A novel image processing pipeline for assessing volumetric changes to grey matter in ex-vivo brain tissue

Browne, Shannon

http://hdl.handle.net/2144/15613

Boston University
A NOVEL IMAGE PROCESSING PIPELINE FOR ASSESSING VOLUMETRIC
CHANGES TO GREY MATTER IN EX-VIVO BRAIN TISSUE

by

SHANNON BROWNE

B.S., Simmons College, 2010

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2015
Approved by

First Reader

Ronald Killiany, Ph.D.
Associate Professor of Anatomy and Neurobiology

Second Reader

Bang-Bon Koo, Ph.D.
Research Fellow of Anatomy and Neurobiology
ACKNOWLEDGMENTS

Thanks to Ronald J. Killiany, for always being there to help me through, and keep me on track, the past two years. Also thanks to Bang-Bon Koo, for helping me with the technical aspects, and being very patient while I tried to understand new techniques. In addition, thanks to my family, especially my parents Edward and Patricia Browne, for always being a phone call away, and supporting me, no questions asked. And to Justin Slawson, for being there through the everything, with a hug and a hot cup of tea.
A NOVEL IMAGE PROCESSING PIPELINE FOR ASSESSING VOLUMETRIC CHANGES TO GREY MATTER IN EX-VIVO BRAIN TISSUE

SHANNON BROWNE

ABSTRACT

Recently, Magnetic Resonance Imaging (MRI) has found great traction in monitoring the effects of Caloric Restriction (CR) on the brain, specifically gray matter. However, there are no streamlined, simple pipelines in existence to analyze data generated from these kinds of MRI studies. Therefore, my hypothesis is two-fold: the first part being the development of a dynamic and straightforward image processing pipeline, which I have tailored to fit the unique needs of the CR data involved in this study. This data brings me to the second part of my hypothesis, which is to use that pipeline to highlight the decreased attenuation in grey matter induced by long-term CR. In order to test the second portion of my hypothesis, T1/MPRAGE scans were collected from 17 male Rhesus Macaques, half of which were maintained on a 30% reduced calorie diet for an average of 22 years, starting around age 3. Using this basis, the inherent properties of the MR images were exploited by the novel pipeline, and used to analyze whether or not CR reduces the attenuation of grey matter atrophy, with regards to aging.
TABLE OF CONTENTS

Title .......................................................................................................................... i
Copyright page ....................................................................................................... ii
Reader Approval Page ............................................................................................ iii
Acknowledgements ................................................................................................. iv
Abstract .................................................................................................................. v
Table of Contents ..................................................................................................... vi
List of Tables .......................................................................................................... vii
List of Figures ......................................................................................................... viii
List of Abbreviations .............................................................................................. ix
Introduction ........................................................................................................... 1
  1.1 Calorie Restriction Research ................................................................. 1
  1.2 Use of Rhesus Macaques in Calorie Restriction Studies ................. 4
  1.3 Use of MRI, and its Effects on Ex-Vivo Tissue .................................. 6
  1.4 Use of Voxel Based Morphometry ...................................................... 10
  1.5 Purpose of this Study ............................................................................ 12
  1.6 Hypothesis ............................................................................................... 13
Materials ............................................................................................................... 14
  1.1 Animals ..................................................................................................... 14
  1.2 Scanner and Sequence .......................................................................... 14
  1.3 Software .................................................................................................. 14
Methods ................................................................................................................. 16
  1.1 Tissue Harvest and Preservation ....................................................... 16
  1.2 Image Post-Processing: Creation of Novel Pipeline ....................... 16
    1.2.1 Template Images ........................................................................... 16
    1.2.2 Calorie Restricted Study Brains .................................................. 17
Results .................................................................................................................... 29

vi
1. Effects of Calorie Restriction ................................................................. 29
   1.1 Calorie Restriction and Grey Matter ............................................. 29
2. Use of a Novel Pipeline ......................................................................... 33

Discussion .................................................................................................. 35

Conclusion ................................................................................................. 38

Appendix ..................................................................................................... 39

References ................................................................................................. 42

Curriculum Vitae .......................................................................................... 47
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summary of Brains through Segmentation</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>Summary of Subjects Included in Study</td>
<td>40</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Figure 1: Brain Flipped into Correct Orthogonal Viewing Panes</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Figure 2: Results from Image Thresholding</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>Figure 3: Results of T-Test, Uncorrected for P-Values</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>Figure 4: Results of T-Test, Corrected for P-Value</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>Figure 5: Demonstration of FSL and MATLAB Synergy</td>
<td>34</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

AVG/avg ........................................................................................................... Average
CR ....................................................................................................................... Calorie Restriction
FIM .................................................................................................................. Final Interpolation Method
FSL .................................................................................................................. fMRI Software Library
I.C. .................................................................................................................. Incorrectly Oriented
IS .................................................................................................................... Inferior/Superior
MPRAGE .......................................................... Magnetized Prepared Rapid Gradient Echo
MRI .................................................................................................................. Magnetic Resonance Image
N.A. ................................................................................................................ Not Aligned
N.C. .............................................................................................................. Normalized Coefficient
NIFTI .......................................................... Neuroimaging Informatics Technology Initiative
PA .................................................................................................................... Posterior/Anterior
RL ................................................................................................................... Right/left
Sac ................................................................................................................ Sacrifice
Stdev .............................................................................................................. Standard Deviation
TOT ............................................................................................................... Total
TRTMNT/trtmnt ........................................................................................... Treatment
VBM .......................................................... Voxel Based Morphometry
YRS/yrs ....................................................................................................... Years
INTRODUCTION

1.1 Calorie Restriction Research

Caloric Restriction (CR) has gained interest in recent years due to its apparent restorative and maintenance properties when it comes to the aging tissues of the body (Anderson, 2009, Colman, 2009, Bendlin, 2011). These, and other studies, have shown that aging takes a toll on all tissues in the body, with the brain being no exception (Grieve, 2005, Bendlin, 2001, Sridharan, 2014). In recent years, many studies have noted that the reduction in caloric intake actually reduces or alleviates many symptoms of aging, such as inflammation, maintenance of tissue quality and function (Colman, 2009, Sridharan, 2014, Bendlin, 2011, Kemnitz, 2011 Wisco, 2008). But even with all this research, the mechanics of how CR maintains tissue health remains unknown.

Overall, the general consensus from these studies is that CR improves and maintains cellular and molecular functions, which have cascading effects on daily functional ability. This can range from maintenance of cognition, improved skeletal muscle health, and reduced systemic inflammation, even as the body gets on in years (Bendlin, 2011, Sridharan, 2014, Kemnitz, 2011, Wisco, 2008, Colman, 2009). This is in addition to the fact that in recent years, some researchers have shown that the reduction in caloric intake actually improves the body’s ability to regulate glucose (Willette, 2012,
Anderson, 2009, Kemnitz, 2011). All of these conclusions have been well illustrated for the past 70 years, when CR was first highlighted (McCay, 1935). In general, the concept of CR has been known to have large positive effects on the aging human, but not much more has been revealed in that same length of time.

The basis of CR is the idea that reducing the overall caloric intake, while keeping a highly balanced nutritional intake, is what reduces inflammation and improves tissue health (Kemnitz, 2011, Colman, 2009). But, what this does to the body, and exactly what goes on during CR, is still very much a mystery. Despite this unknown, it cannot be ignored that the effects are astounding and, in many cases, can be solely attributed to CR (Willette, 2012, Colman, 2009, Wisco, 2008, Anderson, 2009, Sridharan, 2014, Matochik, 2004, Bendlin, 2011). Over these seven decades of research, a mountain of evidence has been amassed, all of which points to CR as being the variable responsible for things like longer life, maintenance of cognition, and improved skeletal muscle health (Willette, 2012, Colman, 2009, Wisco, 2008, Anderson, 2009, Sridharan, 2014, Matochik, 2004, Bendlin, 2011). What gets tricky is finding uncompromised ways of studying CR in humans.

