

NEURAL PLASTICITY IN ACTION RECOGNITION: A VBM AND fMRI STUDY

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Shira Levy
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Committee:

Jim Thompson

Director

Oliver

G. Miller

David Little

Department Chairperson

S. Zyg

Dean, College of Humanities
and Social Sciences

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Arts at George Mason University

By

Shira L. Levy
Bachelor of Science
Columbia University, 2007
Bachelor of Arts
Jewish Theological Seminary, 2007

Director: James C. Thompson, Professor
Department of Psychology

Fall Semester 2010
George Mason University
Fairfax, VA

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LIST OF ABBREVIATIONS

1. MRI.....	Magnetic Resonance Imaging
2. fMRI.....	Functional Magnetic Resonance Imaging
3. VBM	Voxel-Based Morphometry
4. ROI	Region of Interest
5. LTP	Long Term Potentiation
6. LTD.....	Long Term Depression
7. M1	Primary Motor Cortex
8. CSF	Cerebral-Spinal Fluid
9. BOLD.....	Blood-Oxygenation-Level Dependent
10. ANOVA	Analysis of Variance
11. HSRB	Human Subjects Review Board
12. MPRAGE.....	Magnetization-Prepared, Rapid-Acquisition Gradient Echo
13. ISI.....	Interstimulus Interval
14. DICOM	Digital Imaging and Communications in Medicine
15. ANCOVA	Analysis of Covariance
16. NaN.....	Not a Number
17. CMA	Cingulate Motor Areas

ABSTRACT

NEURAL PLASTICITY IN ACTION RECOGNITION: A VBM AND fMRI STUDY

Shira L. Levy, M.A.

George Mason University, 2010

Thesis Director: Dr. James C. Thompson

In our day-to-day interactions, we use our own motor representations to understand the actions of those around us. How we relate to others is an important part of life.

Understanding motor representations can help us to better learn and grow, conduct relationships, assess intent, and increase the quality of our daily lives. Further, understanding how we acquire motor representations can help us better understand this mechanism as we use it. The acquisition of these motor representations can be described by functional changes in the brain as well as structural changes in the brain. The relationship between these expressions of plasticity is not well understood. In this study, I sought to establish if a mediation equation would explain the results of an action recognition study. Participants were taught a novel one ball juggling cascade task either by physically performing it (nonvisual motor group) or by means of observation (nonmotor visual group). The group who physically performed the task learned completely without visual input ensured by blindfolds. The observation group was taught

a visual task with physical practice. Magnetic resonance imaging (MRI) scans were taken before, during, and after training. The functional magnetic resonance imaging (fMRI) results represented functional changes between time points. These results showed the changes in action recognition as a result of training. A voxel-based morphometry (VBM) analysis, performed on the structural MRI images, represented the structural changes. These results were overlaid to determine regions which spatially overlap for both functional and structural changes, with the head of the hippocampus as the only brain region which demonstrated both. A region of interest (ROI) analysis was conducted on the individual images for the head of the hippocampus to obtain mean intensities. These means were entered into Baron and Kenny's (1986) mediation equations to determine if structural changes act as a mediator on the relationship between time and functional changes. While the equations for the relationship between time on functional changes and time on structural changes were significant, the relationship between structural changes on functional changes was not significant. Therefore the requirements of Baron and Kenny (1986) were not upheld, leading to the conclusion that structural changes do not act as a mediator in the relationship between time and functional changes.

1. Introduction

This section discusses the purpose of the study, some background information, and the basic premise of the study.

1.1 Purpose of the Study

Action recognition helps us understand the intentions of other people. There is evidence that as we interact with people around us, we use our own motor representations to understand the actions of others (Rizzolatti & Craighero, 2004; Rizzolatti & Luppino, 2001). This suggests that we are better able to understand the actions of others if we have our own motor representations of those actions. These motor representations have been demonstrated in motor-related brain regions (Karni et al., 1995). These regions are defined by functional activity, defined here as the functional magnetic resonance imaging (fMRI) response using the hemodynamic response. Any change in functional activity should indicate a change in motor representation. Therefore, learning to perform a new motor action, even with no visual input, should change the functional activity in motor related brain regions that occurs when we see someone else performing that action.

Changes in functional activity are representative of the underlying plasticity of the brain as new representations are formed. This represents functional plasticity but does not describe the underlying structural plasticity. In addition to the changes in functional activity, structural changes can also occur while developing new motor representations (Draganski et al., 2004; Draganski et al., 2006). When the underlying brain tissue changes in volume or concentration, structural changes have occurred.

This study investigated how motor skill acquisition influences action observation. It was postulated that structural changes would mediate the relationship between time and functional changes. This was determined by overlaying functional changes on voxel-based morphometry (VBM) changes of the structural data to find jointly affected regions. These regions were then put through the Baron and Kenny (1986) tests of mediation to determine if structural changes mediate the relationship between time and functional changes.

1.2 Plasticity

The concept of plasticity extends back to the 1890s, where the idea that neurons are not arranged in static connections first came to the forefront of scientific debate. Studies were conducted on the neuronal connections, showing that reorganization of the neuron systems took place during the life of the neuron (Rabl-Ruckhard, 1890; Weidersheim, 1890). This early research led to the current definition of plasticity as the

modification of the neural structure in response to the demands of the environment (DeFelipe, 2006).

Despite knowing that plasticity exists, it is still not certain which neurophysiological change it represents. It has been suggested that plasticity may be a change which is related to functionally relevant behavior (Leocani & Comi, 2006). A second possibility is that plasticity changes are maladaptive changes, related to difficulty in performing a task (Leocani & Comi, 2006) rather than learning one. However, the exact basis is yet unknown. A widely recognized candidate for the explanation comes from Hebb (1949). Now referred to as Hebbian learning, this theory proposed that the strength or effectiveness of synapses changes as a result of activity. Under this theory, repetitive stimulation of the postsynaptic cell by the presynaptic cell causes an increase in synaptic efficacy (Hebb, 1949). This has grown into the theory of long term potentiation (LTP), whereby Hebbian learning is expressed. LTP occurs when cells that have been firing synchronously show long lasting improvement in communication. Long term depression (LTD) can also occur, whereby the synapse is weakened by inactivity. Despite all of these possibilities, plasticity is expressed in literature as changes in functional activity or structure, as the exact mechanisms are unknown. When functional activation shows a change from its previous state, usually from learning, experience, or injury, it is due to plasticity.

1.3 Plasticity Expressed from Motor Skill Acquisition

Plasticity due to motor skill acquisition can be expressed by changes in functional activity. In this study, functional changes specifically refer to the differences in the pattern or strength of fMRI activity in a given region, before and after motor skill training. Plasticity can be seen as the ability of the brain to adapt to changes in the environment, expressed through functional changes. As motor representations are acquired through skill acquisition, the functional activity in motor-related regions changes in response to the adapted environment. While basic finger movements can be performed in a scanner, most motor skills cannot. In order to investigate the functional activity, action observation is used. Viewing an action provides a similar response in the mirror neuron system as if the subject was performing the action himself.

Motor skill acquisition is reflected in the mirror neuron system. The mirror neuron system is a set of neurons originally recorded from the monkey premotor cortex. These neurons responded when the monkey performed a particular action and also when the monkey saw someone else perform that same action (Rizzolatti & Luppino, 2001). This same phenomenon was shown to exist in humans (Cohen-Seat, Gastaut, Faure, & Heuyer, 1954; Fadiga, Fogassi, Pavesi & Rizzolatti 1995; Gastaut & Bert, 1954). This discovery led to the conclusion that the mirror neuron system is involved in action recognition (Rizzolatti & Luppino, 2001). Additionally, it was found that understanding action causes greater activation of the mirror neuron system than merely imitating action

(Grezes, Costes & Decety, 1998). Specifically, it was shown that the mirror neuron system will respond with greater fMRI activity when viewing motor actions that the subject is an expert in performing, rather than an action that he or she has never performed (Calvo-Merino, Grezes, Glaser, Passingham & Haggard, 2006). From this, it is concluded that having a motor representation of an action will lead to greater understanding and subsequent mirror neuron activity under appropriate circumstances. As expertise in a skill is gained, it should affect action recognition, which will be displayed in the mirror neuron system through changes in the functional activity.

Early research into the functional effects of motor skill learning shows that the primary motor cortex (M1) undergoes changes as a result of learning (Karni et al., 1995, Vidoni & Boyd, 2008). The cortical motor representation of the proximal muscles involved in a specific skill increases with expertise and repetition of that skill (Tyc, Boyadjian & Devanna, 2005). Practiced sequences show a greater response in M1 compared to non-practiced sequences (Karni et al., 1995). This increased response can be seen even in the absence of visual feedback (Vidoni & Boyd, 2008).

