OPTIMIZATION OF IMAGE PROCESSING FOR MOUSE PHARMACOLOGICAL MAGENTIC RESONANCE IMAGING DATA

By

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Has been approved

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ABSTRACT

New imaging techniques, such as pharmacological magnetic resonance imaging (phMRI) permit measurement of *in vivo* changes in the brain after drug administration. However, there are numerous challenges when trying to apply this technique to commonly used animal models of disease, such as the mouse. This capstone project focuses on some of the data analysis issues faced when trying to analyze high resolution mouse phMRI data. Many of the problems encountered were due to the small size of the mouse brain in comparison to the human brain and the fact that most available MRI software is designed for human data. This work is part of a larger project designed to measure changes in the mouse brain after alcohol administration.

Introduction and Purpose

There is extensive data in the field of alcohol research on both the behaviors associated with alcohol ingestion and the role of genetics on these behaviors [1, 2]. There is less information available on how these genetic influences (genotype) are translated physiologically into the observed alcohol-related behaviors (phenotype). One potential approach to studying this problem is to use *in vivo* imaging techniques such as pharmacological magnetic resonance imaging (phMRI), a specialized form of functional magnetic resonance imaging (fMRI), to study metabolic changes after alcohol exposure in mice known to have different behavioral responses to alcohol based on their genetic make-up. Recent advances in magnetic resonance imaging (MRI), which include new protocols for studying non-anesthetized animals [3] and the increased availability of higher strength scanners make these types of studies feasible.

Main Project

The main project that is the basis for this capstone project is a pharmacologic magnetic resonance imaging study designed to measure *in vivo* changes in the mouse brain after alcohol administration. The central hypothesis for the main project is that *in vivo* metabolic changes that occur after alcohol administration can be measured using pharmacologic magnetic resonance imaging and that changes in response to alcohol between different mouse strains can be quantified. The methodology for this project involves the scanning of awake mice that have been acclimated to scanning [4] on a high-field instrument (12 Tesla) before and after intraperitoneal injection of alcohol using a pump system so the mouse does not need to removed between scans (a key step that

facilitates data analysis). The initial data analysis for this project was done using BrainVoyager™ QX. It is an ongoing project that is continually changing and improving the methods for obtaining data and for performing the data analysis. As of the writing of this capstone, the main project is still in progress.

Capstone Project

The purpose of this capstone project is to determine the optimal processing paradigm for mouse phMRI data. This includes an overview of the image processing issues encountered thus far, a discussion of how the problem was resolved, or if unresolved, current approaches being tried for the resolution of the issue. There will be a special focus on the advantages and disadvantages of available processing programs and the challenges of adapting these methods to mouse studies.

A significant problem in the field of phMRI is determining an optimal and appropriate processing paradigm. It would be best to adapt current processing procedures to be used in mouse studies. The current processing methods are optimized and designed to work with human subjects and trying to fit these processes to mouse subjects presents a number of unresolved issues.

Background

Magnetic Resonance Imaging

MRI, or magnetic resonance imaging, is an imaging modality used in both clinical and research environments. In research it is mainly used to obtain high resolution anatomic

images of brains, whether they are from humans or animals. To obtain these images, MRI uses powerful magnetic fields, radio frequency pulses and a computer. Different radio frequency pulses in different magnetic fields produce different spatial and temporal resolutions.

Functional MRI (fMRI) is a type of MRI that uses BOLD (blood-oxygen-level dependent) contrast to indirectly measure changes in neural activity in the brain [5]. In a classical fMRI experiment, a stimulus such as an image or sound is presented repeatedly and then changes in BOLD signal between the no stimulus and stimulus condition are compared to determine brain response to the activity. Three main parameters contribute to BOLD contrast: cerebral blood flow, cerebral blood volume and cerebral metabolic rate of oxygen extraction. For most standard fMRI experiments cerebral blood flow is the major contributor to the BOLD signal.