People are breeding grounds for confounding variables, creating an ambiguous setting where results of a particular lifestyle cannot be attributed to one factor alone. This gets even more complicated when it comes to measuring CR, as there is no control group, or baseline, by which to measure the reduction of percent calories, which is how most CR studies are conducted (Bendlin, 2011, Anderson, 2009, Sridharan, 2014, Sridharan, 2013,
Colman, 2009, Willette, 2012). However, there are certain island populations, remote and restricted, which have incorporated voluntary CR into their lifestyle for generations (Anderson, 2009). These populations demonstrate the effects of CR, which have been repeatedly reproduced in the lab. One of these is an island in Japan (Anderson, 2009). Unfortunately, there are still too many variables, which could comprise, mask, or accentuate the effect of CR, making it impossible to attribute any effects directly to the effects of reduced caloric intake.

Excluding the aforementioned study (Anderson, 2009), there are not many instances of populations undergoing CR, and living until they are centurions. Why exactly long term CR has this effect is not well understood. However, it has been demonstrated that maintaining a reduced calorie diet while keeping up optimal nutrient intake has been shown to maintain function in memory tasks, muscle tasks, and various types of cell types in the brain (Bendlin, 2011, Sridharan, 2014). While the methods behind this premise are not well understood, many think that it has to do with the reduced rate of oxidative inflammation, which is commonly associated with the reduced calorie intake (Matochik, 2004, Bendlin, 2011, Sridharan, 2014). Something that is addressed by these paper is that normal caloric intake can induce inflammation, which can cause cascading damage to cells, and their associated molecules. In fact, this is thought to be the major source for most of the “aging” (Matochik, 2004, Willette, 2012).

Therefore, in order to isolate, and study, the effects of CR, and only CR, the environment needs to be controlled and manipulated in a way that researchers are sure
that CR is the only variable responsible for the effects being seen. In the past, CR as been performed in cells, and rodents such as mice and rats (Willett, 2012, Colman, 2009, Anderson, 2009), but none of these models can be easily translated to humans. This is where non-human primate research comes into play, specifically involving Rhesus Macaques.

### 1.2 The Use of Rhesus Macaques in CR Studies

Rhesus Macaques (*Macaca mulatta*) are an Old World monkey native to India and parts of China (Kemnitz, 2011, Kemnitz, 1998). Classified as a Non-Human Primate (NHP) and commonly used in experimental designs, this model has the advantage of being a primate model distinct from humans, but with certain undeniable characteristics which can be extended to humans. Rhesus Macaques are used because of their reaction to environmental stimuli, and availability in well-maintained and controlled research facilities due to the easy upkeep (Kemnitz, 2011, Kemnitz, 1998, Colman 2009).

In particular, the details of their species are well documented, which makes using them for research extremely easy. The males weigh about 7.5kg, grow to be about 52cm, and live to be about 40 years old in captivity (Kemnitz, 2011, Kemnitz, 1998). What’s most important is that their aging pattern can be extrapolated almost as a 3:1 ratio in relation to humans; for every three years the monkey ages, a human ages one (Kemnitz,
2011, Colman, 2009, Kemnitz, 1998). This implies that they go through the same level of cellular atrophy, be it grey matter or white, and are susceptible to cerebrum shrinkage (Sridharan, 2012). This makes Rhesus Macaques the perfect subjects not only to monitor aging, as it relates to humans, but also to monitor any effects that CR have on a typical aging pattern.

However, reducing the caloric intake of animals on study is more complicated than it may seem. It requires measuring not only the animal’s baseline food intake, but also taking into account the baseline food intake of the control group, and reducing the calories based on that amount. In many cases, the food intake needs to be constantly monitored and revaluated for effectiveness (Willette, 2012, Bendlin, 2011, Matochick, 2004). In these cases, many of the control Macaques spontaneously stopped eating their allotment of food, meaning that the restriction of the variable group was closer to 40%, not 30% as required by the study (Matochik, 2004). In these cases, the food and calorie allotment had to be altered to meet, and not go under that 30% reduction.

However, as well as these studies are designed, the problem of looking at the tissue, in a method other than is common with the destructive nature of histopathology, there is really only one option: MRI. This modality is of specific interest to this study as it is the perfect modality to image the ex-vivo tissues, which were too valuable to use only for pathology. With particular interest paid to the effect of CR on the grey matter of the brain, the goal of this study is to help pull apart exactly what happens to the brains after a long period of CR restriction. Rhesus Macaques are the perfect subject for
this because, not only does CR leave its mark on the brain, but any results can be extrapolated to humans. For these purposes, 17 male Rhesus Macaques were put on long term CR, with the idea that the effects of this lifestyle would be monitored in the brain post-mortem via histology and MRI.

1.3 Use of MRI, and Its Effect on Ex-Vivo Tissue

In recent years, MRI has grown in popularity with its ability to monitor the quality and type of tissue inside a brain, without using invasive or harmful chemicals. Specifically, it can visualize various tissue types with relatively high resolution, and produce image files, which can be analyzed easily with experienced readers or the appropriate software (Smith, 2004, Sridharan, 2012). With this study, this was particularly important, because while the brains had all previously been harvested, the samples were too valuable to use for one study. While histology can get highly detailed images of cell types, including the organelles inside the cells, it is hard to get a whole, holistic perspective about how these cells interact with each other to form whole tissue sets (Sridharan, 2012, Pfefferbaum, 2000, Grieve, 2005).

In addition, it is possible to visualize different tissue types, damage and lesions in MRI without cutting tissues, or even applying harsh contrast agents. MRI, in its purest form, only manipulates the endogenous water molecules in a sample, and generates tissue
contrast which is reflective of the amount of water present in each cell type (Wisco, 2007, Gunning-Dixon, 2009, Matochik, 2004, Pfefferbaum, 2000), and therefore the type of tissue present. This generated contrast is ideal for looking at specific tissue types in the brain, and allows researchers a way to look at what’s actually going on inside a tissue is a non-invasive manner, unlike the techniques involved in histology. Because, in histology, once a tissue is sliced, or stained, it can never be used for any other purpose. Therefore, for the purposes of this study, MRI was the best solution to the problem of wanting to visualize the brain as whole, while maintaining tissue integrity for other purposes.

MRI is the ideal form of analysis for this tissue sample, as it is non-invasive and non-destructive, while giving detailed images about all the tissues in a certain region (Pfefferbaum, 2000, Grieve, 2005, Bendlin, 2011, Matochik, 2003, Gunning-Dixon, 2009, Wisco, 2007, Kennedy, 2009). While this is highly advantageous for living subjects, it gets a little complicated with ex-vivo tissue, as far fewer studies are preformed on dead tissue. With this study, the only tissue to which we had access was a single hemisphere of a brain suspended in solution. This meant that certain parameters had to be changed, or taken into account when it came to collecting and analyzing the remaining samples.