The magnitude of motor skill acquisition can be expressed by a learning curve, which depicts the skill level at any given point during training. This learning curve does not follow a uniform slope. Instead it falls into several phases, each representing a different stage of learning (Buitrago, Schulz, Dichgans & Luft, 2004; Doyon & Benali, 2005; Kleim et al., 2004; Ungerleider, Doyon & Karni, 2002). According to one theory

of learning, the first stage is characterized by initial rapid learning, followed by a slower learning phase (Kleim et al., 2004). Reorganization of the primary motor cortex is shown in the slower learning phase and not in the initial rapid learning stage (Kleim et al., 2004). Thus, acquisition of expertise may be essential for reorganization to take place; the initial burst of learning may not be sufficient. It was also shown that the changes in regions such as the cerebellum, striatum, and motor-related cortical regions may evolve rapidly, while other regions take over during latter stages of learning (Ungerleider, Doyon & Karni, 2002). Moreover, Doyon and Benali (2005) suggest several additional stages of learning, including consolidation, automatization, and retention. They propose that different neural networks take over during each of these steps. In both theories, it is the late stages of learning that are essential for studying plasticity since long-term functional or structural changes would be expressed during the later stages.

Another view of plasticity and learning supports the idea that the specific stage of learning affects motor representations, but that changes occur as a result of time, not the stage of learning. The reorganization of M1 may be a slowly evolving phenomenon, only showing significant reorganization after sufficient time has passed (Ungerleider, Doyon & Karni, 2002). This contrasts with the previously stated view in which the motor representations are dependent on the learning stage. Here the emphasis is not on the stage of learning, but the amount of time that has passed. Since learning occurs over time, these theories are not necessarily exclusive, but the emphasis remains different. These theories may explain current literature, which does not always agree on learning

dependent functional activity. When studies report that certain regions are not active during a stage of learning, it may be due to the timing and phase of learning rather than a lack of response.

1.4 Plasticity Expressed through Observational Learning

In addition to learning a motor skill through physical acquisition, observation can lead to functional expressions of plasticity. The mirror neuron system makes mental representations of our own actions and the actions of others by visual input and existing motor representations. From this we can conclude that vision plays an important role in action recognition. The effect of observation on learning can be seen in fMRI data, which demonstrate learning related changes. For example, Itoh et al. (2008) found that learned visual patterns will activate relevant brain regions for the task. Further, it has been specifically shown that motor skill acquisition can occur by purely visual means (Meegan, Aslin & Jacobs, 2000). If skill learning can occur through purely observational means, resulting in fMRI changes, then mental representations of the motor task will also form. Based on this, it can be concluded that observational skill learning potentially causes the same type of functional activation changes that result from motor practice.

While these results look promising for observational learning, opposing results have demonstrated that the functional changes are not identical to motor learning changes (Calvo-Merino, Grezes, Glaser, Passingham & Haggard, 2006). Calvo-Merino et al.

(2006) showed that the functional response to observational learning was less than the response to movements that had been physically learned. They studied dancers, and compared fMRI activation from two conditions representing observational learning and motor learning. Observational learning was tested using dance steps performed exclusively by the opposite gender. The stimuli for the motor learning group were limited to dance steps that the dancer was personally familiar with performing. Dance steps that the participants had actually performed had a greater fMRI response in the mirror neuron system than the steps of the opposite gender. Calvo-Merino et al. (2006) did not control for observational learning of the physically performed movements. Dancers frequently observe themselves or others of the same gender performing motions. It is possible that these motions elicited greater activation due to learning by means of several sensory inputs. Complete isolation of the two types of learning is necessary to draw valid conclusions. Casile and Giese (2006) isolated motor learning without the aspect of observational learning by blindfolding all participants and found that visual recognition improved. They did not test their results with functional neuroimaging, however, and did not attempt to compare these results to purely observational learning. A firm conclusion on the representation of functional plasticity by observational learning is lacking throughout literature. In this study, both types of learning are taken into account.

1.5 Structural Changes from Learning

There is evidence that in addition to functional changes, learning to perform a new motor task leads to structural changes in the brain. These are changes in volume or concentration of the grey and white matter and cerebral-spinal fluid (CSF). Volume is assessed when the Jacobian determinants of the deformation field are taken into account following spatial normalization during analysis (Ashburner & Friston, 2000).

Concentration can be evaluated when the deformations from spatial normalization are ignored, leaving only the proportion of grey matter to other tissue types within a given region (Ashburner & Friston, 2000). Structural changes are based on the physical expression of plasticity from skill acquisition, as demonstrated in recent literature.

Structural plasticity does not provide any additional evidence for action recognition as a result of training. If structural plasticity mediates the relationship between time and functional plasticity, then it may be possible with further research to establish the role structure plays in action recognition.

In Draganski et al. (2004), subjects were trained to juggle a classic three-ball cascade over the course of three months. An MRI scan of naïve participants was taken prior to any training. A second MRI scan was conducted immediately following training. Presumably, at this point, the subjects were at the highest level of expertise in their task. Structural changes were calculated using VBM. The effects of expertise were shown in the physical structure of the brain. Changes were found in task specific brain regions,

including expansions in area MT/V5 and the intraparietal sulcus. Finally, a third MRI scan was conducted three months following all experimental manipulation and training. The previously documented expansions had decreased at that time. These data led the authors to conclude that the changes in grey matter which occurred after physical learning, were task dependent, but transient.

Draganski et al. (2006), investigated subjects who were studying for a specific exam, involving intensive abstract learning. The schedule remained the same as above, with three MRI scans, each three months apart. This study confirmed previous findings of significant task related changes in the grey matter between the naïve first scan and the second scan. The hippocampus, parietal cortex, and occipito-parietal lobe were affected - again only in the experimental condition. Contrastingly, the results of Draganski et al. (2006) showed that not all of the demonstrated changes were transient in the limited trial period. The posterior and inferior parietal lobule showed an increase in grey matter between the first and second scans, which only decreased insignificantly at the time of the third scan. These data seem to provide evidence that these changes, resulting from abstract learning, are less transient. Therefore, depending on the type of learning, the resulting grey matter changes may be either transient or less transient, but still remain task specific.

While the extent of the changes of grey matter can be determined, both Draganski et al. 2004 and 2006 make note of the fact that the nature of the change cannot. It has

been demonstrated that synapses are plastic through LTP and LTD. However, the relationship between this synaptic effect and the amount of grey matter has not been determined. Additionally, increased cell genesis may be the cause of enlarged regions of grey matter. It is also unclear if changes in grey matter represent cell genesis or an increase in other cellular elements. Current literature seems to favor the idea of synaptogenesis and increased dendritic branching. It is assumed that any variation of the physiological structure of the brain does rely on the addition, subtraction, or reorganization of cellular elements (Bansal, Gerber & Peterson, 2008). Most literature now shies away from mentioning cell genesis as the cause of grey matter increases. Draganski et al. (2004) does mention cell genesis as a possibility, however, and since it has not been disproven, the theory is included here. Animal research supports the idea of synaptogenesis as the driving factor behind grey matter changes (Kleim et al., 2002; Kleim et al., 2004). Increases in the number of synapses have been shown in layer V in rat literature (Kleim et al., 2002; Kleim et al., 2004). This in fact may be the valid explanation if human anatomy follows the animal research. The only conclusion that can be drawn at present, however, is that any changes in the grey matter are due to the addition or subtraction of cellular elements. Further research will lead to conclusions about the basis of grey matter changes.

1.6 Mediation

Since both functional and structural changes stem from learning over time, these three variables can be tested for mediation by insertion into a series of equations. Specifically for this experiment, the test for mediation determines if the following relationship can be ruled out: structural changes mediate the relationship between time and functional changes. If the mediation relationship is shown to exist, it would indicate that the extent to which functional changes occur is determined by the extent to which the underlying structure is changed by skill acquisition.

A formal description of mediation was created by Baron and Kenny (1986). When the effect of variable X on variable Y is decreased to zero when variable M is taken into account, perfect mediation occurs. In this scenario, M completely explains the variation that is also accounted for by X. Baron and Kenny (1986) specifically define M as a mediator “to the extent that it accounts for the relation between the predictor and the criterion” (Baron & Kenny, 1986, p. 1176), with variable X being the predictor (independent variable) and variable Y being the criterion (dependent variable). This relationship is depicted in Figure 1.

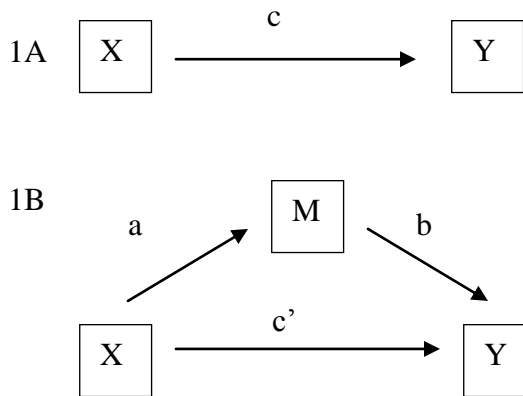


Figure 1: Basic Mediation Model. Figure 1A shows the total effect between the independent variable (X) and the dependent variable (Y), denoted as c . Figure 1B shows the mediation relationship between the independent variable (X) and the dependent variable (Y) and the mediator (M). Here, the direct effect is denoted as c' , with a and b being simple relationships between variables.