Pharmacologic MRI is a type of functional MRI study that is designed to evaluate the impact of a drug on brain function. While BOLD contrast has been used for these experiments, the assumption that cerebral blood flow is the major contributor to the BOLD signal may not be accurate due to drug impact on cerebral blood volume and cerebral metabolic rate of oxygen extraction that is independent of the neural response to the drug. Therefore, an alternate MRI method that uses an administered contrast agent to measure changes in cerebral blood volume is increasingly being used for these studies. The changes in cerebral blood volume are measured before and after drug administration.

An additional task, such as a motor activity, may also be added to further help define the drug effect.

Mouse Models

In most scientific research animal models are used to study disease states, disease progression, and in the case of this study, pharmacological effects. The mouse is a very common animal model. Mouse models are regularly used because they are reasonably inexpensive, have short gestation periods, and are easy to care for. Inbred strains have been created so that each animal subject is as similar to each other as possible. This is desired so that each mouse has the same genetic and physical characteristics and can be appropriately compared to each other in data analysis. In alcoholism research some phenotypic traits that are studied are tolerance, withdrawal, motivational effects, self-administration models, and level of response [6]. Different inbred mouse strains display these phenotypic traits in different ways. Each of these mouse traits can be used to test different diagnostic criteria in human alcoholism. For example, in humans withdrawal symptoms alleviated with alcohol can be related to the withdrawal seizure paradigm in mice ~~Table 1 shows which mouse models are used for which diagnostic criteria~~ [6].

~~Table Table 1 [6]~~
~~This table shows the Diagnostic and Statistical Manual of Mental Disorders criteria for alcoholism and the matched anim~~The inbred strain used in the main project was the DBA/2J, also known as D2, mouse strain. The D2 mouse strain is one of the most widely used mouse models for studying alcohol response. It is a low ethanol-preferring strain and some of its characteristics include a high level withdrawal severity and a consistent

voluntary ethanol consumption of approximately 0.2 g/kg per day [6]. The D2 mouse model has also been used to research other conditions such as age-related hearing loss, glaucoma, epilepsy, and diet-induced atherosclerosis [7].

Imaging Data Tools

There are many different image processing and analysis tools that can be used to evaluate fMRI images. This section will include a description of two software packages that have been used in the current project.

The first piece of software that will be described is BrainVoyager™ QX. This is commercial software that was originally created to analyze images of different modalities for human brain studies. The program is fairly user friendly if the user has sufficient knowledge about image data capture, interpretation and analysis. It includes an easy to learn user interface and is supported by good documentation and an online wiki for information resources.

The second piece of software that is described is MATLAB®. MATLAB® is software that is used for many different purposes. It can include many different modules called toolboxes that are used for individual situations. The image processing toolbox is the module that provides the graphical tools and algorithms used for image data analysis. The toolbox can be used to develop in-house data analysis scripts. There is also an analysis package that has been created to work with the MATLAB® framework called Statistical Parametric Mapping (SPM). SPM is a piece of software that is freely available and can be

used to analyze images from many different modalities similar to BrainVoyager™ and is a very powerful tool. Unlike BrainVoyager™, SPM has a less than optimal user interface and has a high learning curve for new users.

Image Data Processing

Data Processing Steps

The procedure for the imaging analysis usually begins with the transfer of the images from the scanning terminal to the analysis computer. After the images are transferred, they are reviewed to ensure the data was transferred properly and that the data is complete. Two types of images are generated. The first is a set of anatomic scans and the second is a set of functional scans (the latter are most commonly generated using the BOLD technique that represents changes in cerebral blood flow, volume, and oxygen extraction). The fMRI data is then pre-processed. ~~then undergoes a pre-processing protocol.~~ This ~~protocol~~ typically includes a 3D motion correction and slice scan time correction. In the analysis for ~~of~~ the main project, the pre-processing was conducted using BrainVoyager™ QX. The images were motion corrected to reduce the artifacts in the image due to head movement and the slice scan time correction was a process that corrected for the differences in the individual slices' acquisition time. Then a general linear model was applied to the data, after specifying which of the continuously acquired images were pre-alcohol injection and which were post-injection, in order to determine which areas of the brain showed changes after alcohol injection. The last step in the typical data analysis is the overlaying of the lower spatial resolution functional dataset onto the anatomic data set to help localize where the ~~the~~ most prominent changes in the

brain occur. However, some problems were encountered with this last step and are~~is~~ discussed below. After the image overlay step, it is customary to look at specific ~~-~~regions of interest (ROI) for further analysis. This permits the comparison of changes in a specific region across time points. The steps of preprocessing, data reconstruction, image overlay, statistical analysis, and ROI capture can either be conducted in BrainVoyager™ or the data can be exported from BrainVoyager™ and opened in a different image analysis software such as MATLAB® to be conducted by a home-developed MATLAB® script or conducted using SPM.