In particular, it had to be taken into account that ex-vivo tissue reacts differently in the MRI environment than living tissue (Kanawaku, 2014). Because of the difference in water contents (between living and dead tissue) and the fixative used, all T1 sequences
(like those gathered for this study) have T2-like properties (Kanawaku, 2014). This means that any pre-set parameter settings in the typical post-processing pipeline, which would normally be applied to a T1-weighted scan, can’t be used in this setting. Instead, unique parameters for these scans were derived and applied for our purposes (see Methods).

This is based on the idea that a T1 sequence and a T2 sequence apply different kinds of magnetic force on the water in the brain, thereby generating different images. In T1 scans, fluid appears dark, whereas in T2 scan, fluid appears bright (Kanawaku, 2014, Macura, 2014). There are more sequences, which can be run, but these are the main ones, which will be addressed in this paper. Clinically, T2 scans are used to highlight fluid related abnormalities (bleeds, edema, etc.) (Kanawaku, 2014, Macura, 2014). But because it’s based on the amount of water in a certain area, it means that it’s affected by things like death and fixative, just like T1.

In fact, when tissue dies, and is subsequently fixed, the water molecules become reduced and the remaining tissues react differently to the MRI magnetic pulses (Macura, 2014, Kanawaku, 2014). Effectively, T1 and T2 signals appear reversed on MRI scans. This means that in a T1, fluid appears bright, making it look like a T2 (Macura, 2014, Kanawaku, 2014). This is important for the study addressed in this paper because these Macaques were kept on study until death, and upon death, their brains were extracted (see Methods) and half of the hemisphere was placed in fixative (10% neutral buffered
formalin) until scanning time. These two factors result in the “bright fluid” T1 scans detailed in this paper.

There are advantages to ex-vivo MRI, over in-vivo, some of which are the increased resolution and decreased motion artifacts that come from working with dead tissue (Kanawaku, 2014). However, motion cannot be eliminated in studies like this one, as the tissue was independently suspended in a solution filled tube. This was then inserted, in its floating state, into the core of the MR scanner, so that the tissue would remain equally hydrated across the scanning session. So while it was possible to generate scans with increased resolution, due to the fact that longer, more detailed saturated scans could be run (Kanawaku, 2014), some motion still occurred.

However, even if the most detailed images can be gathered, it still takes a third party (either man or machine) to analyze the data present in the images. Up until fairly recently, most often, the analysis was done via manual segmentation (Kennedy, 2009, Callaert, 2014, Ashburner, 2009). This meant that a trained individual would go through a subset of the images and pull out the different tissue types based on the grey scale values, which had been visually assessed to be part of one tissue type, or another. This means that that image analysis could vary between people, and even between labs, leaving questions about whether or not data was reproducible, even if they followed the same protocol (Ashburner, 2009, Calleart, 2014). This is where more automated computer analyses come into play.
There are computer programs built specifically to work with these MR images based on the tissue’s intrinsic grey scale values, which can be programmed to identify tissue classes. These computer programs have been built on the concept of Voxel-Based Morphometry (VBM). This program uses lines of code to analyze the MRI voxel by voxel, (Li, 2011, Ashburner, 2009, Ashburner, 1999) and depending on the grey scale value, sort it into grey matter, white matter and CSF.

### 1.4 Use of Voxel-Based Morphometry

The basis of Voxel-Based Morphometry (VBM) is the idea of using a few lines of code to look at a digital image, and sort it based on predefined parameters (Ashburner, 1999, 2009, Sawiak, 2013). This comes into play in the study outlined in this paper because the details for which images were being combed could not have been detected with the naked eye, no matter how good the analyst. In particular shifts in total amount of grey matter across two groups of animals were being tracked. In many cases, it was projected that the shift would be subtle, and not contain enough of a difference to be picked up by a human.

While this will be addressed in this paper, it is pertinent to note that these details were overcome by using a computer based process, which utilized VBM. The basis of this program comes from analyzing voxels as they correlate to tissue types (Ashburner,
1999, 2009). The computer identifies, based on probability maps, the most likely tissue class the voxel belongs to, and assigns it a value (typically 0 to 2) based on its internal statistics.

VBM analyzes the MR image voxel by voxel, and classify each voxel based not only what the grey scale value of that individual voxel, but also based on the values of the surrounding voxels (Ashburner, 1999, 2009, Sawiak, 2014). Therefore, it takes into consideration not only the assumed value of the voxel currently being analyzed, but also the values of the neighboring voxels (Ashburner, 1999, 2009). This means that the voxel is based on a probability value, which increases the chance of accurately labeling the voxel while at the same time reducing the chance of inaccuracy due to machine or computer error. This idea of probability based values is the basis of what makes VBM so unique.

While nothing can replace the human eye when it comes to analyzing MR images for damage and lesions, computers do trump manual analysis when it comes to analyzing global changes in tissue types and amounts (Sawiak, 2014, Smith, 2004, Callaert, 2014, Kennedy, 2009). It has been documented in many studies about how computers produce more accurate, consistent and reproducible results, compared to manual raters (Smith, 2004, Callaert, 2014, Kennedy, 2009). VBM really comes into play with this paper because it allows for an accurate way to analyze global changes in brain grey matter.
1.5 Purpose of This Study

In this paper, an alternative and flexible image processing pipeline was developed to handle exclusively the hurdles presented by MRI scans of fixed, half-hemispheric brain tissue from Rhesus Macaques. In the following methods section, the pipeline will be detailed from start to finish, from converting the data to segmentation and analysis.

There are very few studies, which use extracted ex-vivo tissue for MR analysis. Some studies will use the samples, still in the body, post-mortem, which allows them to use things like contrast and sequences, which generate a higher resolution (Kanawaku, 2014). But very few studies deal with tissue that has already been extracted. It was felt that this was an oversight, as extracted ex-vivo tissue can be just as valuable and informative as intact ex-vivo tissue and in-vivo imaging (Macura, 2014, Kanawaku, 2014). Therefore, it was decided that an image processing pipeline needed to be developed.

We developed this pipeline using applicable parts and functions of MRI Software Library (FSL) and MATLAB, both of which are useful when it comes to analyzing images down to the voxel level (Smith, 2014). We use the strengths of FSL to align and segment the tissue and MATLAB scripts to exclude and highlight only the information, which was pertinent to the second portion of my hypothesis.
The purpose of this study was to develop a novel pipeline to handle to the samples from this long-term CR study, in order to view the effect of CR on grey matter density over the course of a significant portion of a lifetime, with respect to aging. A handful of studies have already outlined potential effects on grey matter aging (Callaert, 2014, Wisco, 2004, Kennedy 2009). But where there’s room for exploration is looking at these respect to CR’s impact on typical aging.

1.6 Hypothesis

In this study, we decided to test two separate hypotheses; the first being that existing scripts, techniques, and computer programs could be adapted from living tissue sequences in order to generate a novel pipeline to analyze single-hemisphere, ex-vivo tissue. The second portion of the hypothesis is to see whether long-term CR reduces the loss of gray matter with regards to aging, as suggested in previous literature, which suggest the idea that CR attenuates the volumetric atrophy gray matter, preventing the reduction of gray matter with age. To my knowledge, while this concept has been alluded to in other studies, it has not been able to be fully explored in a quantitative manner, which is what helps make this a unique and significant study in the budding world of MR image analysis.
MATERIALS

Subjects

Single hemisphere brain tissue from 17 male Rhesus Macaques (*Macaca mulatta*), were available for use in this study. These animals were part of a longitudinal study being conducted at the National Institutes of Health (NIH), and were individually housed at an NIH facility in Poolesville MD. They were kept on a 30% calorie reduced diet for an average of 23 years. Most animals started the study when they were approximately 7 years of age, with the start age ranging from juvenile (1-2 years), adolescent (3-5 years) or old (16-23 years). In total, 8 animals were fed *ab litum*, and 9 animals were calorie restricted (CR).