Baron and Kenny (1986) proposed a simple model to determine if mediation exists. Variable M can only be considered a mediator if all of the following main criteria are met: (1) X significantly predicts Y (2) X significantly predicts M and (3) M significantly predicts Y, when X is controlled for. Additionally, Y should not cause M. Accurate measurement of all variables is essential for the model.

There are four steps necessary to conclude a mediation relationship under the Baron and Kenny (1986) model. The first is to determine that the independent variable (X) has a significant effect on the dependent variable (Y). Following that, the independent variable (X) should have a significant effect on the mediator (M). Next, the mediator should be proven to have a significant effect on the dependent variable. Finally, the effect of the independent variable on the dependent variable should be reduced when

controlling for the mediator. These four steps can be accomplished with three regression equations: (1) the dependent variable is regressed on the independent variable, (2) the mediator is regressed on the independent variable, and (3) the dependent variable is regressed on both the mediator and the independent variable (Weinstein, Woodward & Ngan, 2007). Perfect mediation occurs when the third equation results in a coefficient of zero.

There are several important effects in the mediation model that are described by the above regression equations. The effect of X on Y is known as the total effect, denoted as c (Figure 1). The direct effect is the effect of X on Y after the effect of M has been accounted for, denoted as c' . If the coefficient for the direct effect is zero, then the relationship is a complete mediation, known as perfect mediation. If the direct effect is equal to the total effect, then there is no mediation relationship. If however, the direct effect is significantly lower than the total effect, mediation can still be concluded, but not a perfect mediation. This is known as partial mediation.

The Sobel test is a more rigorous statistical test for mediation. Developed in 1982 by Sobel, it is described in great detail in Baron and Kenny (1986). The Sobel test is a test of the indirect effect. Here, the indirect effect of X on Y is defined as the product of the effect of X on M and M on Y. Usually, this is calculated as $(c - c')$, or ab (Figure 1). For the Sobel test, the indirect effect is divided by the standard error of the indirect effect. This result is a ratio that is compared to values from the standard normal distribution for a

given alpha level, and a resulting description of the indirect effect. This method is known for its rigorous nature and high power. However, Preacher and Hayes (2004) discuss issues with the test, and propose an alternative.

Preacher and Hayes (2004) are suspicious of the usage of normal distributions to compute the resulting p value. They postulate that the sampling distribution of the indirect effect may not be normal. Therefore, imposing a test that requires normality will invalidate the results. Since Preacher and Hayes (2004) do support testing the indirect effect of X on Y through M, they propose an alternative to the Sobel test.

Preacher and Hayes (2004) propose a new model for mediation, in which the only requirements for mediation are (1) that there is a total effect and (2) that the indirect effect is not only significant, but significant in the direction predicted by the mediation hypothesis. The sampling distribution of ab is bootstrapped to derive a confidence interval with the empirically derived bootstrapped sampling distribution. By using a nonparametric approach to effect size estimation, Preacher and Hayes (2004) are able to obtain more accurate results. This method can be used in addition to the Baron and Kenny (1986) method. Additionally, based on the structure of the data, it is not always appropriate to use this method as it may skew results.

1.7 Methods for Determining Functional Changes

In order to conduct the mediation analysis, the data must be analyzed to show overlapping regions of functional and structural data. One of the most common methods for determining functional activity is by using fMRI. Cerebral blood flow has been correlated with brain activity to the extent that we can reliably conclude that areas which show a decrease in deoxygenated blood in fMRI represent brain activity. Specifically, the activity measured is the metabolic correlates of neuronal activity. Using blood oxygenation levels to determine brain activity is known as the blood-oxygenation-level dependent (BOLD) contrast. A BOLD contrast will show hemodynamic activity in response to a set of stimuli, and therefore the functional activity.

One particular challenge that fMRI research faces is artifact that comes from the scanner. Preprocessing the data will reduce the noise by increasing the signal to noise ratio. This clarifies the signal and allows a stronger response to appear through analysis. Following correction, a model is created based on each individual experiment. Based on the contrasts inherent in every fMRI experimental design, significance is determined, and the resulting activation is recorded as the functional activity.

1.8 Voxel-Based Morphometry

Analysis of structural changes is conducted by using VBM (Ashburner & Friston, 2000). Developed by Ashburner and Friston (2000), VBM is a technique which allows regional comparisons of changes in grey and white matter and cerebral-spinal fluid (CSF) of the brain from high resolution MRI images. It is a several-step process that involves normalizing images to the same stereotactic space, segmenting the images into grey matter, white matter, and CSF, smoothing the segmented images, and finally applying statistics to determine significant change in each voxel. The images are input into statistical tests, usually an analysis of variance (ANOVA). The output shows areas of significant change in the concentration of grey and white matter and CSF. VBM is specifically useful to define changes in small, irregular structures (Allen, Bruss, Brown & Damasio, 2005), though it can be used over the entire brain.

VBM is highly vulnerable to error. Firstly, all images that will be used in the analysis must be acquired on the same MRI scanner. Intensity values that would be classified as grey matter in an image from one scanner might be classified as white matter on another, particularly around borders of grey and white matter. In these borders, voxels are never entirely composed of grey or white matter. This is known as a partial volume. Generally, the voxel is classified as whichever tissue holds the majority. As a result, slight differences due to different scanner sensitivities can lead to completely different classifications throughout the brain. It should also be noted that during spatial

normalization, the brain is not exactly registered to the template. Rough structures are matched between the individual subject's image and the template. However, VBM assumes that a single voxel in the standardized version represents the same region across all brains (Bansal et al., 2008). Smoothing may help, but using too large of a kernel can lead to the loss of small differences that would normally have shown up in a VBM analysis (Allen et al., 2005; Bansal et al., 2008). When a small region of the brain is smoothed, if it is small enough, it will take on some of the greyscale values of the surrounding voxels. If a structural change is extremely small, smoothing will cause the surrounding greyscale values to overwhelm the region, causing the change to be obscured (Lagopoulos, 2007). Therefore, smoothing must be used carefully to ensure proper results from the analysis.

As described above, a vital concern using VBM is the assumption that the images are properly segmented. Scanner inhomogeneity can also cause grey matter to be classified as white matter and vice versa (Bansal et al., 2008). Proper classification is essential for VBM to be an effective tool. Good et al. (2001) add an additional normalization to the regular VBM protocol to create an optimized VBM protocol. In the extra normalization, the segmented images are normalized to the templates of grey and white matter of the group. This additional step increases the ability of the analysis to successfully take care of partial volume effects and misclassification.

Good et al. (2001) introduced the idea of modulation in order to preserve the ability to calculate volumetric differences. Modulation is a further processing step in the protocol. Voxel values of the segmented images are multiplied by Jacobian determinants derived from spatial normalization. This leads to preservation of volume which would otherwise have been distorted as a result of nonlinear spatial normalization.

1.9 Experiment Summary

Using data from an existing study on the effects of expertise on action recognition, the mediation potential of structural changes on the relationship between time and functional changes was tested in the head of the hippocampus. Participants were trained to juggle, and tested in an fMRI task with structural images also acquired before, during, and after training. Functional and structural changes were calculated based on the scans before and after training. These changes represent the plasticity in these modalities, and how they relate to one another. In order to test the relationship between the changes, the mean intensity values from the functional and structural changes in the head of the hippocampus were input into a mediation equation. Structural changes did not mediate the relationship between time and functional changes.

2. Methods

This section explains in detail the study from which the data were taken, including training and behavioral and imaging data acquisition. Additionally, the analysis of the data specific to this thesis is detailed.

2.1 Subject Information

All subjects were healthy undergraduate or graduate students enrolled in George Mason University. There were 23 subjects total, ages 18-40 ($M = 21.61$), which were split between men ($N=11$) and women ($N=12$). All subjects were prescreened for normal or corrected-to-normal vision, right handedness, and strict MRI safety. All subjects were asked to sign informed consent prior to any study activity, following regulations of the Human Subjects Review Board (HSRB) approval. Subjects were placed into group by random assignment, either the nonvisual motor learning group ($N=12$) or the nonmotor visual learning group ($N=11$).

Subjects were paid \$15 per hour, given at completion of the study. There was an additional financial bonus each week given to one subject based on randomized criteria. An example of the criteria is highest number of consecutive catches for the nonvisual

motor learning group, or lowest number of errors in counting for the nonmotor visual learning group. This ensured that the participants were actively trying to obtain expertise and not merely going through the motions of the study.

Based on responses during the fMRI task, it was found that one subject had fallen asleep in the scanner. This subject was removed from analysis. Additionally, following the region of interest (ROI) analysis, it was found that the functional mask did not extend to the head of the hippocampus where the ROI was located in two subjects. These two subjects were also removed from analysis.