Data Acquisition

All data for the main project was acquired on a 12 Tesla MRI scanner at the Advanced Imaging Research Center (AIRC) at the Oregon Health & Science University (OHSU). All studies were pre-approved by the Institutional Animal Care and Use Committee (IACUC) at the Portland VA Medical Center where the animals are housed and were acclimated. Animals were acclimated to conscious scanning by Dr. Gang Chen from Dr. Kari Buck's laboratory at the Portland VA Medical Center. The following imaging parameters were used: ~~A~~ high resolution anatomic scan with a single 0.5 mm slice acquired with TR = 2770 ms, TE = 16, and voxel size of 100 micrometers and ~~was performed prior to functional scanning. The~~ functional scanning was obtained using 0.5 mm thick slices acquired with TR = 3500 ms, TE = 15, and voxel size of 300 microns. Functional scanning was performed continuously for 60 to 90 minutes with 15 minutes of pre-injection data and a hypnotic-sedative dosage of alcohol was used (4 gm/kg).

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Data Processing Issues

While adapting processing methods for mouse phMRI studies, several issues became apparent that needed to be addressed and solved to move forward with the project. In this section, these issues will be discussed by describing the problem that was encountered, explaining why it was encountered, describing how it was solved or describing a proposed resolution for the issue.

1) Motion Correction

The first issue identified was within the data pre-processing step of motion correction. This step was conducted to remove noise in the data caused by any subtle motion that the animal made, for example motion associated with respiration, in the MRI scanner at the time of data acquisition. While continuous scanning of the mouse without removal from the instrument facilitated the comparison of images taken at different time points, even small movements introduce errors in the analysis. BrainVoyager™, during the motion correction step, produces a graph that shows the amount of motion that was corrected for in all six directions of motion. These six directions are movement along the x axis (red), along the y axis (green), along the z axis (blue), rotation around the x axis (yellow), rotation around the y axis (magenta), and rotation around the z axis (cyan). These axes refer to the 3-dimentional space within the MRI magnet. The issue that was specifically identified was that a large amount of motion correction was taking place and was causing an unacceptably high amount of noise in the data. This indicated that some type of larger

amplitude movement was occurring during scanning. While this was not a data processing problem, the large amount of motion correction applied by the program indicated an experimental issue that needed to be addressed before any further data collection analysis could be performed. It was determined that the motion artifact was caused by excessive vibration of the animal holder inside of the bore of the magnet due to the low weight of the mouse (typical mouse weight is~~was~~ 30 gm). This issue was resolved by adding weight to the holder which reduced the amount of vibration that occurred. Figure 1 shows a comparison between two motion correction graphs. Figure 1a is the graph that was produced before the holder had a weight added to it ~~issue was resolve~~d and Figure 1b is the graph of the motion correction after the holder was weighted and the issue was resolved. The colored lines on the graph signify the six directions of motion and each color is defined in parentheses above. These graphs clearly illustrate that much less motion correction was necessary after the weighting of the animal holder with the result that an acceptable signal-to-noise ratio was achieved