Scanner and Sequence

All samples were scanned in the Phillips 3T, Achieva whole body imager located at the Center for Biomedical Imaging at Boston University School of Medicine. Scanning consisted of a Magnetized Prepared Rapid Gradient Echo (MPRAGE), using the 8-channel phase array coil. The sequence TE = 3, TR = 7, slice thickness = 0.6, matrix = 256 x 256, FOV = 20 x 20, flip angle = 8, nex = 1.

Software

The software package used was the fMRI Software Library (FSL) v.2.9. This program was used to analyze the MPRAGE NIFTI (nii) files after they were converted
with MRIconvert (v.1.0). It was then used to change the orientations, threshold and segment the tissue in a manner in which it could be analyzed and compared to an atlas, done by using MATLAB (R2012b, 8.0.0.783, 64bit win64).
METHODS

Tissue Harvesting and Preservation:
Animals were euthanized with an overdose of sodium pentobarbital. The brain tissues were harvested fresh and placed in 10% formalin before they were shipped to Boston University School of Medicine. Upon arrival, the brains were transferred to 10% glycerol solution, and then to a 20% glycerol solution.

Image Post Processing: The Generation of a Novel Pipeline

Template Images

1. Template masked to match study brain:

   a. Previously generated average brain image of normal Rhesus Macaques were used in order to extract the information from the sample images. An average brain was formatted to match the sample brains. In this case, because the experimental brains were only half a hemisphere, the template brains needed to have the “extra” hemisphere masked out. This was done through the MATLAB script described below:

   ```matlab
   filename = 'template.nii';
   tlinfo = spm_vol(filename);
   T1 = spm_read_vols(tlinfo);
   xdim = 120;
   ydim = 135;
   ```
zd = 95;
x=1:xdim;
y=1:ylim;
z=1:zdim;

pT1_half_vol= T1;
pT1_half_vol(1:60,:,: )= 0;

Note: Image file name and dimensions may vary between experiments and labs.

b. The script reads the two brains, the template and the experimental, in parallel. As the script passes through the study image, and everywhere there’s a black voxel (value: 0), it replaces the same voxel at the same x, y, z coordinate with black(0). This effectively masks out the “extra” hemisphere on the template brain, making it identical to the study brain.

Calorie Restricted Monkey Brains

2. Converting the files:

The brain tissue was scanned and the images were saved and exported from the scanner in the dicom format. The files were converted to the Neuroimaging Informatics Technology Initiative (NIFTI) format for use in the post- processing pipeline using MRIconvert. NIFTI (or .nii) is used because of its ability to be used by multiple programs, while retaining the specific properties of the MRI scans.
- WhyMRIconvert: it is consistently the most used program when converting DICOM files to NIFTI format as it is simple and effective, producing few to zero errors during the process of conversion.

3. Creating an “average” brain from the scans:

Each half hemisphere sample went through a series of scans (anywhere from 4 to 10), creating a sampling of images from the same brain. These images were then combined into one “average” brain image for further analysis via an averaging function in FSL, described below:

a. **Averaging** was completed using the terminal window on the lab’s Mac computer. After setting the path to the images, the command “fslmaths” was run by inputting the file, an “-add” function, a second file, and then the resulting file name of the two combined images, illustrated in the below command:

   Fslmaths inputvolumename -add inputvolume2 combinedvolumename

b. This function was repeated until all the images from a particular subject were combined into one file.

c. Images were then saved as a compressed nii.gz file extension for further use
4. **Cropping the images to tighten the focus of the information:**

Once the average brain for each monkey was created, the extraneous area surrounding the brain was cropped away. This allows the focus of information to be solely on the brain. This subsequently highlighted the tissue contrast to the point where grey and white matter becomes distinguishable.

a. **Cropping:** This was done in the terminal window on the lab’s Mac, after setting the location to the new averaged nii.gz image via the terminal window. The command “fslroi” was run. This creates a bounding box, generated by the max and min values for the X Y and Z dimensions of the brain, not the image. This is what allows FSL to cut the image at all three dimensions, focusing the brain to the center. In addition, this cuts down on the processing that will have to be done in later steps, because much of the extra information has been removed. The bounding box was created as follows:

```
Inputvolumeputvolumexminxsizeyminysizezminsize
```

b. Once created, the image was saved into a new file.
5. Orientation of the Brain:

In order to ensure that the brain tissue images were displayed correctly, the images had to be flipped into the correct orientation: coronal in the top left, axial in the bottom left and sagittal in the top right, as seen below:

![Brain Flipped into Correct Orthogonal View Panes](image)

**Figure 1, Brain Flipped into Correct Orthogonal View Panes:** Above is an image of CR006 after is has been flipped and aligned into the correct space in the orthogonal viewer.

a. **Orientation:** This was done through the terminal window by tracing the path to the correct images and, once there, typing in “fslswapdim”. For the study images, the directional labeled had to be changed, instead of just rotating them around x, y, or z axis’. This caused them to flip between view panes, instead of just flipped in their view panes. The command was input in the following sequence:
First:    fslswapdim inputvolume RL PA IS outputvolume1
Second:  fslswapdim outputvolume1 LR IS PA outputvolume2
Third:   fslswapdim outputvolume2 –x y z outputvolume3

Note: not all of these flipping sequences were required to get all the images in the correct plane. Which flipping sequences were applied to each sample is illustrated in Table 1 (in Appendix). Typically, no matter how many flipping sequences were applied, the necessary ones were applied and checked for accuracy before proceeding to the next step. Overall, the sequences caused the right left orientations to become flipped and for them to end up in the correct view panes, so they could be correctly processed further.

b. In certain cases, because of the way the images were placed in the scanner, an additional set of sequences was required in order to get the brains in the correct orientations. This is illustrated in Table 2 (in Appendix)

6. Removing the directional labels:
In many cases, once the brain was correctly oriented in the orthogonal viewer, the directional labels were still incorrect. For the study purposes, this information was deleted.

a. Deleting: The terminal window was again used, and after a path to the images was set, the following commands were run:
fslorient –deleteorient outputvolume3or2fromstep5

b. Careful track of all directional information was kept while completing this step.

Note: There is no need to save this file outside of its current name, as the information is just being deleted, and no other information is changing.

7. Thresholding the image:

In an attempt to remove as much “extra” information as possible, while improving the processing, the brain images were **thresholded** to eliminate the background noise.

a. **Thresholding:** Images were opened in fslview (either through the program or through the terminal window) and the min scroll bar, located with all the other tools at the top of the window, was used to determine the minimum threshold to eliminate the noise.

b. Once a value has been determined, it can be input into the terminal function as follows:

```
fslmaths outputvolumefromstep6 trthresholdnumberoutput volume
```
Figure 2, Results from Image Thresholding: Image A (on left) is of Cr012 before being thresholded to appear as image B (on left).

Note: In some cases, this had to be repeated in order to maximize the amount of background noise that was eliminated. This is illustrated in Table 1 (in appendix)

8. **Aligning the images to the template:**

In order to correct for the alignment of the image, the images were run through the FLIRT process in FSL, against the previously prepared template image (described in previous section). This process matches the alignment of the study brains to that of the template brains.

a. **Aligning:** Images were opened via FSL FLIRT function, which uses a window in which parameter can be se. The parameters were run are as follows:
9 parameters controls
normalized mutual information
incorrectly oriented
correlation ratio

b. Some of the images did not respond well to these parameters, and therefore required an additional flip, and second FLIRT process. This is outlined in Table 1 (in appendix).