2.2 Data Collection

2.2.1 Behavioral Data

All data were taken from an ongoing study, investigating the effects of expertise on action recognition. Subjects were taught a novel one ball cascade juggling technique by either nonvisual motor learning or nonmotor visual learning, assigned randomly. Both groups underwent twenty minutes of training per day for five weeks, five consecutive days each week. Interspersed with the training were three MRI scans: one prior to the first training session, one following the first week of training, and one at the end of the fifth week of training.

During the nonvisual motor training task, subjects were taught to physically perform the novel one ball cascade juggling task. All training was conducted with no visual input, ensured by blindfolds. Prior to any training, subjects were given a set of guidelines to follow and given a blind practice throw to enable them to experience the motion performed properly. Guidelines included throwing the ball too low or too high, holding on to the ball too long before initiating a new throw, and catching the ball against the body. The rules were enforced to ensure that the juggling task was sufficiently difficult so as to not gain expertise immediately, but also kept the task from being overly difficult so that expertise could be acquired over the course of five weeks. Only one ball was used to perform the task because multiple balls were too difficult to master blindfolded over the course of five weeks. Subjects juggled for 20 minutes per day, uninterrupted unless the subject was making errors. If errors were being made, subjects were stopped after 10 minutes, given verbal instruction to improve technique, and then restarted immediately. Following training, subjects were given verbal feedback on their performance. Highest number of consecutive catches and mean number of consecutive catches were recorded. This paradigm continued throughout the entire five weeks.

During the nonmotor visual learning task, subjects viewed five minute video clips of actors performing the novel one ball cascade juggling task. These videos were created specifically for this study by the experimenters. The actors were blindfolded to maintain a technique identical to that of the motor learning subjects. During taping, the actors were observed by experimenters, who enforced the guidelines of the motor task. This

ensured that the videos were as close to the nonvisual motor paradigm as possible. Observational subjects had an additional task to ensure that their attention was directed at the screen. They were instructed to count the consecutive catches of the ball in each sequence and report them into an excel spreadsheet. This was used to calculate the percentage error and as a result the learning progress of these subjects. These subjects were trained and given the guidelines for counting to ensure that calculation of their error was accurate. The guidelines for the observational subjects were the same as the guidelines given to the motor learning subjects. Each training session was approximately 20 minutes long. Three five-minute long videos of actors performing the task were played during each session. Feedback was given to these subjects following each video for their performance in the counting task. The number of errors in counting was recorded. This paradigm continued throughout the entire five weeks.

Rather than counting the number of errors, skill acquisition for observational subjects was based on the percentage error made during counting. The number of consecutive catches and total catches was highly variable between videos. Therefore, any increase or decrease in the number of errors made could be based on the probability of making any given number of errors in any given number of possible catches, rather than skill in counting. Analyzing the subject's percentage of errors eliminates this confound. The percentage of errors was calculated by the number of total errors divided by the total number of catches.

Every video that the subjects watched had a different skill level. Experimenter experience indicated that as the actor's skill increased, the ability to successfully count cascades also increased. If videos were presented in chronological order, any increase in the ability to count errors might have been based on a decrease in the difficulty of the video. Additionally, the different juggling styles of the actors varied the ease of counting. Therefore, the videos were presented randomly to all observational subjects. All accuracy increases can be inferred to be a result of skill increase, not due to any confounds of video order presentation.

2.2.2 Imaging Data Acquisition

The Siemens Allegra 3T scanner in the George Mason University Krasnow Institute for Advanced Study was used to acquire all images on all scans, both structural and functional, for every participant. Since movement creates huge artifact in images, subjects were restrained in the scanner using foam in the head coil. Due to potential discomfort in the scanner, subjects were given a squeeze ball attached to an alert which would indicate to investigators that it was necessary to remove the subject from the scanning environment. Subject comfort was confirmed in between each run of the MRI session. Subjects also held a button press in their right hand to give responses as necessary in the fMRI paradigm. All stimuli were displayed on a rear projection screen, which was viewed by means of a mirror mounted on the head coil.

A total of three MRI scans were taken for each subject. The first MRI session occurred prior to any training, a second followed the first week of practice, and then a third scan at the end of the testing period. The blood-oxygen-level dependent (BOLD) signal was acquired using a gradient-echo, echo-planar pulse sequence. The whole brain volume was obtained in 33 axial slices every two seconds (TR = 2; 64 x 64 matrix; 4mm slice thickness with a 1mm gap). In each run, 189 volumes were collected, the first five of which were thrown out due to scanner inhomogeneity at the start of every run. Each scanning session consisted of four runs of functional data collection, with 52 stimuli in each run, and one three-dimensional, magnetization-prepared, rapid-acquisition gradient echo (MPRAGE) structural sequence (160 1mm thick slices; 256 x 256 matrix, TR/TE = 2300/3ms).

The fMRI stimuli consisted of moving videos of actors performing the novel one ball cascade juggling task, static images of actors during the task, and a blank screen. A randomized interstimulus interval (ISI) was used in between each stimulus. These stimuli were presented in a randomized event-related design using Presentation software (Neurobehavioral Systems, www.neurobs.com). During stimuli presentation, subjects were required to respond on the button press box how well they believe they would have performed on the cascade. During the static images and blank screen, where a determination could not be made, subjects were asked to alternate responses. These responses were recorded by Presentation in log files, which were later analyzed to ensure full participation and attention by subjects. These log files also recorded the event onset

and which type of stimulus was presented for later analysis. This paradigm was repeated at all three MRI scans that a subject underwent.

2.3 Imaging Data Analysis

The data taken at time points one and three were used for the functional and structural analysis. Using pilot data, it was determined that the robustness of the signal was greater in the analysis comparing times one and three. In the interest of having maximum potential overlap for the later overlap analysis of the functional and structural responses, it was necessary to use the most robust analysis.

2.3.1 fMRI Analysis

The CDs obtained from each scanning session contained the data in the digital imaging and communications in medicine (DICOM) format. These images were then sorted by run using the software DicomWorks (<http://dicom.online.fr>). Following this, the images were transformed to ANALYZE format for use in SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/>) using DCM2NII (<http://www.cabiatl.com/mricro/mricron/dcm2nii.html>). The fMRI data underwent preprocessing steps using SPM5, including realignment, slice timing correction, coregistration, spatial normalization, and spatial smoothing. Each run was analyzed separately for each individual subject. The runs were then averaged, creating one set of data per scan per subject. Contrast images were obtained, contrasting the moving videos

with the rest of the scan. These contrast images were input into an ANOVA. A 2x2 ANOVA was run for the data. The factors for the ANOVA were group and time. Group had two levels: motor learning and observational learning. Time was also split into two levels: time point one, prior to any manipulation, and time point three, the final scan after all training was complete. In the ANOVA, the main effects of group, time, and the group by time interaction were recorded.

2.3.2 Voxel-Based Morphometry Analysis

The MPRAGE structural scans were also analyzed using SPM5. SPM5 is set up for the optimized VBM protocol with no extra steps required. Optimization was done in the segmentation process, where the grey and white matter and CSF were segmented, and three new images were created. During segmentation, SPM5 warps a set of tissue probability maps, overlaying the images to segment the grey and white matter and CSF with improved accuracy. In total, images underwent the entire VBM process, which included normalization, segmentation and smoothing. A 2x2 ANOVA was performed on the modulated grey matter images. The ANOVA was created with the same effects as the fMRI ANOVA analysis, with group and time as the factors. Again, group was split into two levels, motor learning and observational learning. Time contained two levels as well, time point one and time point three. The results of the group by time interaction as well as the main effect of time and group were recorded.

2.4 Overlap

To test the mediation model, the results for functional and structural changes were overlaid to establish regions of overlap. MarsBaR (Brett, Anton, Valabregue, & Poline, 2002) was used to overlay the images. In this step, the analyses for both functional activity and structural activity were identical. SPM activation clusters were imported into MarsBaR from SPM5 to create individual masks for every active voxel cluster based on the ANOVA previously outlined ($p < .01$, uncorrected). For each analysis, the individual cluster masks were then combined in MarsBaR to create a single binary mask which corresponded to all of the SPM clusters. Using this method, there were six masks total. This included three masks each for fMRI and VBM results, representing the SPM clusters for the group by time interaction, the main effect of time, and the main effect of group. For each of the three analyses separately, the fMRI and VBM masks were overlaid in MarsBaR to determine if any voxels were present in both the SPM cluster masks of both the fMRI and VBM. This was done with a simple spatial overlap. Since both the fMRI and VBM results were normalized to a standard template, the voxels for each correspond to the same location in the brain. When voxels from each of the maps occur in the same location, overlap from the fMRI results and the VBM results can be concluded. The main effect of time showed overlap in the head of the hippocampus, and so these voxels were chosen as a region of interest (ROI).

2.5 Mean Intensity Values

In order to calculate the mediation equations, mean intensity values for the fMRI and VBM individual subjects were necessary. MarsBaR was used to calculate mean values within the ROI using the Extract ROI data (full options) option. The mean timecourse for each fMRI image was calculated and then mean intensity for the ROI was extracted. For the VBM images, the mean intensity values were calculated for the region within the ROI. These means were saved in an excel file to be input into the mediation equations.

