"
```

```
44   120    0   62        1  1  1    "Calcarine_R"
45     0  122   40        1  1  1    "Cuneus_L"
46     0  122   40        1  1  1    "Cuneus_R"
47    46  212  110        1  1  1    "Lingual_L"
48     0  123   65        1  1  1    "Lingual_R"
49     0  149  125        1  1  1    "Occipital_Sup_L"
50     0  149  125        1  1  1    "Occipital_Sup_R"
51   123  117    0        1  1  1    "Occipital_Mid_L"
52   123  109    0        1  1  1    "Occipital_Mid_R"
53     0  175  165        1  1  1    "Occipital_Inf_L"
54     0  175  165        1  1  1    "Occipital_Inf_R"
55   255  190   84        1  1  1    "Fusiform_L"
56   244  155   71        1  1  1    "Fusiform_R"
57     0  123  140        1  1  1    "Postcentral_L"
58     0  123  140        1  1  1    "Postcentral_R"
59   152    0  152        1  1  1    "Parietal_Sup_L"
60   152    0  150        1  1  1    "Parietal_Sup_R"
61   187   87    0        1  1  1    "Parietal_Inf_L"
62   187   68    0        1  1  1    "Parietal_Inf_R"
63    25  172    0        1  1  1    "SupraMarginal_L"
64    13  208   16        1  1  1    "SupraMarginal_R"
65     0  114  181        1  1  1    "Angular_L"
```

```
66     0  114  181        1  1  1    "Angular_R"
67   203   60   55        1  1  1    "Precuneus_L"
68   208   53   61        1  1  1    "Precuneus_R"
69   140   52  255        1  1  1    "Paracentral_Lobule_L"
70   140   52  248        1  1  1    "Paracentral_Lobule_R"
71   255  243    0        1  1  1    "Caudate_L"
72     0  206  209        1  1  1    "Caudate_R"
73     0  255  127        1  1  1    "Putamen_L"
74   128    0  128        1  1  1    "Putamen_R"
75   255  250  205        1  1  1    "Pallidum_L"
76   250  128  114        1  1  1    "Pallidum_R"
77   148    0  211        1  1  1    "Thalamus_L"
78   178   34   34        1  1  1    "Thalamus_R"
79   113   66  206        1  1  1    "Heschl_L"
80   113   66  206        1  1  1    "Heschl_R"
81   190   63  198        1  1  1    "Temporal_Sup_L"
82   190   63  198        1  1  1    "Temporal_Sup_R"
83   129   55  202        1  1  1    "Temporal_Pole_Sup_L"
84   129   55  202        1  1  1    "Temporal_Pole_Sup_R"
85   199  105  240        1  1  1    "Temporal_Mid_L"
86   199  105  240        1  1  1    "Temporal_Mid_R"
87    80   99  220        1  1  1    "Temporal_Pole_Mid_L"
```

```
 88    80  117  220        1  1  1    "Temporal_Pole_Mid_R"
 89   160   21  183        1  1  1    "Temporal_Inf_L"
 90   160   21  183        1  1  1    "Temporal_Inf_R"
 91   115  255    0        1  1  1    "CorpusCallosum"
 92     0  191  255        1  1  1    "Lateral_Ventricle_L"
 93    98    0  255        1  1  1    "Lateral_Ventricle_R"
 94   210  105   30        1  1  1    "Midbrain_L"
 95   255  248  220        1  1  1    "Midbrain_R"
 96    47   79   79        1  1  1    "Pons_L"
 97    72   61  139        1  1  1    "Pons_R"
 98   204  176  238        1  1  1    "Medulla_L"
 99   128  128    0        1  1  1    "Medulla_R"
100    76  135    0        1  1  1    "Cerebellum_L"
101   255  240  245        1  1  1    "Cerebellum_R"
102     4   30  175        1  1  1    "Vermis_Ant_L"
103   175  130    4        1  1  1    "Vermis_Ant_R"
104   255  255  115        1  1  1    "Vermis_Post_L"
105    41  173   34        1  1  1    "Vermis_Post_R"
106   255    0  230        1  1  1    "Vermis_Cent_L"
107    62   76  202        1  1  1    "Vermis_Cent_R"
108    72  209  204        1  1  1    "Subthalamic_Nuc_L"
109   255    0  255        1  1  1    "Subthalamic_Nuc_R"
```

```
110   199   21  133        1  1  1    "Hippocampal_Comm"
111   255  102    0        1  1  1    "Fornix"
112   167   70   22        1  1  0    "Cortical_Plate_L"
113   255   92   95        1  1  0    "Cortical_Plate_R"
114    37  185    0     0.61  1  1    "Subplate_L"
115     0  115  255     0.61  1  1    "Subplate_R"
116   250  210  170     0.61  1  1    "Inter_Zone_L"