9. **Masking the extraneous background:**

In order to remove the extraneous background information, the images were **masked** as follows:

a. **Masking:** These images (experimental and template) were imported into MATLAB and the script below was run as follows:

```matlab
filenameT = 'template.nii';
t1info = spm_vol(filenameT);
T1_avg = spm_read_vols(t1info);

filenameCR = 'calorie_restricted_sample.nii';
t1info = spm_vol(filenameCR);
T1_CR = spm_read_vols(t1info);

xdim=120;
ydim=135;
zdim=95;

x=1:xdim;
y=1:ydim;
```
z=1:zdim;

T1_CR_mask = T1_CR;
  for x=1:xdim;
    for y=1:ydim;
      for z=1:zdim;
        if T1_avg(x,y,z) < 15;
          T1_CR_mask(x,y,z) = 0;
        end
      end
    end
  end
end

Note: This is just a sample of the script, which was run, all file names, dimensions and threshold levels should be adjusted for specific purposes.

b. The above script is doing a “read and replace” job. It reads both the experimental and template brains in parallel, and where it detects a black voxel in the template brain (value: 0), it replaced the voxel at the same x,y,z coordinate with that value (0). This effectively masks out all the background noise.

10. Segmentation:

Once the images have been aligned and masked, they are ready to have different tissue classes segmented by the FAST process in FSL. Because the tissue was imaged post-mortem, and due to the nature of imaged ex-vivo tissue, these T1 images appear as if they are T2. Because of this, even though they had T1 sequences run, they were run under T2 parameters in FAST.
a. 3 tissue classes were chosen as end points for segmentation (with the goal being to pull out grey, white matter, and CSF). In order to pull out grey matter specifically, for the question being asked, the a-priori map should be used to optimize the grey matter segmentation. Overall, the parameters used were as follows:

- Image Type: T2
- Number of classes: 3
- MRF: 0.1
- Biased Field Removal: 4
- FWHM: 20.0

Note: All the segmentation files generated by this function should be double checked for accuracy. In this case, the “pveseg” files might be of most use.

b. FAST produces a lot of varying image files, but for the purposes of this study, only the Grey Matter (GM) .pve file was used. For our use, the grey matter file was labeled “pve_0”, which was used for all further statistical analysis.

Note: On these images, pve_0 appeared as the “paler” of the two tissues in question, even though it is grey matter. This is due to the tissue contrast reversal caused by taking an MRI of extracted ex-vivo tissue kept in formalin for over a week.
11. Image Analysis: Two sample unpaired T-Test:

Once the 0_pve file was obtained, the statistical files needed to be generated in order to determine whether the hypothesis was supported or not. For this, a two sample unpaired T-test was run via the following steps:

a. **Create 4D data:** This involves the combination of all the data generated, across all dose groups. Usually done through fsmerge using the pve_0 files (these are the GM files generated by FAST, even though they are the paler of the two tissues classes. Because of the ex-vivo nature of the tissue, the T1 signal is an inversion of what it would normally be, visually). The following command was used:

   Fslmerge –t crab_merge.nii in1 in2 ...inN

i. “GLM_gui” (General Linear Model) was run, which will generate all files for future models, by typing glm_gui into the terminal, and after designating the correct number of total inputs, run the higher-level analysis. After which the wizard was run, with an unpaired two-sample test, and set the number of subjects in the first group.

ii. The EV “map” was created by designating the first digit, which should indicate the dose group; the second in the series should designate the
Once saved, it will become the .mat files to run “randomise” in the terminal window with the below code:

```
r -randomiseicrAB_merge.nii -o c rAB_merge_rand.nii -d design.mat -t design.con -n 5000
```

Note: n = number of permutations to be run

iii. 4 files will be generated from this, and the following T stat will be generated:

- **T stat 1**: greatest difference A-B, A>B
- **T stat 2**: greatest similarity: B-A, B>A
- **T stat 3**: average A
- **T stat 4**: average B

Note: These will all generate different images, to be used how you need them

b. These were opened in FSL to view and “add” them to each other to create a more complete picture of where there are deficits and where areas overlap. Which can be better visualized by changing the thresholds (max, min)
RESULTS

1. Effect of Calorie Restriction:

When most of the animals began the study, they fell within the “young” age group, described in Table 1. All brains were kept in formalin for about the same time before being analyzed. Once the images were generated from the FSL segmentation process, an additional set of statistical files had to be created in order to interpret the results (detailed in Methods section). Once these files were generated, and run with the data images, the results were generated in a visual format, illustrated below, and highlight the effect of CR on grey matter.

1.1 CR and Grey Matter:

Once the images were merged to create a master file, they were run through a two sample, unpaired t-test, to test for directionality with the change in grey matter. Specifically, we wanted to see if grey matter was maintained with respect to CR and aging, so a single direction t-test was chosen for the analysis. In this case, as was mentioned before, group A is the control group, and group B is the Calorie Restricted group. In order to analyze the difference between these groups, the statistical program “randomise” was run on the terminal window on the Mac.
The randomize program in FSL is a non-parametric inference tool to be used on neuroimaging data (Winkler, 2014). Randomise is used to threshold when the null distribution is not known (either because non-standard statistics are used or because the data does not follow simple distribution) (Winkler, 2014). In addition, it has multiple output modes, one of which is Threshold-Free Cluster Enhancement (TFCE) based, and can correct for p-values. Below is the command line used with randomise in order to generate the difference maps:

```
Randomise -icrAB_merge.nii -o crAB_merge_rand.nii -d design.mat -t design.con -n 5000 -T
```

In the above command line, the listed sections call the file into the line, give it a new output name, and uses two generated stats files (previously mentioned). In this case, the “-T” function runs the TFCE, while remaining a voxel-wise comparison (Winkler, 2014), and specifically calls up a two sample unpaired T-test with the input data. Multiple image files are produces, some of which are corrected p-values, and some which do not. Below are the resulting images, before and after the correction for the P-value:
Output 1: Results of T-test, Uncorrected for P-Values

Figure 3: Above the merged file (black and white) overlaid with the original map of difference between the two groups, A and B (green). The green file illustrates the “tstat1” file form the output of the statistics program “randomize”. In essence, while it appears that there are some changes here, it’s hard to tell if it’s significant.

The above image only shows where there’s maintenance in grey matter volume attenuation, not what’s statistically significant. The areas of difference include cortical areas like the poscentralgyrus, supramarginalgyrus, all the way deeper into areas of the brain like the thalamus. It also includes some areas in deeper parts of the brain, such as the lateral geniculate nucleus, the peduncle, and the red nucleus. However, because the file only shows the areas of general grey matter maintenance, not statistical difference, whatever interest these areas may hold needs to be curtailed until the significant
difference is determined. This is where the image file highlighting “corrp” is used. The files produced with this same line of code, with the “-T” added in to call in files generated by the two sample T test, with the corrected p-value of 0.05.

**Output 2: Results of T-Test, Corrected for P-Values:**

![Figure 4](image)

**Figure 4:** the black and white file contains the merged image of the brain, while the red file (seen as three small red dots in the cortical region) are all that’s left of the data when the p-value is adjusted to 0.05.

As can be seen, once a p-value of 0.05 is implemented, most of the areas of interest disappear, leaving only three (at most), which border between the supramarginal gyrus and the superior temporal gyrus. Here, it can be determined that because of the
single direction, two sample t-test, these areas maintain their grey matter volume over time with respect to CR.