2.6 Mediation Model

In order to establish mediation, the four steps of Baron and Kenny (1986) were applied to the mean intensity data for both fMRI and VBM. The mediation model suggested that the relationship between learning and functional changes is mediated by the structural changes (Figure 2). Here, time is the independent variable (X). The dependent variable (Y) is the functional changes. Structural changes, proposed to mediate the relationship between learning and functional changes, are the mediator (M). For Baron and Kenny's (1986) regression requirements, three equations fulfill the four requirements. The following analyses were conducted: (1) functional changes were regressed on time, (2) structural changes were regressed on time, and (3) the functional changes were regressed on both time and the structural changes.

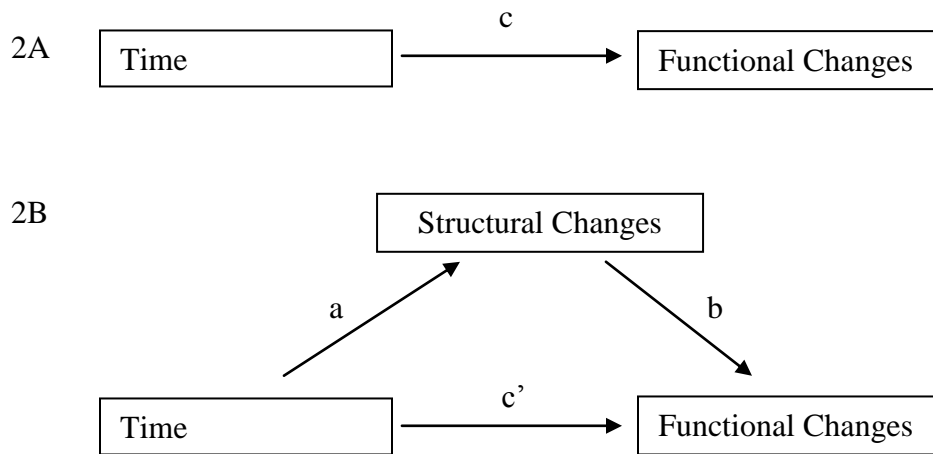


Figure 2: Study Specific Mediation Model. Figure 2A shows the direct effect with the variables from the study. Figure 2B shows the mediation relationship with the variables from the study.

In order to establish mediation, the mean intensity data for both the fMRI and VBM images were input into SAS (SAS 9.0, SAS Institute Inc., Cary, NC, USA). Based on how the program handles repeat measure data, the variable for time was inherent in the design and was included in all analyses. For the first equation, pathway c (Figure 2A) must be significant to satisfy the first requirement, that there is a direct effect. A repeat measure t test was conducted on the differences between time point one and time point three for the functional changes. This takes into account time and functional changes, as shown in Figure 2A, path c. The second equation demonstrates path a (Figure 2B). As with the first equation, this path was determined by a repeat measure t test on time points one and three, but here with structural changes. Again, time was taken into account through the setup in SAS. The third equation concerns pathways b and c'. In order to satisfy this equation, an analysis of covariance (ANCOVA) was conducted on the data.

Again, time was implied in the model, functional data was input as a repeat measure factor, while structural data was input as a repeat measure covariate. The output of these equations was used to determine significance of all of the steps for Baron and Kenny (1986). Due to the fact that the Baron and Kenny (1986) requirements were not upheld, further tests of the indirect effect were not conducted.

3. Results

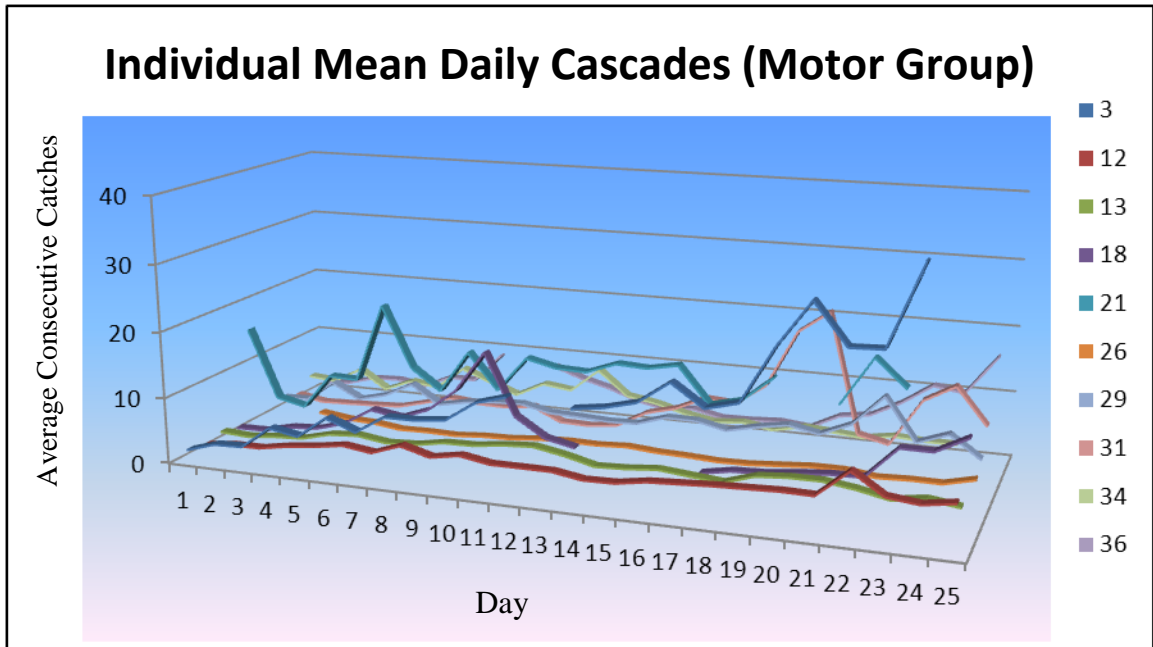
This section describes the results from the previously described experiment.

3.1 Behavioral Results

3.1.1 Motor Group Results

An analysis of the nonvisual motor group behavioral data showed that training was successful. Average daily cascades were significantly greater after twenty-five days of training, $t(9) = -2.16, p < 0.059$. Investigators counted the consecutive catches from start to finish. Subjects began with a low daily average ($M = 3.27$). As a result of training, subjects acquired expertise and ended with an increased daily average ($M = 7.32$). These data show an upward trend in the average number of consecutive catches per day (Figure 3). This indicates that training is successful. Subjects had a wide variety of capability. Even from the beginning scores varied, with the lowest subject at an average of 1.23 catches and the highest subject at an average of 15.90. As expertise was acquired, personal skill played a greater role. The highest average of consecutive catches per day was 38.07, and the lowest was 2.78.