Figure 1a

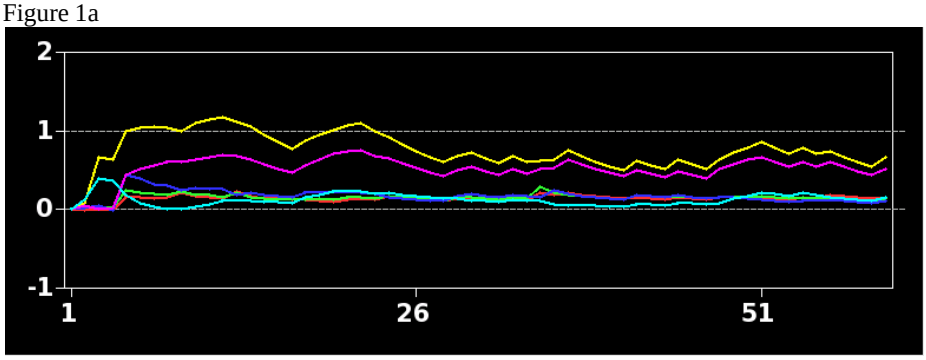

Figure 1b

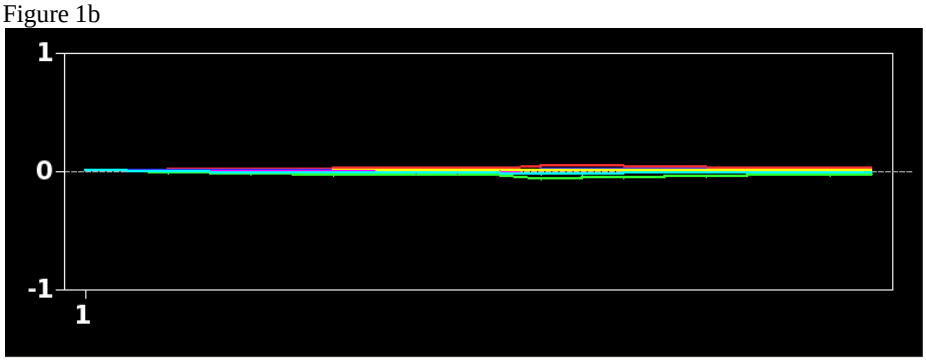

Figure 1
These graphs show the comparison between the motion correction that occurred before and after the issue of the animal holder vibration in the MRI scanner. Each number on the x-axis corresponds to a complete set of contiguous scans through the brain and took approximately 1 minute per set to acquire. The numbers on the y-axis are~~is~~ a general unit representing the top of the image to the bottom of the image in image space.

2) Data Reconstruction

Typically data is acquired in an isovoxel fashion which means that the individual voxels in the image are aligned with each other across the slices, which permits reconstruction after data acquisition in any plane. This is important because data analysis in rodents is

often done in the axial plane to minimize the number of slices needed to get good coverage of the brain and thus reduce the scanning time. At the time of initial data acquisition it was not clear what temporal and spatial resolution would be required to observe a significant difference between the pre- and post-alcohol slices, so a decision was made to maximize the resolution in the axial direction at the expense of the other planes (non-isovoxel data collection). However, t~~T~~his was a problem because the data was not able to be reconstructed in the coronal plane, which is the plane used for most mouse brain atlases. In the future this problem will be addressed by acquiring the data isovoxelly, even though this ~~there~~ will be at the ~~an~~ expense of temporal and spatial resolution. However, Isovoxel acquisition will permit reconstruction in any plane and ensure that we will be able to overlay the anatomic and functional data.

3) Image Overlay

Image overlay is the process of aligning a functional image onto a higher resolution anatomic image (this scan is typically acquired at the same time as the functional image or can be a composite of anatomic scans obtained from multiple animals). ~~brain atlas (which is usually an anatomic image of the mouse brain) over the functional MRI image of the mouse brain.~~ This permits better localization of the functional signal because the anatomic image has better spatial resolution. However, it was discovered after data acquisition was complete, that because of the lower resolution in the non-acquisition planes (coronal and sagittal) that BrainVoyager™ would not permit the data analyst to perform this overlay. ~~know where in the brain the functional contrast intensities are located. Generally functional images are of lower spatial resolution and the image~~

~~overlay onto anatomic images allows for better localization of the signal changes.~~ Figure 2 shows two images of a mouse brain in the axial plane. Figure 2a is the anatomic image and Figure 2b is the functional image. This figure ~~also~~ illustrates the lower spatial resolution of the functional scan. ~~s.~~