117   250  250  130     0.61  1  1    "Inter_Zone_R"
118    42    0  255     0.61  1  1    "Vent_Zone_L"
119     0  108    5     0.61  1  1    "Vent_Zone_R"
120   250  210  170     0.61  1  1    "White_Matter_L"
121   250  250  130     0.61  1  0    "White_Matter_R"
122    29  123  255     0.61  1  1    "Internal_Capsule_L"
123   255  156  249     0.61  1  1    "Internal_Capsule_R"
124   136  161  230     0.61  1  0    "CSF"
```

Appendix E: MATLAB Script for Regional Atlas Parcellation

```
CONVERT SINGLE ROI (WITH >1 NUMBERS) IMAGE INTO MULTIPLE ROI IMAGES
(CODED 0/1)
im=spm_select(1,'image','Select ROI atlas image...','',pwd,'.*');
V=spm_vol(im);
atlas=spm_read_vols(V);

for i = 79:80,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Heschl_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 100:101,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Cerebellum_',in,'.nii');
    spm_write_vol(V,tmp);
end

for i = 102:103,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Vermis_Ant_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 104:105,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Vermis_Post_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 106:107,

    in=mat2str(i);
```

```
    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Vermis_Cent_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 108:109,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Subthalamic_Nuc_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 110,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Hippocampal_Comm_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 112:113,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Cortical_Plate_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 114:115,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Subplate_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 116:117,

    in=mat2str(i);
```

```
    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Inter_Zone_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 118:119,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Vent_Zone_',in,'.nii');
    spm_write_vol(V,tmp);
end
for i = 96:97,

    in=mat2str(i);

    tmp=atlas;
    tmp(find(atlas~=i))=0;
    tmp(find(atlas==i))=1;

    V.fname=strcat('Pons_',in,'.nii');
    spm_write_vol(V,tmp);
end
```

Appendix F. Fetal Workflow Recreation Steps

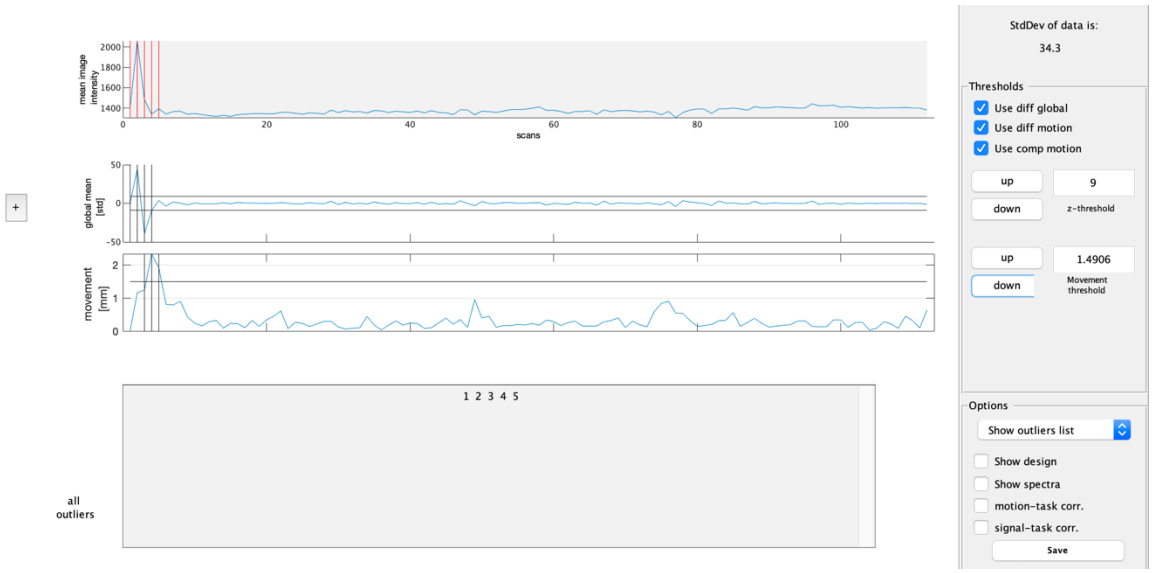
1. DICOM to .nii Conversion:
    a. Launch dcm2niix
    b. Select in drop down menu SPM 8 (3D NIFTI nii)
    c. Then go to file
    d. Select DICOM to NIFTI in drop down menu
    e. A popup will come asking you to select you DICOM images you would like to convert
    f. Find the file you saved your DICOM files in
    g. Once you have selected the file click ok
    h. Now go to the file you selected to make sure your .nii files are saved there
    i. Stack 3D data by using the SPM/FSL selection in the drop down menu
    j. Select stack and select the 3D volumes that will need to be stacked into 4D (this is just done to visualize motion and must be discarded to avoid confusion of motion and brain extracted version later on)
2. Download the Computational Radiology Lab Gestational Age Atlases
3. Launch FSL
    a. Select FSLeyes
    b. Go to file and select add file
    c. Click on .nii file of interest (4D file first)
    d. Once loaded in, select movie mode
    e. Go through and mark down each volume with motion
4. Launch SPM 12
    a. Open the Batch Editor – typically this module realigns the volumes but since we do not want this, we use the 4D volume stack to see where the motion is as a double check
    b. Load the 4D volume stack into the module
    c. Leave the quality the same, the separation is 4 mm for this study, smoothing remains the same, num passes is changed to register to first image (typically what is done for fMRI), interpolation, wrapping and weighting remains the same as what is in the module.