3A



3B

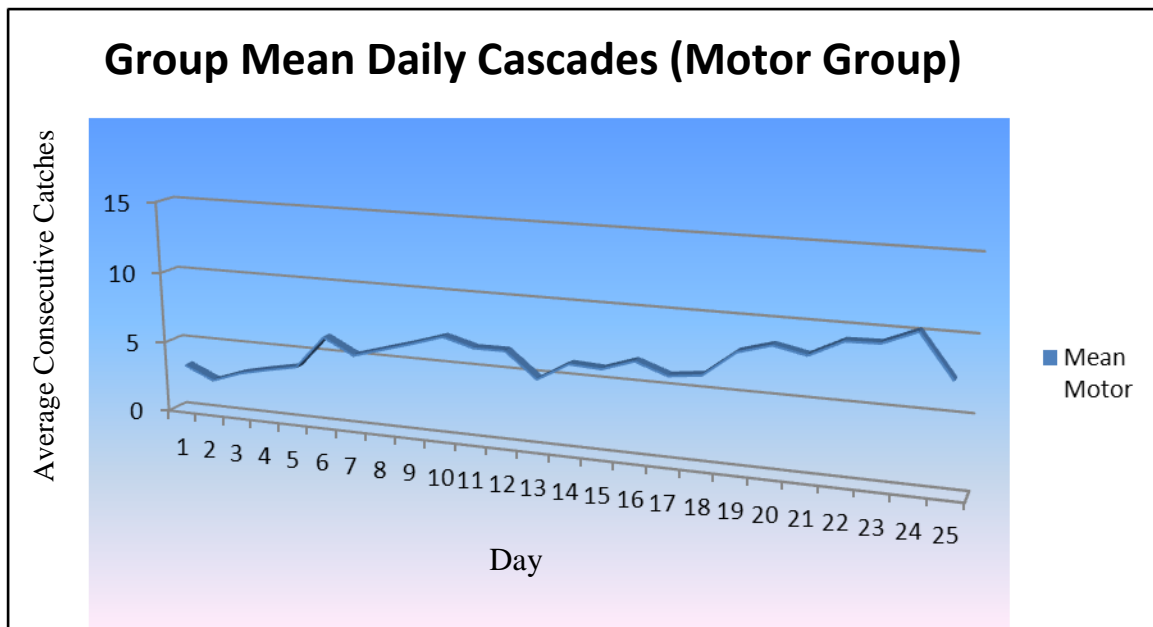
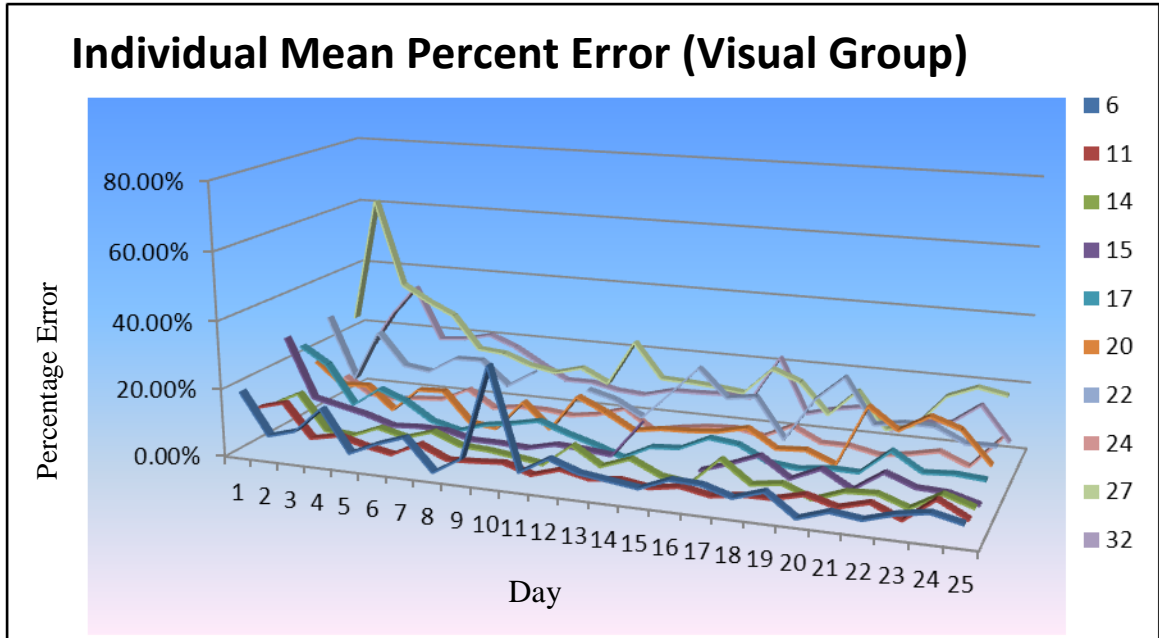


Figure 3: Nonvisual Motor Group Behavioral Progress. Figure 3A shows the individual progress made by the motor group participants. All participants show upward progression. Figure 3B shows the upward progression of the motor group as a whole.

3.1.2 Observational Group Results

The nonmotor observational group showed successful training and expertise acquisition (Figure 4). Subjects were successfully able to learn how to count catches and thereby focus their attention on the skill of the actor in the video, $t(9) = 4.39$, $p < 0.002$. Initially, subjects made errors at a high rate (mean percentage error = 18.19%). Following training, subjects increased their skill at identifying successful cascades (mean percentage error = 6.69%). As with the motor skill group, individual skill was variable across subjects. At the first training session, the most skilled subject demonstrated high accuracy in identifying successful cascades (percentage error = 7.08%) while another subject tended towards a lower accuracy (percentage error = 28.67%). At the completion of training, the highest accuracy (percentage error = 1.37%) and the lowest accuracy (percentage error = 19.32%) show a clear improvement.

4A



4B

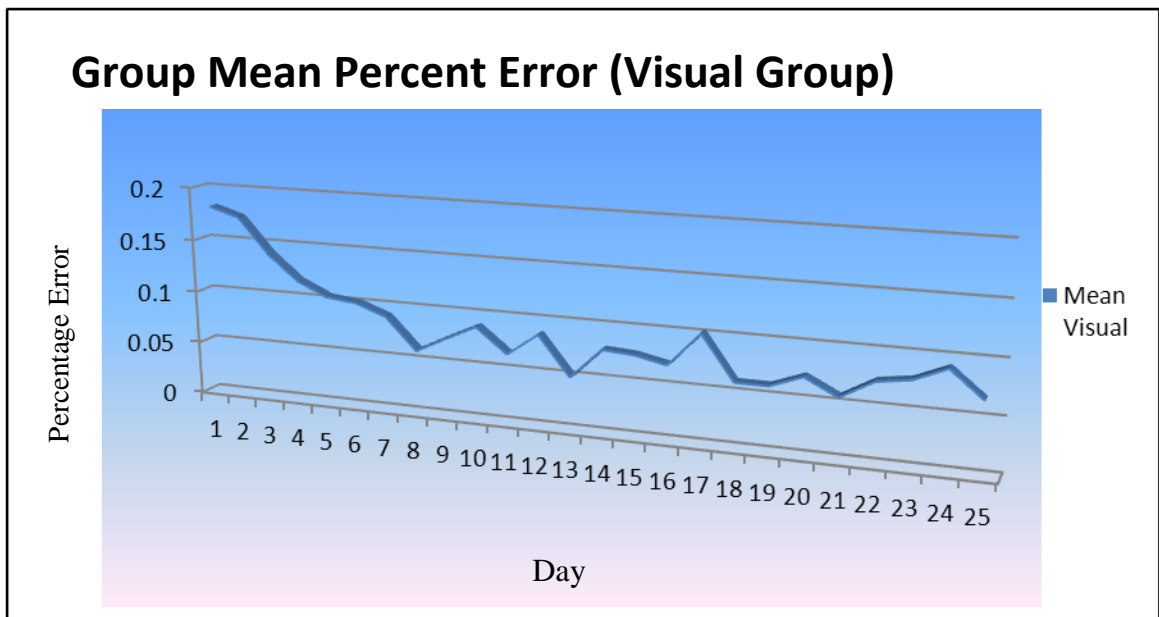


Figure 4: Nonmotor Visual Behavioral Progress. Figure 4A shows the individual scores for the observational subjects. The Percentage of error clearly decreases over time. Figure 4B shows the visual group's mean percentage error over time, also decreasing.

3.2 fMRI Results

All data obtained were analyzed using the aforementioned fMRI methods detailed in section 2. These data represent the images of the moving contrast, where the trials containing a video of active juggling are contrasted with the null trials and static images. A 2x2 ANOVA was conducted on the contrast images, resulting in several main effect results. Two factors were used in the analysis, group and time. Group refers to whether the subject was in the observational learning group or the motor learning group, while time indicates data taken from either time point one, the naive fMRI scan, or time point three, the fMRI scan taken at the conclusion of the five week training period.

3.2.1 Initial Analysis

To begin the analysis, a t test between groups at the initial time point was performed to ensure that random assignment had resulted in an averaged brain and to ensure that any group affects were due to training. Above chance differences were present in the two groups prior to any training ($p < .01$, uncorrected). These regions included the right precentral gyrus, right cerebellum, right fusiform gyrus, and the bilateral parieto-occipital fissure (Figure 5). To maintain the integrity of the data, these regions were subtracted out of the subsequent analyses where appropriate by applying an exclusion mask.

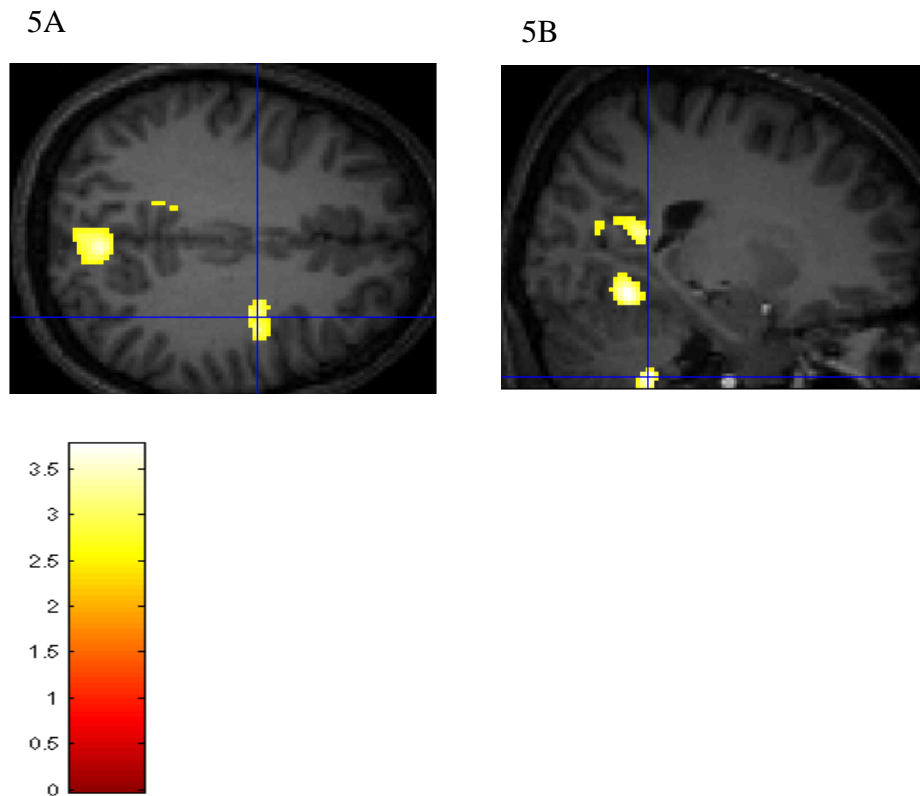


Figure 5: fMRI Initial Analysis. Figure 5A shows activity in the precentral gyrus and in bilateral parieto-occipital fissure from the initial analysis of time point one of the fMRI data. Figure 5B depicts activity in the right cerebellum, the right fusiform gyrus, and the parieto-occipital fissure from the right side from the initial analysis of time point one of the fMRI data.