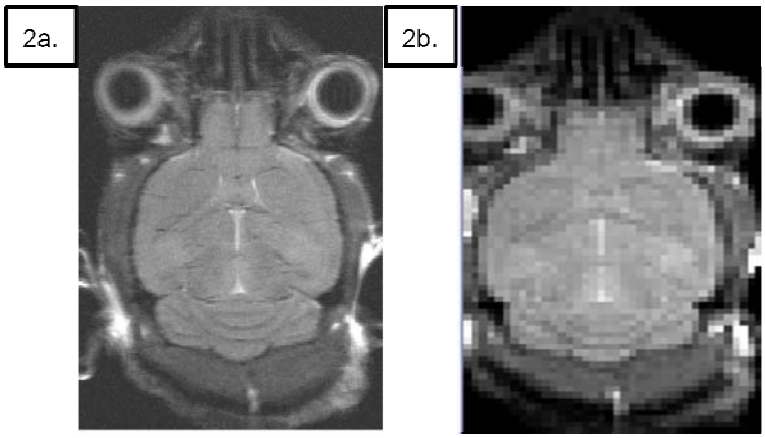

Figure 2
These pictures show a comparison between a MRI anatomic image and a MRI functional image. Figure 2a is the anatomic image of a mouse in the axial plane. Figure 2b is the functional image of a mouse in the axial plane.

4) Region-of-Interest Analysis

Region-of-interest analysis is the process of defining a particular section of an image for further analysis. There are a number of ways in which ROIs can be used in MRI studies. These include drawing the boundaries of an abnormal structure such as a tumor to determine its volume or other characteristics, identifying a specific region on a functional scan so how that region changes across different experimental conditions can be determined, or even trying to compare how a specific region varies from subject to subject. ~~In the main project there is a need to obtain a region-of-interest (ROI) because~~

~~only specific parts of the brain are of interest for comparison of the pre- and post-alcohol states. If the ROI is not used in analysis, the entire image would be included, which in the case of mice, would include the entire head.~~ The issue that arises in ROI capture when adapting methods designed for ~~from~~ human images ~~subjects~~ to mouse images~~subjects~~ is mainly ~~purely~~ due to the difference between the sizes of each species' brain. Since the mouse brain is so small (about 0.5 gm) even subtle changes in the placement of the ROI from slice to slice could potentially introduce large errors by ~~potentially~~ comparing brain regions that are slightly different from scan to scan. ~~not functionally equivalent.~~ Currently the methods for obtaining ROIs are relatively rudimentary and the method that needs to be used for obtaining ROIs in mice requires a high degree of accuracy and precision, especially if the smaller structures are to be evaluated. Work on this issue is still underway. Some preliminary efforts at ROI capture are outlined in the next section.

Preliminary Research on ROI Issue

Some Current Methods of Obtaining ROI

There are few methods of obtaining ROI. The most common and easiest to use is the manual method. There are also automated methods that may make the process of obtaining ROIs more accurate and precise.

Manual Method

In this method the researcher draws a ROI by looking at the image and visually drawing the ROI around the desired location (usually a structural entity or abnormality such as a

tumor). This hand drawn ROI is then used for analysis. When using BrainVoyager™ QX, the ROI tool is a box that the user draws around the region of interest, which makes this a manual method. Figure 3 shows a snapshot of an image with an ROI manually drawn in BrainVoyager™.

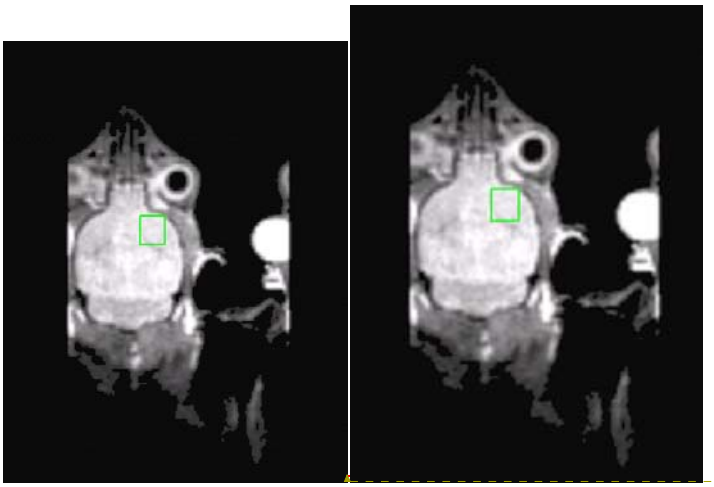

Figure 3
This is a functional MRI image of a mouse brain with an example a manually drawn ROI using BrainVoyager™.