    d. Estimation maps will pop up and indicate the amount of rotation and translational movement the image may have
5. Launch FSLeyes again
    a. Open FSLeyes
    b. Load in 3D volumes
    c. Load in correct gestational age atlas
    d. Each volume is manually reoriented to the atlas by reorienting to the atlas using the Nudge Tool in FSLeyes and the coordinates provided from the estimation map in SPM
    e. Save each file
6. Launch MATLAB
    a. Launch the ART tool
    b. Input the all the volumes
    c. Input the estimation map file provided by the realignment tool earlier on in the process

    d. The volumes that need to be removed are indicated
    e. Volumes that need to be removed are marked and not included in the analysis or 4D stack
7. BET
    a. Input each volume through BET twice to produce a sufficient brain extraction

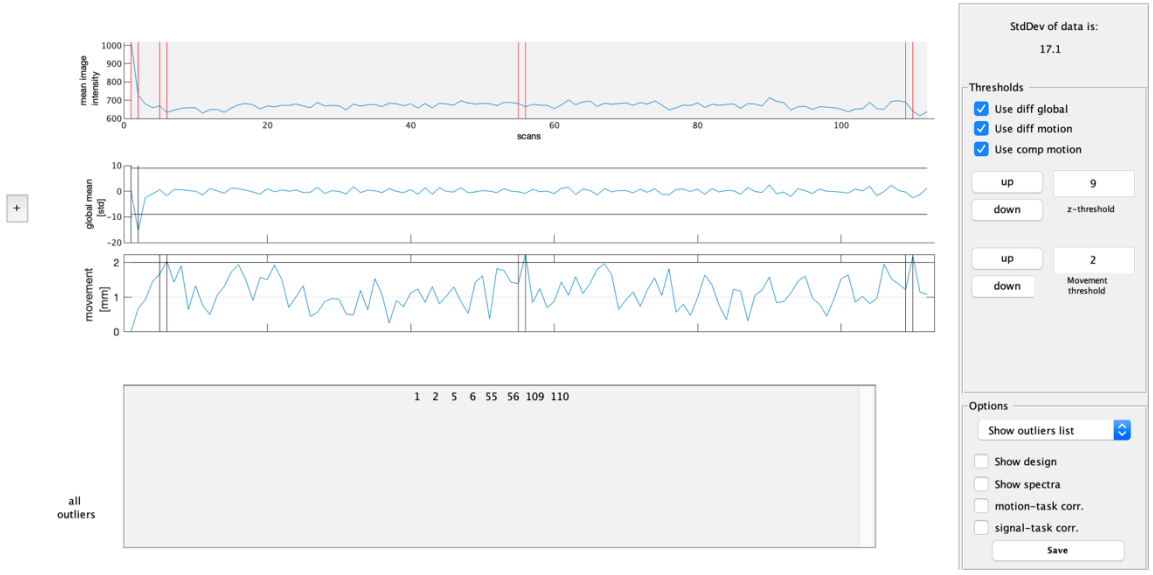
8. Dcm2niix again
    a. Stack brain extracted 3D volumes into a 4D dataset and delete previous 4D stack
9. Launch SPM 12 again
    a. Using the co-registration module in SPM, co-register the 4D stack to the correct gestational age atlas
    b. View the registration to assure each region is localized in the brain accurately
10. Atlas Parcellation
    a. Open new script in MATLAB
    b. Use script in appendix E
11. Analysis
    a. Select first level single subject analysis
    b. Input co-registered, realigned, and segmented data
    c. Input image parameters
    d. Obtain a T- contrast
12. Launch FSLeyes again
    a. Open new SPM file with analyzed data
    b. Open correct fetal gestational age atlas
    c. Open areas of interest from parcellated atlas
    d. Overlay atlas, region, and analyzed functional data