3.2.2 Group by Time Interaction

The first main effect concerns the analysis regarding a group by time interaction. A lenient p value was used ($p < .01$, uncorrected), with the rationale that a more stringent value would decrease the likelihood that the results for the fMRI analysis and the VBM analysis to overlap. After taking into account the initial group differences, regions

displaying an interaction between group and time included: right cerebellum, right parieto-occipital fissure, right fusiform gyrus, right superior temporal sulcus, and the left parahippocampal gyrus (Figure 6).

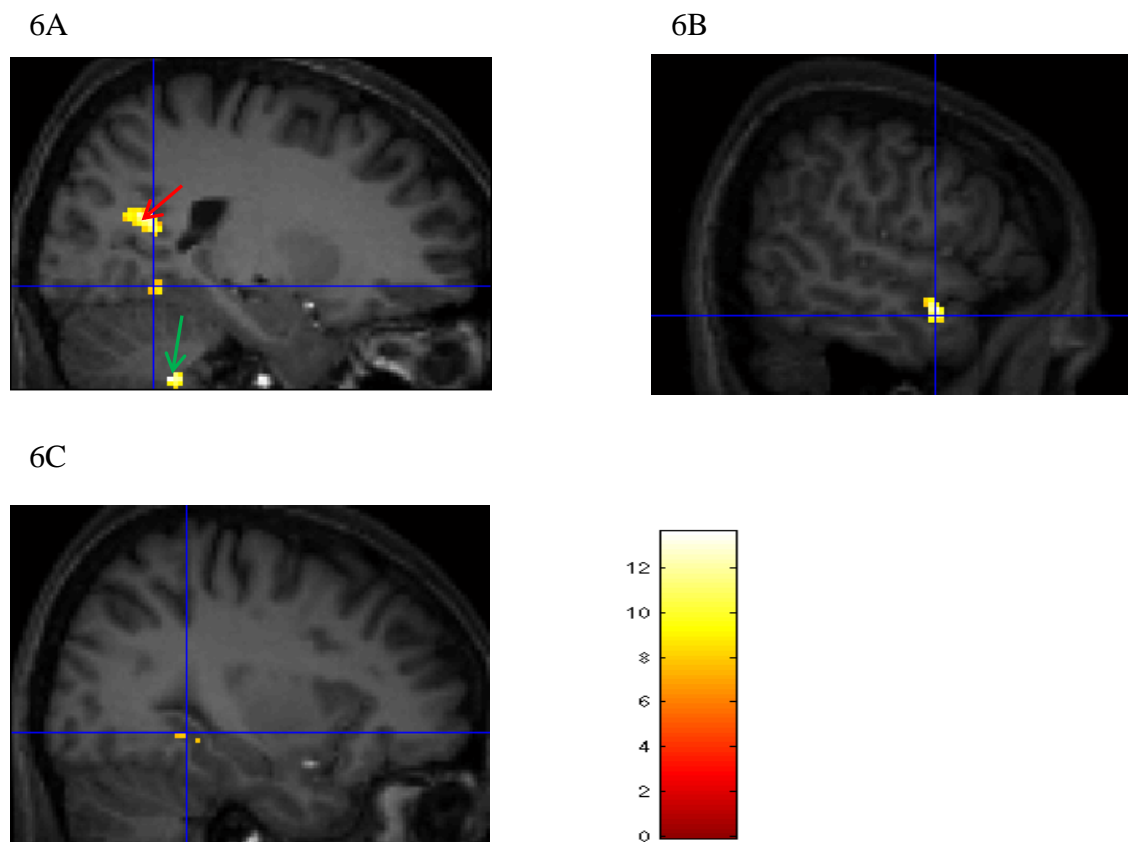


Figure 6: fMRI Interaction of Group by Time Results. Figure 6A shows the blue crosshairs on the right fusiform gyrus, the red arrow on the right parieto-occipital fissure, and the green arrow on the right cerebellum activity from the group by time interaction. Figure 6B shows the blue crosshairs on the right superior temporal sulcus for the group by time interaction. Figure 6C shows the blue crosshairs on the activity in the left collateral sulcus.

3.2.3 Group Main Effect

The second main effect is the main effect of group. Based on a randomized assignment, subjects were either trained by observational learning or by motor practice. The lenient p value ($p < .01$, uncorrected) was maintained while obtaining active regions for the main effect of group. Regions including the right precentral gyrus, right cerebellum, right fusiform gyrus, bilateral calcarine sulcus, bilateral cingulate, left anterior cingulate sulcus, left cingulate sulcus, marginal segment and the left angular gyrus displayed an effect based on group, after the initial differences were removed (Figure 7).

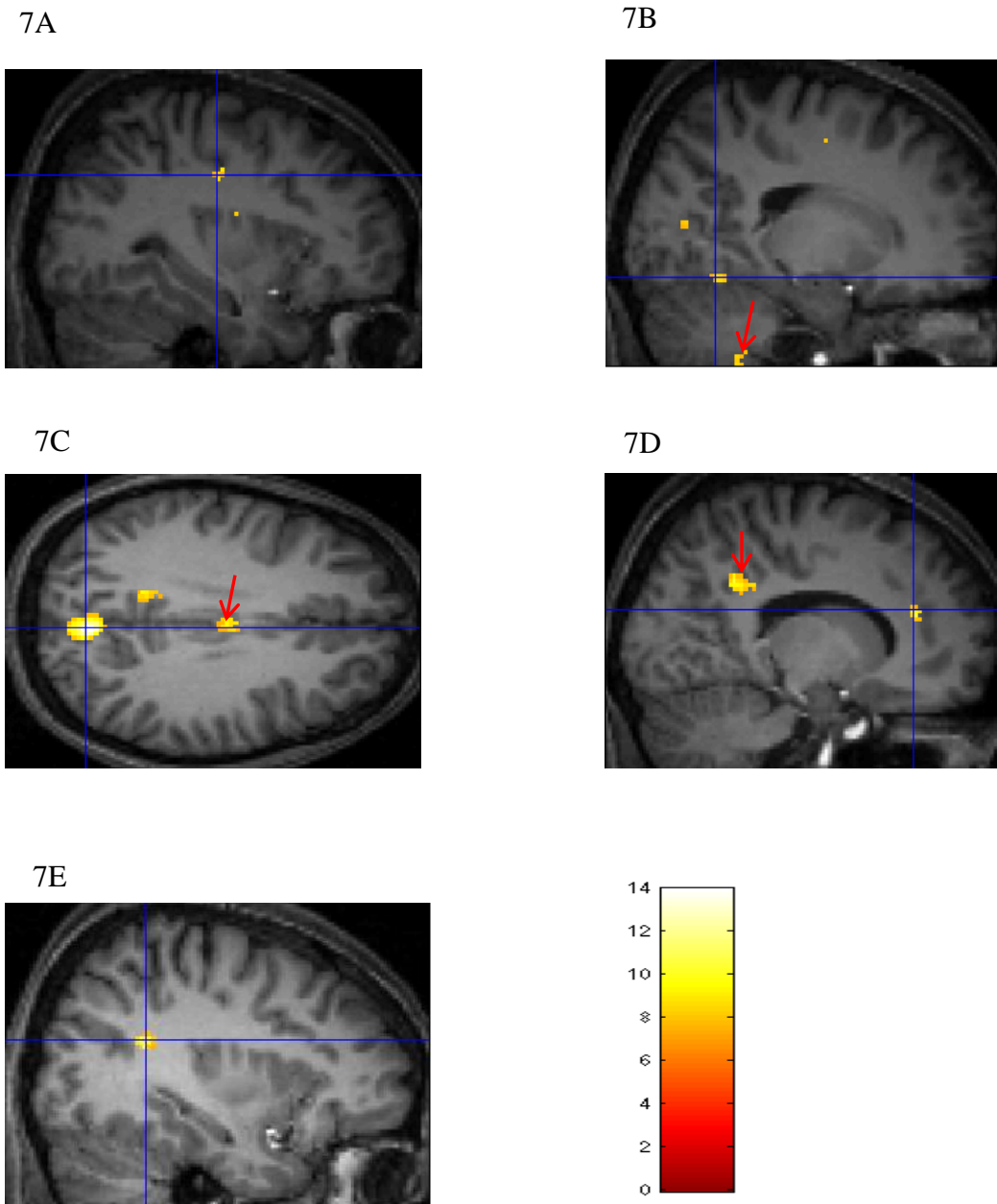


Figure 7: fMRI Main Effect of Group Results. Figure 7A shows the blue crosshairs on activity in the right precentral gyrus from the group main effect. Figure 7B shows activity from the group main effect in the right fusiform gyrus (blue crosshairs) the red arrow points to activity in the cerebellum. Figure 7C shows the bilateral effects in the cingulate (red arrow) and calcarine sulcus (blue crosshairs) from the group main effect. Figure 7D shows the left anterior cingulate sulcus (blue crosshairs) and the cingulate sulcus, marginal segment (red arrow) for the group main effect. Figure 7E shows the blue crosshairs on the activity in the left angular gyrus from the group main effect.