2. Use of a Novel Pipeline

In this study, a novel pipeling was sucessfully designed and implemeted to meet the unique needs of the samples provided and the study requirements. And as can be seen by the results generated, this pipeline perfectly sorted through the a-typical tissue samples to extract the nessisary data. This was done in a way that a typical pipeline wouldn’t have been able to sucessfully complete, as evidece by the results.

Specifically, MATLAB was used in conjuction with FSL to be able to do a lot of functions that either program could not do alone, as is the typical way of usual pipelines. In particular, these programs were used to take out a lot of background noise surrounding the brains. While FSL could do a decent job of this, MATLAB really came in to clean up everything to exclude all extraneous data, which really tailored the area of focus to just the brain tissue. Below is a figure of how these two programs worked:
Figure 5: Above is an example of how FSL and the previously mentioned MATLAB masking script work together. A. is the raw, unthresholded and masked image. B. is the image after is have been thresholded below a certain level in FSL (described in Table 1). As can be seen, there is still some background noise. This is eliminated in C. with the use of a MATLAB script.

As can be seen in Figure 5, the pipeline completely isolated the brain tissue for analysis. In many cases, FSL can be used to threshold the brain to a point where there is a balance between tissue resolution and reduction in background noise. However, background noise is still present, which impedes further processing.

For this pipeline, a MATLAB script was developed to eliminate all background noise, while maintaining the tissue differentiation created in FSL. This generated the best image possible from the ex-vivo brains suspended in solution at the time of imaging. Therefore, because of the dynamicability to use two programs (MATLAB and FSL) together, it was possible to explore fully the first hypothesis.
DISCUSSION

In this study, we’ve reported on a unique post-processing pipeline, which draws upon the strength of the established post processing tools, such as FSL and VBM. We’ve shown that this pipeline can be used to process ex-vivo MRI scans of a single hemisphere of a brain. Samples of 17 subjects, falling into either CR or control groups were used. Differences between these groups were identified in three cortical areas, suggesting that significant preservation is present over the course of over 20 years of CR.

The regions, which differ between the groups, are mainly involved in auditory interpretation and memory. In monkeys, it has been well documented that this part of the brain has a huge interplay with the somatosensory cortex (Seltzer, 1978, Petides, 2004), specifically the supramarginal gyrus. This particular portion of the cortex helps to interpret and relay auditory information to the auditory cortex, and to certain parts of the brain involved with memory (Aggleton, 1980). The supramarginal gyrus is of certain interest as it is part of the parietal lobe, involved with somatosensory input and interpretation, and works with the angular gyrus to link sounds and meanings (Aggleton, 1980, Petrides, 2004). But what’s even more interesting is that there was some grey matter conserved in the superior temporal gyrus.

In many cases it has been documented that the superior temporal gyrus, which is involved with the auditory pathway in ways regarding processing of sound frequency, localization, and visual pathways (Zeki, 1974). It garners a little more interest as it’s not
only involved in sound processing, but memory as well. In particular, the superior temporal gyrus is involved with short-term memory, selective attention, sensorimotor connection and species specific communication based on sound, and sight (Petrides, 2004).

As with any study, there are some inherent weaknesses, which deserve attention as suggestions for further exploration. The sample size was rather small, which limited the assessment to rather large effects. The findings are also meager, and future research should consider using other means for correcting multiple statistical analyses, such as the used of spatial filters, as is typically done with function MRI analyses.

In addition, the present study used a cross sectional design where no baseline scans were obtained. Future studies should consider obtaining these scans, which would better control for individual differences. In this same vein, it might be useful to obtain a “mid-study” MRI. Because these studies are long (about 20 years) it would be crucial, based on the amount of time and energy being spent on this study, to see if any effects are present at earlier bench marks. We found that the pipeline we succeeded to assemble produced the data needed to generate the between group comparisons, to explore the second part of our hypothesis.

The pipeline monopolizes the dynamic ability of both FSL and MATLAB, and overcomes the major hurdle of only having one hemisphere to work with. In this same vein, the other hurdle, which was overcome by this pipeline, is the ability to analyze scans from ex-vivo tissue. These kinds of samples are especially problematic for programs like FSL and MATLAB, as ex-vivo tissue presents very differently when
imaged magnetically. In particular, based on the sequence, the grey/white contrast is flipped. With this study (as is apparent in the included images), all T1 sequences— which result in the grey matter looking grey, and the white matter looking white— appear reversed, and almost like T2 sequences. For most pipelines, this would be a huge problem, however for this particular set of steps, where the programs are designed to work with each other, the reversal of contrast is easily overcome.
CONCLUSIONS

Where the strength of this study lies is in the processing pipeline we developed. Specifically, this pipeline was able to overcome the difficulties present by using a-typical samples (half hemisphere, post-mortem). The results is that we have a dynamic and versatile pipeline, full of steps to handle the hurdles of the tissues used in this study, and the potential to handle additional challenges. Using this pipeline, we were able to assess the degree to which differences exist in the gray matter of the brain of non-human primates maintain on over 20 years of CR, compared to being fed *ad libitum*. Interestingly, we found subtle differences in regions responsible for processing sound and memory.
## APPENDIX

### Table 1, Summary of Brains through Segmentation:

Below is the table which outlines the parameters by which the study images were manipulated. This specifically outlines the parameters used to flip and align (via FLIRT) all the brains included in this study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Trtmnt</th>
<th>Flip (4 occurred postpost thresholding)</th>
<th>Thresholding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CR004</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR PA IS</td>
</tr>
<tr>
<td>CR005</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR006</td>
<td>B</td>
<td>RL PA IS</td>
<td>LR PA IS</td>
</tr>
<tr>
<td>CR007</td>
<td>B</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR008</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR009</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR010</td>
<td>B</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR011</td>
<td>B</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR012</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR015</td>
<td>B</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR017</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR020</td>
<td>B</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR021</td>
<td>B</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR022</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR023</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR024</td>
<td>B</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR025</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
<tr>
<td>CR026</td>
<td>A</td>
<td>RL PA IS</td>
<td>LR IS PA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Trtmnt</th>
<th>Flip</th>
<th>FLIRT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 (flip)</td>
<td>2</td>
</tr>
<tr>
<td>CR012</td>
<td>A</td>
<td>12/L.C./N.C</td>
<td></td>
</tr>
<tr>
<td>CR015</td>
<td>B</td>
<td>12/L.C./N.C</td>
<td></td>
</tr>
<tr>
<td>CR020</td>
<td>B</td>
<td>12/L.C./N.C</td>
<td></td>
</tr>
<tr>
<td>CR021</td>
<td>B</td>
<td>12/L.C./N.C</td>
<td></td>
</tr>
<tr>
<td>CR023</td>
<td>A</td>
<td>12/N.A./N.C.</td>
<td></td>
</tr>
<tr>
<td>CR026</td>
<td>A</td>
<td>12/L.C./N.C</td>
<td></td>
</tr>
</tbody>
</table>

### Key:
- **RL**: Right/Left
- **PA**: Posterior/Anterior
- **IS**: Inferior/Superior
- **FIM**: Final Interpoation Method (reslice only)
- **6/9/12**: Parameter control (traditional, affine)
- **I.C.**: Incorrectly Oriented
- **N.A.**: Not Aligned
- **N.C.**: Normalized Coefficient

Flip (4 occurred postpost thresholding)
FLIRT
Thresholding
Table 2, Summary of Monkeys Included in Study: Below illustrates all the details of the monkeys included in the analysis. Everything including age, time on study and age at sacrifice.