    e. Record z-scores of regions of interest

Appendix G: ART Results After Reorientation for Subjects Included in Analysis

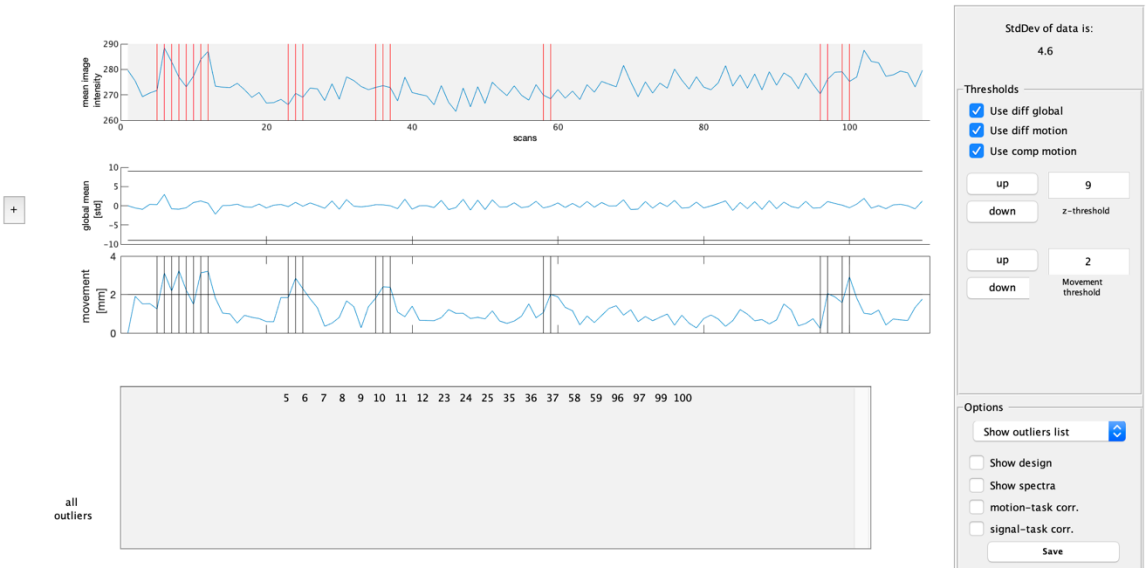
Subject 1



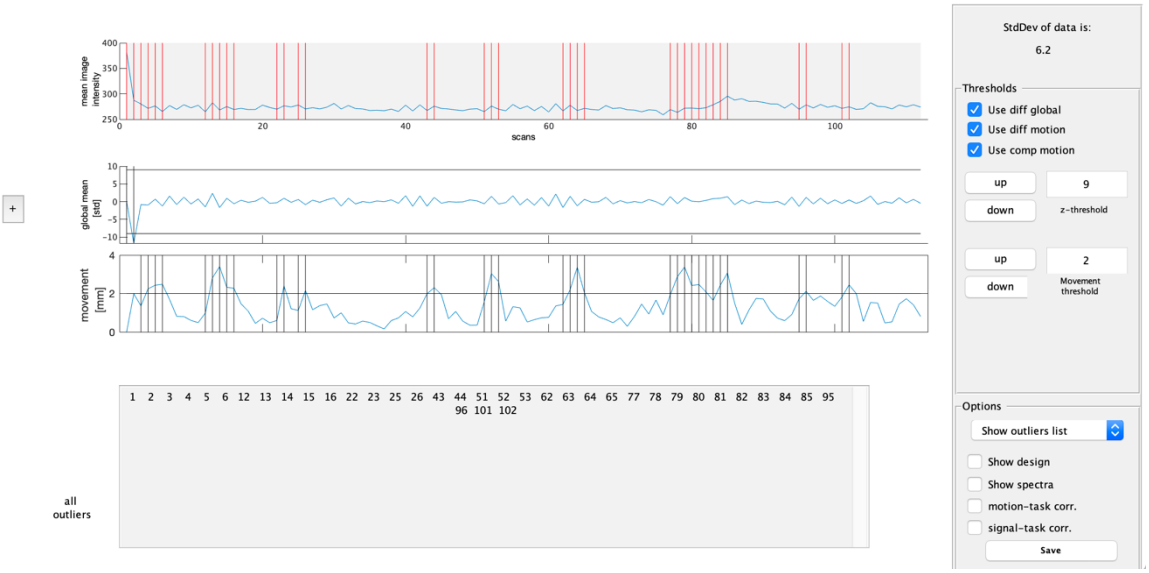
Subject 2



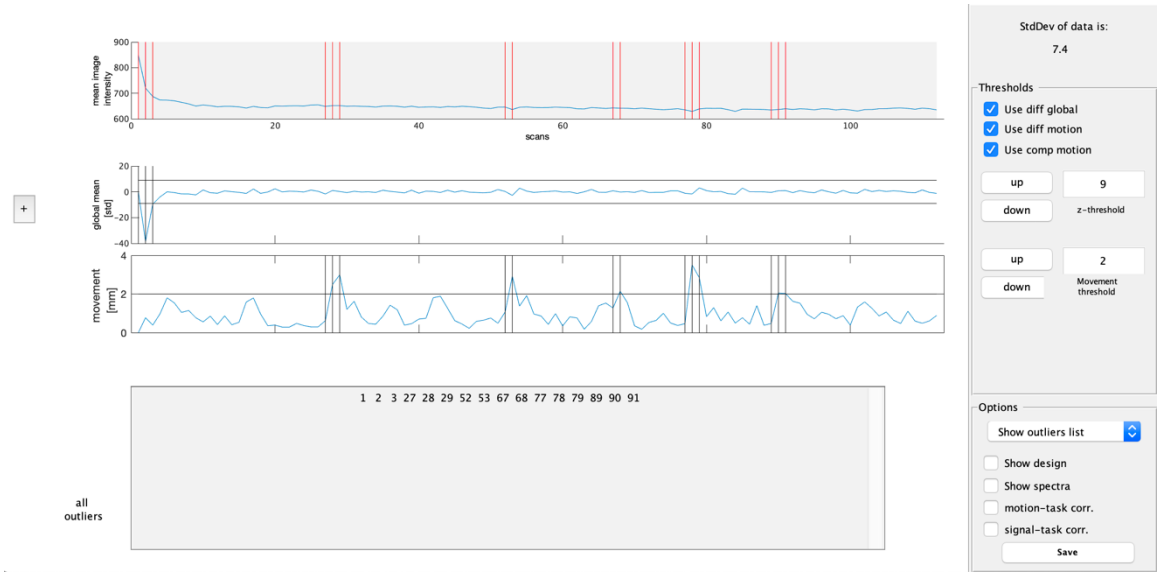
Subject 4



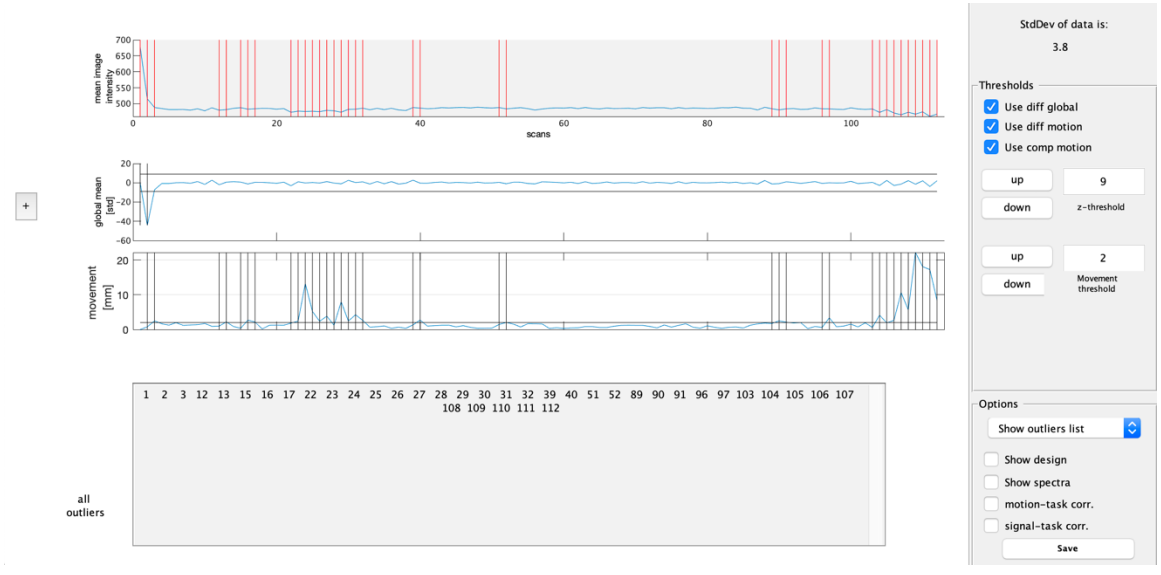
Subject 5



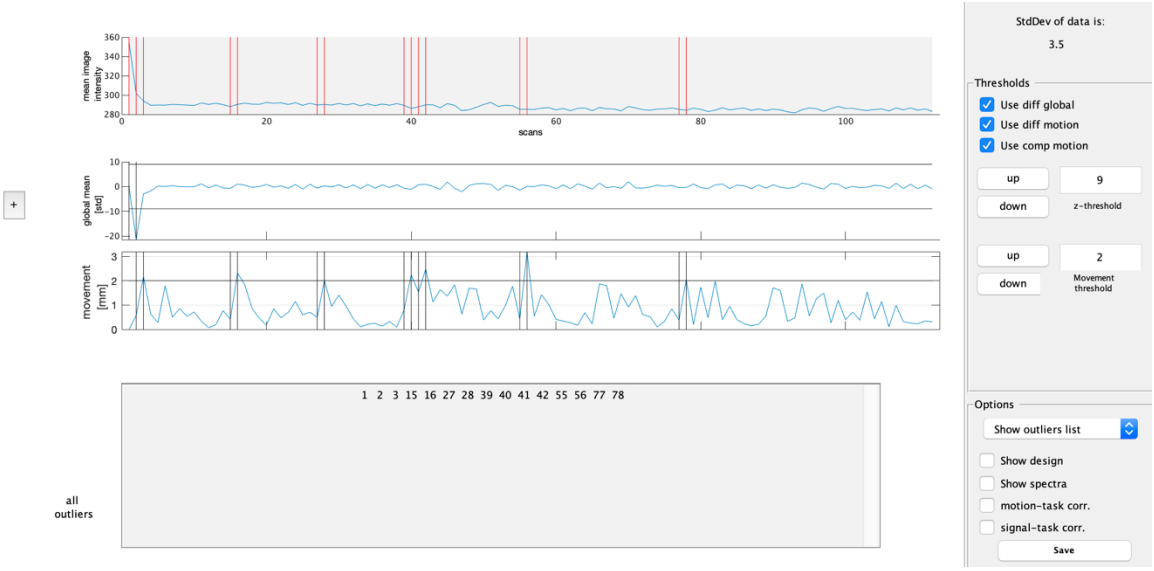
Subject 6



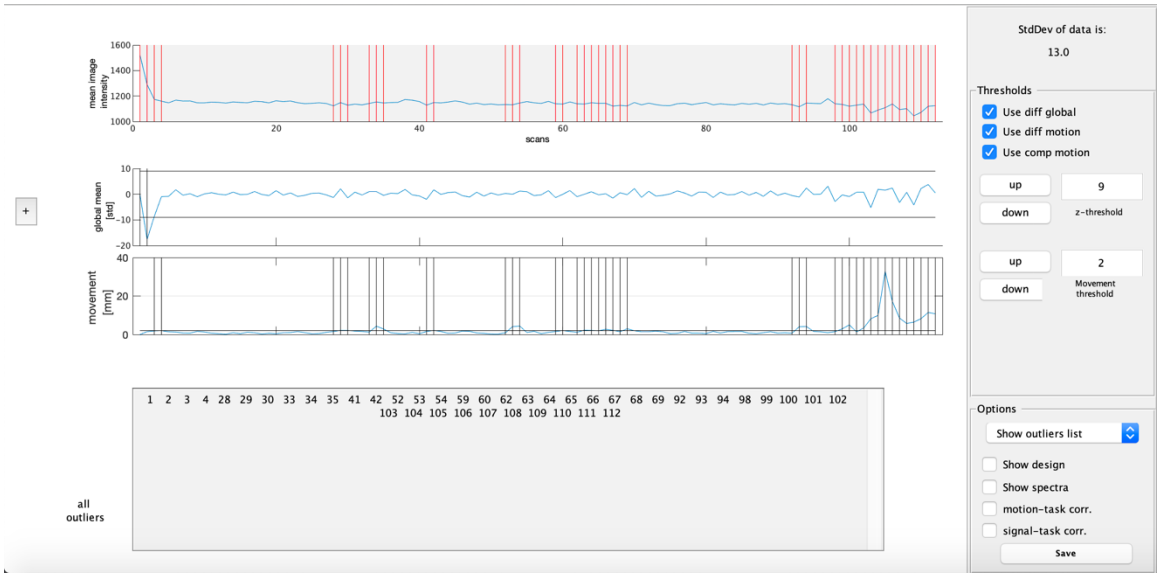
Subject 7



Subject 8

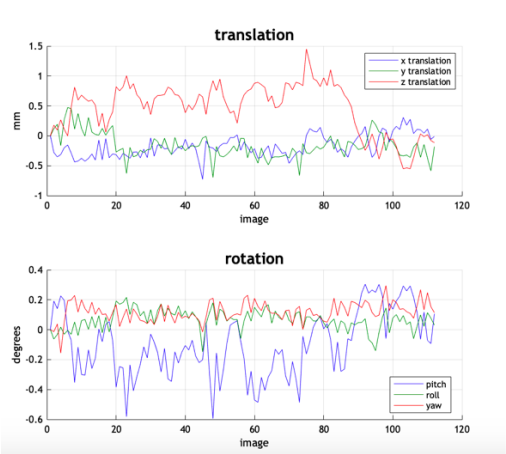


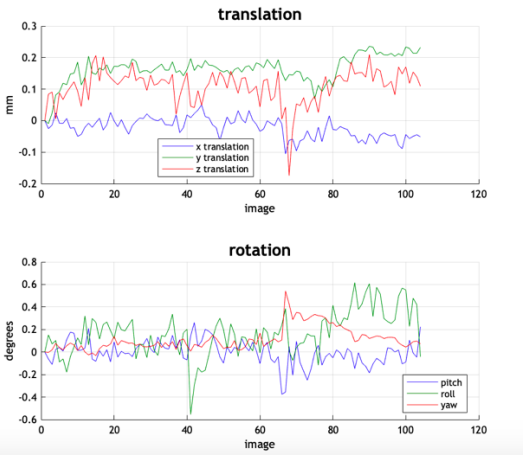
Subject 9



Appendix H: Estimation Maps of Before and After Manual Reorientation

Subject 1





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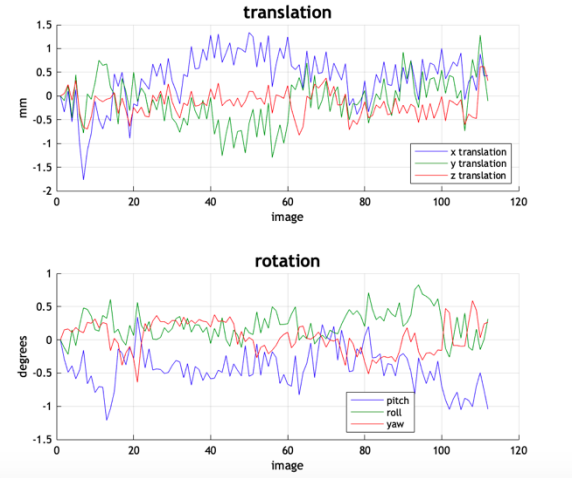

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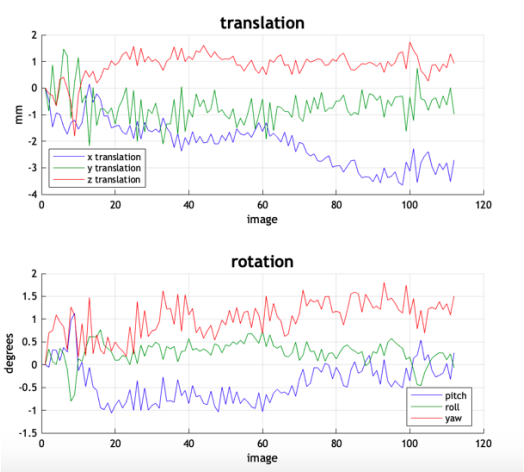

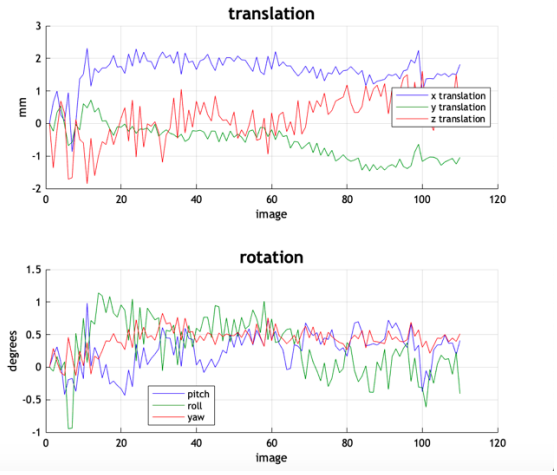