MATLAB® also has a manual ROI tool, but it is not limited to a box shape and can be used to draw any shape.

Limitations of Manual Method

The biggest limitation of the manual method is that there is no way of being sure that each ROI drawn is the same. There could be slight differences between two ROIs, even if they are drawn with the same shape. In regards to the issue of ROI shape, some analysis software only allows ROI to be drawn with a fixed shape, such as the case with BrainVoyager™ only allowing a box shape. If the region a person is trying to capture is

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of a different shape than the ROI tool and a manual ROI is drawn, the analysis would include or possibly exclude a range of voxels that should not be a part of the analysis. Depending on how many voxels that may be, could possibly change the results of the analysis.

An Automated Method

One automated approach utilizes image filters and edge detection tools to find the borders of the ROI. Once the appropriate filters and edge detection tools are chosen for the particular situation, the program is very consistent in determining an ROI from one subject to the next. The user gives the program an initial manually drawn ROI that is used as a starting point and tells the program roughly where to find the ROI borders. This method described is a home-developed program using MATLAB® and the Image Processing Toolbox. It was created by Dr. Jayashree Kalpathy-Cramer, a researcher in the Department of Medical Informatics and Clinical Epidemiology at the Oregon Health Science University in Portland, Oregon.

Limitations of this Automated Method

The main limitation of this method is that the user may need to make filter adjustments depending on the image being analyzed and these adjustments are not necessarily consistent across all situations. For example the filters used to obtain ROIs of tumors in human organs (which is what this tool which was originally created for) may not be the same filters that would be best for obtaining ROIs of mouse brain structures. Also, even

if the same filters worked for both cases, the thresholds of the filters would most likely be different.

Discussion

The major theme that emerged in the data analysis was that there were specific challenges caused by trying to scale from a human brain down to a mouse brain. There is a large difference between the sizes of a human brain which is approximately 1,300 grams and a mouse brain which is approximately 0.5 gram. The first problem encountered, the need for a large motion correction, was the direct result of the mouse not weighing enough to prevent vibration of the animal holder in the magnet. It was not until the animal holder was weighted that this issue was resolved and the needed signal-to-noise ratio achieved.

The small size of the mouse brain required the initial data acquisition to be done using a non-isovoxel approach in order to achieve the needed spatial and temporal resolution. Because of this, it was not possible to reconstruct the data into the coronal plane (which all the mouse brain atlases use) and it was not possible to perform the key step of overlaying the functional scan on top of the anatomic scan.

Lastly, the typical challenges involving accurate placement of ROIs between slices were greatly amplified by the small size of the mouse brain. The tools currently used may be sufficient for most human imaging work because the amount of error introduced by

inconsistencies in ROI selection from slice to slice are small compared to the size of the ROI being investigated. Reducing the size of the area being analyzed increases the area to error ratio and presents a much greater problem in data analysis. The current ROI capture tools are simply not accurate or precise enough for the analysis of mouse phfMRI data.

Overall, the resolution of many of the data analysis issues encountered during this project involved clearly identifying the issue (e.g. large amount of motion correction indicating excessive movement) and making adjustments to the experimental protocol. Further adjustments to both the acquisition and data analysis protocols are expected. One remaining unresolved issue is exactly how to interpret a large, but non-uniform BOLD signal change. Future work on the main project will involve using other acquisition paradigms, such as the use of injected contrast to measure cerebral blood volume changes to determine if this is a better correlate of functional change after alcohol ingestion than the more widely available and easier to measure BOLD changes. The data analysis issues raised in this cCapstone project will continue to require attention and work if the full potential of phMRI is to be realized in mouse models of alcohol response.

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