### A. Years on treatment

<table>
<thead>
<tr>
<th>name</th>
<th>Group</th>
<th>yrs on trtm</th>
<th>diff from tot avg</th>
<th>diff from group avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR023</td>
<td>A</td>
<td>27.03</td>
<td>6.15</td>
<td>6.84</td>
</tr>
<tr>
<td>CR004</td>
<td>A</td>
<td>18.72</td>
<td>-2.16</td>
<td>-1.46</td>
</tr>
<tr>
<td>CR017</td>
<td>A</td>
<td>26.36</td>
<td>5.48</td>
<td>6.18</td>
</tr>
<tr>
<td>CR022</td>
<td>A</td>
<td>25.48</td>
<td>4.60</td>
<td>5.29</td>
</tr>
<tr>
<td>CR012</td>
<td>A</td>
<td>23.23</td>
<td>2.35</td>
<td>3.04</td>
</tr>
<tr>
<td>CR026</td>
<td>A</td>
<td>21.03</td>
<td>0.15</td>
<td>0.84</td>
</tr>
<tr>
<td>CR009</td>
<td>A</td>
<td>19.80</td>
<td>-1.08</td>
<td>-0.39</td>
</tr>
<tr>
<td>CR008</td>
<td>A</td>
<td>15.55</td>
<td>-5.33</td>
<td>-4.64</td>
</tr>
<tr>
<td>CR005</td>
<td>A</td>
<td>16.88</td>
<td>-4.00</td>
<td>-3.31</td>
</tr>
<tr>
<td>CR020</td>
<td>B</td>
<td>21.79</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>CR021</td>
<td>B</td>
<td>26.91</td>
<td>6.03</td>
<td>6.03</td>
</tr>
<tr>
<td>CR010</td>
<td>B</td>
<td>18.64</td>
<td>-2.24</td>
<td>-2.24</td>
</tr>
<tr>
<td>CR024</td>
<td>B</td>
<td>27.04</td>
<td>6.16</td>
<td>25.22</td>
</tr>
<tr>
<td>CR015</td>
<td>B</td>
<td>23.42</td>
<td>2.54</td>
<td>2.54</td>
</tr>
<tr>
<td>CR011</td>
<td>B</td>
<td>20.66</td>
<td>-0.22</td>
<td>-0.22</td>
</tr>
<tr>
<td>CR006</td>
<td>B</td>
<td>16.81</td>
<td>-4.07</td>
<td>-4.07</td>
</tr>
<tr>
<td>CR007</td>
<td>B</td>
<td>18.70</td>
<td>-2.18</td>
<td>-2.18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>averages</th>
<th>TOT</th>
<th>17</th>
<th>20.88</th>
<th>3.89</th>
<th>0.00</th>
<th>2.26</th>
</tr>
</thead>
<tbody>
<tr>
<td>For A</td>
<td>9</td>
<td>20.19</td>
<td>4.19</td>
<td>0.68</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>For B</td>
<td>8</td>
<td>20.88</td>
<td>3.82</td>
<td>0.87</td>
<td>3.25</td>
<td></td>
</tr>
</tbody>
</table>

### B. Age at Start

<table>
<thead>
<tr>
<th>name</th>
<th>Group</th>
<th>age at start</th>
<th>from tot avg</th>
<th>from group avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR023</td>
<td>A</td>
<td>1.82</td>
<td>-5.68</td>
<td>-5.94</td>
</tr>
<tr>
<td>CR004</td>
<td>A</td>
<td>3.73</td>
<td>-3.77</td>
<td>-4.03</td>
</tr>
<tr>
<td>CR017</td>
<td>A</td>
<td>3.75</td>
<td>-3.75</td>
<td>-4.01</td>
</tr>
<tr>
<td>CR022</td>
<td>A</td>
<td>3.24</td>
<td>-4.26</td>
<td>-4.52</td>
</tr>
<tr>
<td>CR012</td>
<td>A</td>
<td>3.9</td>
<td>-3.60</td>
<td>-3.86</td>
</tr>
<tr>
<td>CR026</td>
<td>A</td>
<td>7.66</td>
<td>0.16</td>
<td>-0.10</td>
</tr>
<tr>
<td>CR009</td>
<td>A</td>
<td>19.09</td>
<td>11.59</td>
<td>11.33</td>
</tr>
<tr>
<td>CR008</td>
<td>A</td>
<td>10.25</td>
<td>2.75</td>
<td>2.49</td>
</tr>
<tr>
<td>name</td>
<td>Group</td>
<td>Age at Sac</td>
<td>From Tot Avg</td>
<td>From Group Avg</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>------------</td>
<td>--------------</td>
<td>----------------</td>
</tr>
<tr>
<td>CR005</td>
<td>A</td>
<td>16.38</td>
<td>9.17</td>
<td>8.62</td>
</tr>
<tr>
<td>CR020</td>
<td>B</td>
<td>1.75</td>
<td>-5.75</td>
<td>-6.01</td>
</tr>
<tr>
<td>CR021*</td>
<td>B</td>
<td>1.76</td>
<td>-5.74</td>
<td>-6.00</td>
</tr>
<tr>
<td>CR010</td>
<td>B</td>
<td>3.32</td>
<td>-4.18</td>
<td>-3.89</td>
</tr>
<tr>
<td>CR024</td>
<td>B</td>
<td>3.88</td>
<td>-3.62</td>
<td>-29.12</td>
</tr>
<tr>
<td>CR015</td>
<td>B</td>
<td>3.55</td>
<td>-3.95</td>
<td>-3.66</td>
</tr>
<tr>
<td>CR011</td>
<td>B</td>
<td>4.76</td>
<td>-2.74</td>
<td>-2.45</td>
</tr>
<tr>
<td>CR006</td>
<td>B</td>
<td>19.58</td>
<td>12.08</td>
<td>12.37</td>
</tr>
<tr>
<td>CR007</td>
<td>B</td>
<td>19.09</td>
<td>11.59</td>
<td>11.88</td>
</tr>
<tr>
<td>stdev</td>
<td></td>
<td></td>
<td>7.50</td>
<td>6.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.53</td>
<td>-1.58</td>
</tr>
<tr>
<td>For A</td>
<td></td>
<td></td>
<td>7.76</td>
<td>6.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.40</td>
<td>0.00</td>
</tr>
<tr>
<td>For B</td>
<td></td>
<td></td>
<td>7.21</td>
<td>7.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.53</td>
<td>-3.36</td>
</tr>
</tbody>
</table>