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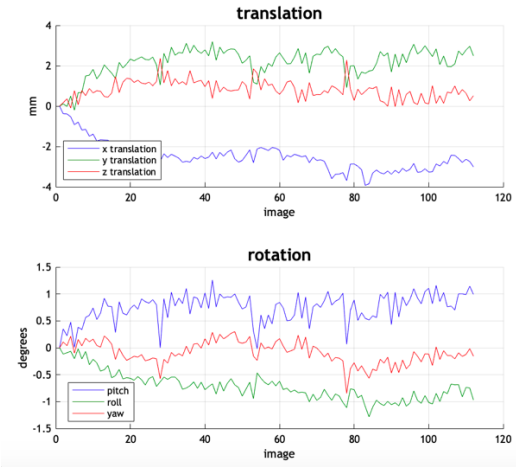

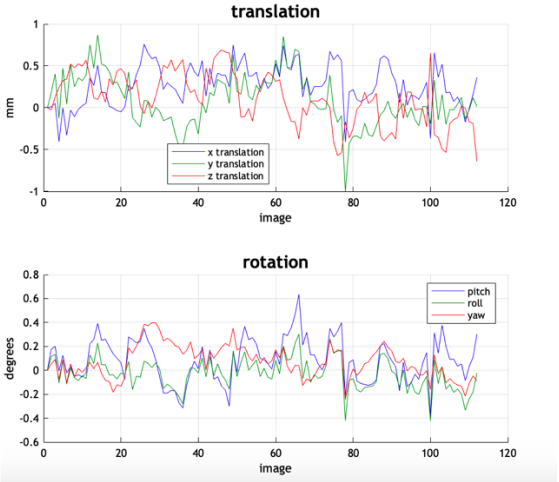

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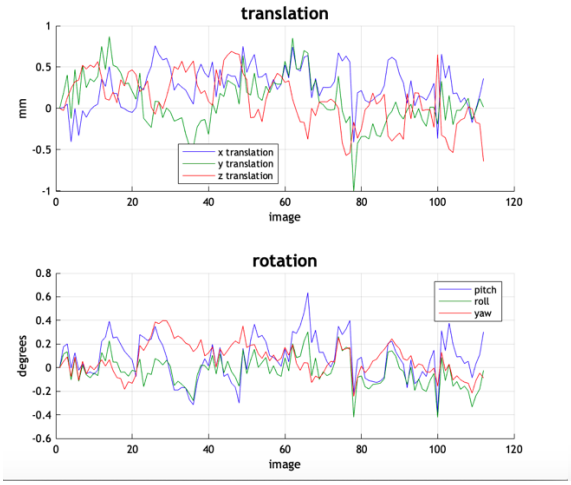

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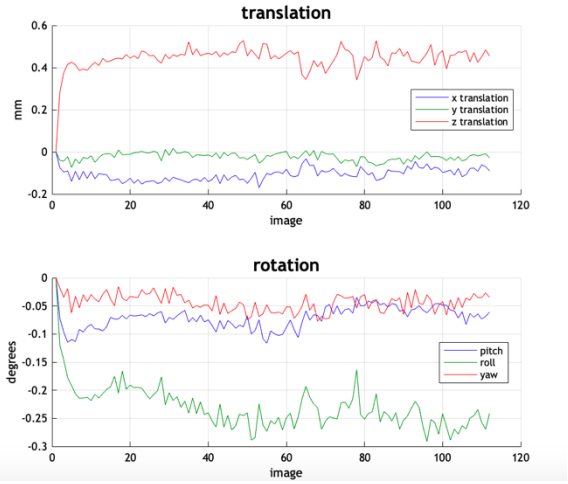

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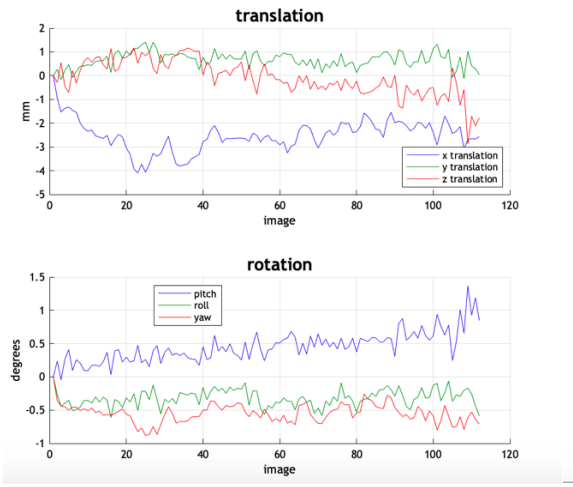

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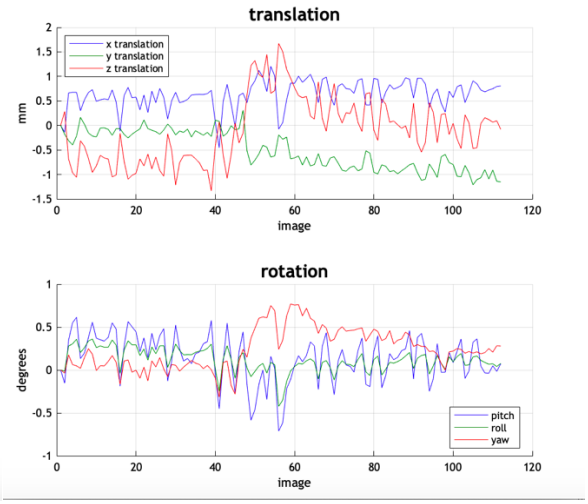

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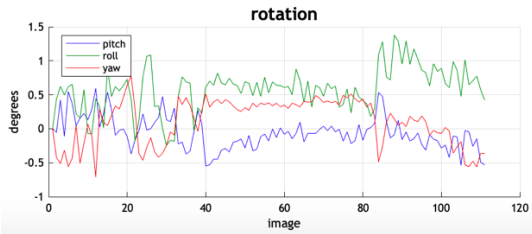

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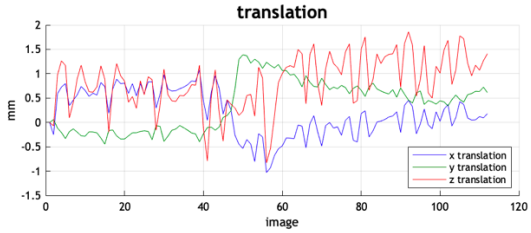

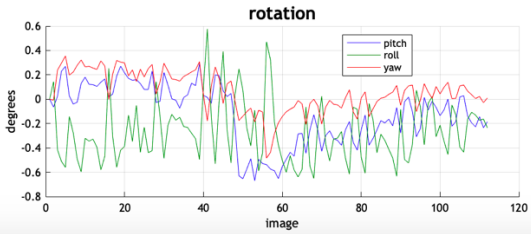

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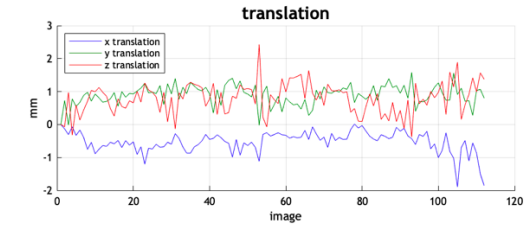

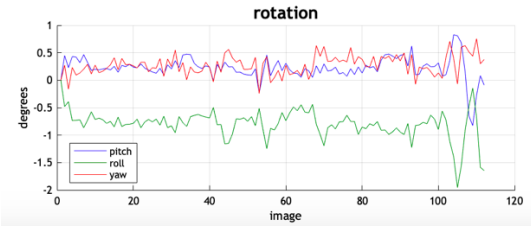

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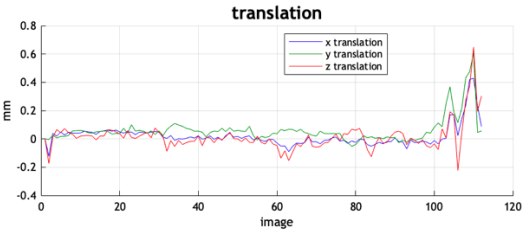

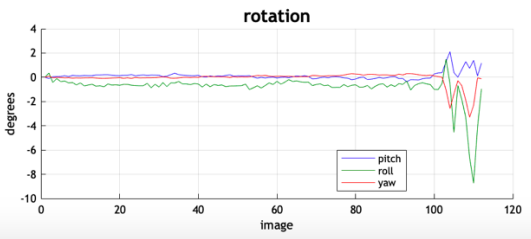

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Appendix J: Curriculum Vitae

Estee Goldberg, BSc

MESc Candidate, School of Biomedical Engineering, Western University

Education

Master of Engineering Science, *Biomedical Engineering* 2018–Present
Western University, London, Canada

Bachelor of Science, *Biology* 2013–2017
Western University, London, Canada

Ontario Secondary School Diploma 2009–2013
Tanenbaum CHAT Kimel Family Education Centre, Richmond Hill, Canada

Research Positions

Research Assistant - Biomedical Engineering 2018–Present
Western University, London, Canada - Under the supervision of Dr. Sandrine de Ribaupierre
- Topic: Fetal Response to an Internal Auditory Stimulus.