C. Age at Sacrifice:

<table>
<thead>
<tr>
<th>name</th>
<th>Group</th>
<th>Age at Sac</th>
<th>From Tot Avg</th>
<th>From Group Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR023</td>
<td>A</td>
<td>28.8</td>
<td>-0.85</td>
<td>-1.24</td>
</tr>
<tr>
<td>CR004</td>
<td>A</td>
<td>22</td>
<td>-7.65</td>
<td>-8.04</td>
</tr>
<tr>
<td>CR017</td>
<td>A</td>
<td>30.1</td>
<td>0.45</td>
<td>0.06</td>
</tr>
<tr>
<td>CR022</td>
<td>A</td>
<td>28.7</td>
<td>-0.95</td>
<td>-1.34</td>
</tr>
<tr>
<td>CR012</td>
<td>A</td>
<td>27</td>
<td>27.00</td>
<td>-3.04</td>
</tr>
<tr>
<td>CR026</td>
<td>A</td>
<td>28.6</td>
<td>-1.05</td>
<td>-1.44</td>
</tr>
<tr>
<td>CR009</td>
<td>A</td>
<td>39</td>
<td>9.35</td>
<td>8.96</td>
</tr>
<tr>
<td>CR008</td>
<td>A</td>
<td>26</td>
<td>-3.65</td>
<td>-4.04</td>
</tr>
<tr>
<td>CR005</td>
<td>A</td>
<td>33</td>
<td>3.35</td>
<td>2.96</td>
</tr>
<tr>
<td>CR020</td>
<td>B</td>
<td>23.5</td>
<td>-6.15</td>
<td>-6.15</td>
</tr>
<tr>
<td>CR021*</td>
<td>B</td>
<td>28.7</td>
<td>-0.95</td>
<td>-0.95</td>
</tr>
<tr>
<td>CR010</td>
<td>B</td>
<td>22</td>
<td>-7.65</td>
<td>-7.65</td>
</tr>
<tr>
<td>CR024</td>
<td>B</td>
<td>30.9</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>CR015</td>
<td>B</td>
<td>27</td>
<td>-2.65</td>
<td>-2.65</td>
</tr>
<tr>
<td>CR011</td>
<td>B</td>
<td>25</td>
<td>-4.65</td>
<td>-4.65</td>
</tr>
<tr>
<td>CR006</td>
<td>B</td>
<td>36</td>
<td>6.35</td>
<td>6.35</td>
</tr>
<tr>
<td>CR007</td>
<td>B</td>
<td>37</td>
<td>7.35</td>
<td>7.35</td>
</tr>
<tr>
<td>stdev</td>
<td></td>
<td></td>
<td>29.65</td>
<td>4.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td>-0.84</td>
</tr>
<tr>
<td>For A</td>
<td></td>
<td></td>
<td>30.04</td>
<td>4.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-2.74</td>
<td>-0.80</td>
</tr>
<tr>
<td>For B</td>
<td></td>
<td></td>
<td>29.65</td>
<td>5.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.85</td>
<td>-0.89</td>
</tr>
</tbody>
</table>
REFERENCES


Francis, Peter J., Binoy Appukuttan, Emily Simmons, Noelle Landauer, Jonathan Stoddard, Sara Hann, Jurigott, Betsy Ferguson, Michael Klein, J. Timothy Stout, Martha Neuringer. (2008) “Rhesus Monkeys and Humans Share Common Susceptibility Gene for Age-Related Macular Disease” Human Molecular Genetics 17(17):2673-80. doi: 10.1093/hmg/ddn167


Mike, Andrea, Erzsebet Strammer, Mihaly Aradi, Gergely Orsi, Gabor Perlak Andras Hajnal,


CURRICULUM VITAE

SHANNON BROWNE smbrowne@bu.edu

Simmons College, B.S., 2010 Year of birth: 1988

Current Position: Associate Scientist I, Pharmacology, Biogen Idec

Education

Majors: Biology, Psychology           French Immersion Program, 11 years
Minor: Chemistry

Research and Work Experience


Biogen Idec,
Thierry Bussiere, Ph.D.
The Pharmacology group is aiming to discover compounds that affect endogenous proteins involved in neurodegeneration.

- Runs chronic and acute studies in mice to determine how effective pre-determined compounds are clearing specific proteins from the brain.
- Removes and preserves brains of AAV injected rats and mice to be sectioned for IHC, and biochemical analysis, in order to view the effectiveness of various treatments and help define spreading models.
- Paraffin and frozen brains are sectioned and analyzed using previously defined algorithms in the program VisioPharm.

Drug, Protein Analysis Using Western Blot and ELISA Techniques in Relation to Molecular Expression of Neurodegenerative Diseases: 06/2010 – 06/2011

Research Associate
Link Medicine, Contractor through SmartStaffing
Berkley Lynch, Ph.D.

Link Medicine was developing a compound designed to alleviate the symptoms associated with neurodegeneration on a molecular level.

- Using Western Blot and ELISA techniques, completed protein immunoblot staining and testing with related antibodies, and quantified results using the LiCor/Odyssey system.
- Fractionated mouse cranial tissue for experimental use as needed. Maintained cell lines, practiced sterile techniques, analyzed cell growth.
- Developed projects to further determine drug and dosing effectiveness.
Research Assistant  
Simmons College  
D. Bruce Gray, Ph.D.

Dr. Gray’s Lab studies theroots of Alzheimer’s Disease using various methods. Used the avian choroid model to simulate synaptic dysfunction characteristic of early Alzheimer’s Disease (AD).

- Preformed required embryo micro-dissection twice a week, harvested the choroid layer of the eye.  
- Designed studies to manipulate beta-Amyloid induced transmitter suppression using radiolabeled Choline.  
- Analyzed Nitric Oxide pathways using various molecular inhibitors to determine their roles in modulating evoked transmitter release.

Serum-Free Media Development for Medical Tissue Graft, Growth and Development: 2008-2010 (Summers and Winters)  
Process Development Intern  
Organogenesis, Inc.: Process Development  
Katie Faria

Organogenesis studies and develops biocompatible tissue grafts for surgical/medical use.

- Assisted with tissue and media studies to help bring research to product.  
- Helped maintain cell lines, practiced aseptic techniques, and analyzed post-growth tissue quality.  
- Helped design and maintain studies viewing the effects of media alterations, including developing and testing serum-free media products.

Optoelectronic Properties of iTMC Complexes, Ruthenium and Iridium: 09/2006 –05/2009  
Research Assistant  
Women In Materials: Optoelectronic Research  
Michael Kaplan, Ph.D.

Studied device applications of ionic Transition Metal Complexes (iTMC.)

- Organized, processed, prepared and analyzed dilutions of Iridium and Ruthenium.  
- Organized and created interim reports and graphics including the results of testing.

Competencies

Cell Culture and Laboratory techniques

- Sterile Media Prep  
- Sterile Solution Prep  
- ELISA  
- Sterile Cell Culture  
- Sterile Cell Harvesting  
- BCA Analysis  
- Frozen Brain Sectioning  
- Paraffin Brain Sectioning  
- Tissue Fractionation
- Aseptic Tissue Culture
- Cell counting:
  i. Trypan-blue method
  ii. Vi-Cell counter
- Western Blot
- Sterile Cell Freeze-Down
- Cell based Assays

**Animal Skills**
- Embryonic Avian Oculus Microdissection
- Neonatal and Adult Rodent Cranial Removal and Dissection
- Rodent Embryo Removal and Dissection
- Rodent Euthanasia via CO2 and Ketamine/Xylazine Cocktail
- Rodent Anesthesia with Isoflurane
- Rodent Techniques:
  i. I.P. Injections
  ii. Cardiac perfusions
  iii. Sub. Cutaneous Injections
  iv. Cardiac Puncture

**Technical Computational Skills**
- Fluorescent Microscope
- HNMR
- Odyssey and LiCor Imaging Systems
- ELE, ELN
- PRISM
- SpectraMax5 System
- ImageJ
- VisoPharm
- Adobe Photoshop (CS6)
- SPM5, SPM8
- MIRIConvert
- MATLAB

**Professional Affiliations and Memberships**

**Women’s Innovation Network (WIN), Biogen Idec Chapter,** Member, 2012-Present
**BETA BETABETA, National Biological Honors Society:** Chapter President, 2008-2009
**Sigma Xi, National Scientific Research:** Member, 2010-Present
**Biology Liaison, Simmons College:** Vice President, 2009-2010, Secretary, 2008-2009