- Topic: User Friendly Motion Correction and Segmentation Workflow.

Research Assistant - THETA Collaborative 2016–2017
University Health Network, Toronto, Canada - Under the supervision of Dr. Girish Kulkarni
- Topic: Development of a utility weighting function for the Bladder Utility Symptom Scale (BUSS-U).

Student Researcher - Robarts Research Institute 2016
Western University, London, Canada - Under the supervision of Dr. Aaron Fenster
- Topic: Segmention and analysis of prostate ablation zone using MRI images after treatment to improve accuracy of the ablation zone.

Student Researcher - Genito-Urinary Bio-Bank 2016
University Health Network, Toronto, Canada - Under the supervision of Dr. Neil Fleshner and Dr. Nathan Perlis
- Topic: Defining a cohort of men who may not require repeat prostate biopsy based on PCA3 and MRI.

Peer Reviewed Journal Publications

Goldberg, E., McKenzie, C.A., de Vrijer, B., Eagleson, R., de Ribaupierre, S. Fetal Response to a Maternal Internal Auditory Task. Journal of Magnetic Resonance Imaging, January 2020.

Perlis N., Al-Kasab T., Ahmad A., **Goldberg, E.**, Fadak K., Sayid R., Finelli A., Kulkarni G., Hamilton R., Zlotta A., Fleshner N. Defining a cohort of men who may not require repeat prostate biopsy based on PCA3 and MRI: The double negative effect. Journal of Urology, November 2017.

Conference Presentations

Goldberg, E., McKenzie, C.A., de Vrijer, B., Eagleson, R., de Ribaupierre, S. "Using an internal auditory stimulus to activate the developing primary auditory cortex: A fetal fMRI study". *Oral Presentation at London Imaging Discovery Day*, June 12th 2019, London, ON.

Goldberg, E., McKenzie, C.A., de Vrijer, B., Eagleson, R., de Ribaupierre, S. "Can an Internal Auditory Task Stimulate the Primary Auditory Cortex? A fetal fMRI Investigation". *Poster Presentation at the Canadian Assosiation for Neuroscience*, May 22-25 2019, Toronto, ON.

Goldberg, E., McKenzie, C.A., de Vrijer, B., Eagleson, R., de Ribaupierre, S. "Fetal Brain Response to an Auditory Stimulus". *Oral Power Pitch Presentation at the International Society for Magnetic Resonace in Medicine*, May 11-16 2019, Montreal, QB.

Goldberg, E., McKenzie, C.A., de Vrijer, B., Eagleson, R., de Ribaupierre, S. "Fetal Brain Response to an Auditory Stimulus". *Digital Poster Presentation at the International Society for Magnetic Resonace in Medicine*, May 11-16 2019, Montreal, QB.

Goldberg, E., McKenzie, C.A., de Vrijer, B., Eagleson, R., de Ribaupierre, S. "Can an Auditory Task Stimulate the Fetal Primary Auditory Cortex? An fMRI investigation". *Poster Presentation at London Health Research Day*, April 30th 2019, London, ON.

Goldberg, E., McKenzie, C.A., de Vrijer, B., Eagleson, R., de Ribaupierre, S. "User Friendly Fetal Brain Image Segmentation Pipeline". *Oral Presentation at the Imaging Network of Ontario Symposium*, March 28-29 2019, London, ON.

de Ribaupierre, S., **Goldberg, E.**, McKenzie, C.A., de Vrijer, B., Eagleson, R. "Fetal Brain Response to an Internal Auditory Stimulus". *Poster Presentation at the Alpine Brain Imaging Meeting*, January 6-10 2019, Gevena, Switzerland.

Perlis N., Al-Kasab T., Ahmad A., **Goldberg, E.**, Fadak K., Sayid R., Finelli A., Kulkarni G., Hamilton R., Zlotta A., Fleshner N. Defining a cohort of men who may not require repeat prostate biopsy based on PCA3 and MRI: The double negative effect. *Poster Presentation at the American Urological Association 112th Annual Meeting*, May 12-16 2017, Boston, MA.

Perlis N., Al-Kasab T., Ahmad A., **Goldberg, E.**, Fadak K., Sayid R., Finelli A., Kulkarni G., Hamilton R., Zlotta A., Fleshner N. Defining a cohort of men who may not require repeat prostate biopsy based on PCA3 and MRI: The double negative effect. *Poster Presentation at The 32nd Annual European Association of Urology Congress*, March 24-28 2017, London, England.

Teaching Assistantships

ES1036: Programming Fundamentals for Engineers — 2019
Western University, London, Canada

Biomedical Engineering Seminar — 2018–2019
Western University, London, Canada

Awards and Distinctions

Western Graduate Research Scholarship — $4,500
Scholarship of Excellence — $2,000
Third Place for Fetal and Placental Power Pitch at ISMRM — $100
First Place for Oral Presentation at London Imaging Discovery Day
Deans Honour List

Relevant Courses

Concepts of MRI	Human Physiology
Medical Imaging	Statistics
Electromagnetic Physics	Calculus

Qualifications and Technical Skills

Visual Basic for Applications (VBA)	MATLAB
Statistical Parametric Mapping (SPM)	3D Slicer
Java	ImageJ
FSL	Python

Societal Memberships

Canadian Assosciation for Neuroscience
International Society of Magnetic Resonance Imaging
Alpha Phi International Women's Fraternity

Certifications and Training

Laboratory Safety and Hazard Certification	Accessibility in Service
Worker Health and Safety Awareness	Animal Ethics, Care and Use Certification
Standard First Aid and CPR C	WHMIS Certification
Standard Operating Procedure for Clinical Research	Safe Campus Community