3.2.4 Time Main Effect

The final main effect is the effect of time. Two of the three time points were included in the analysis: the initial time point, prior to any training, and the third time point, following the completion of training. It should be noted that for this analysis it was not necessary to exclude the initial differences found between groups as groups were not differentiated for the analysis. Regions which displayed differences over time regardless of group ($p < .01$, uncorrected) were the right circular insular sulcus, right supplementary motor area, right cingulate sulcus, right cerebellum, right head of hippocampus, right parahippocampal gyrus, and the left cerebellum (Figure 8).

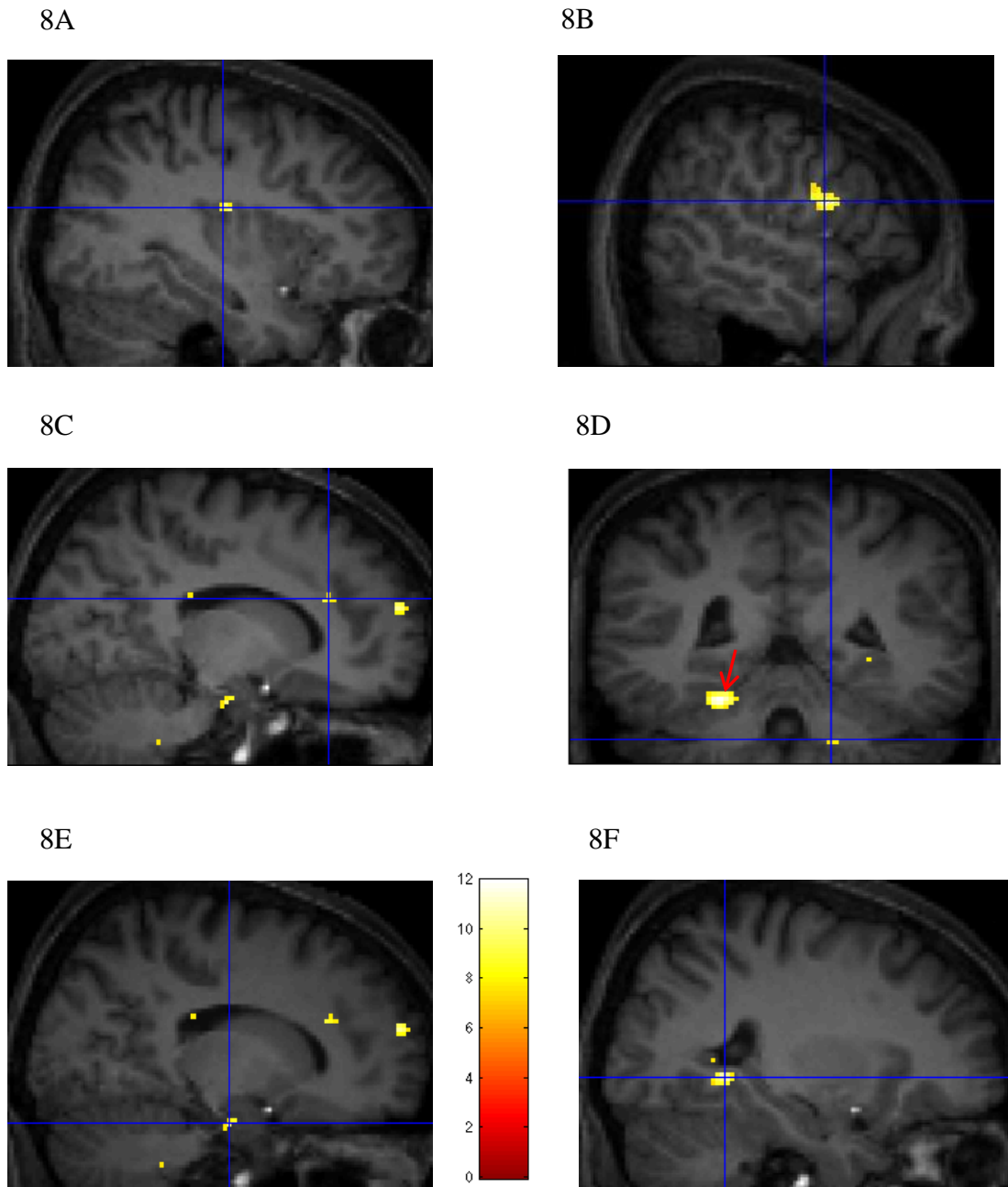


Figure 8: fMRI Main Effect of Time Results. Figure 8A shows the right circular insular sulcus for the main effect of time. Figure 8B shows the activity for the main effect of time in the right supplementary motor area. Figure 8C shows activity in the right cingulate sulcus for the main effect of time. Figure 8D shows cerebellum activity in the right (blue crosshairs) and left (red arrow) hemispheres for the main effect of time. Figure 8E shows the blue crosshairs on the head of the hippocampus for the main effect of time. Finally, Figure 8F shows activity in the parahippocampal gyrus for the main effect of time.

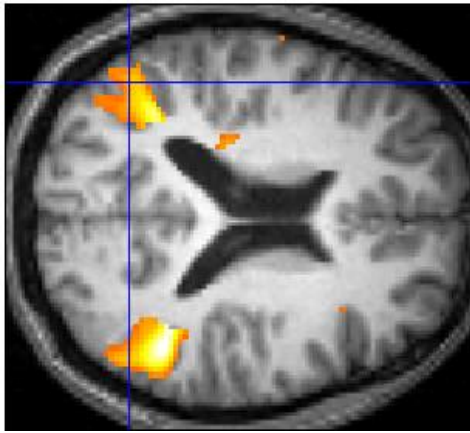
3.3 Voxel-Based Morphometry Results

A VBM analysis was performed on the data obtained from the MPRAGE of each scan. The images from the modulated grey matter segmentation were chosen to be fully analyzed and compared to the fMRI results. These images maintain volumetric capabilities and show expansion or contraction in the grey matter. A 2x2 ANOVA was performed on the data with factors of group and time. This analysis was identical to the ANOVA conducted on the fMRI data. The two groups, observational learning and motor learning, were used as the factors of group. The two levels of time correspond to the initial time point, time point one, and the final time point, time point three.

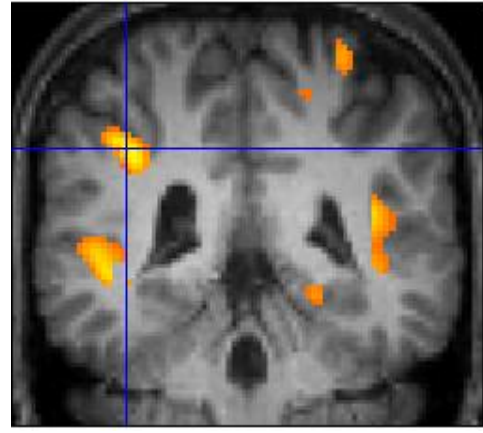
3.3.1 Initial Analysis

As with the functional data, the initial group differences were calculated. Similarly to the functional data, group differences occurred in the VBM data at time point one, prior to any experimental manipulation ($p < .01$, uncorrected). These differences were apparent in the bilateral superior temporal sulcus, left intraparietal sulcus, left precentral gyrus, and in the right cingulate sulcus (Figure 9). These regions were taken out of all subsequent analyses where group assignment plays a role by means of an exclusion mask.

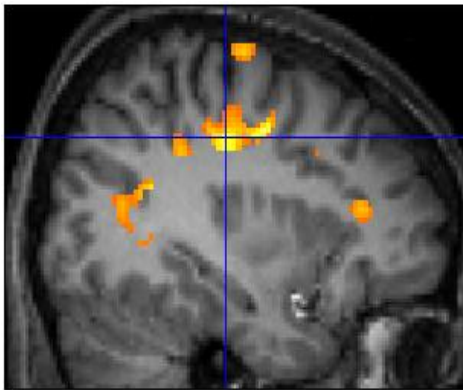
9A



9B



9C



9D

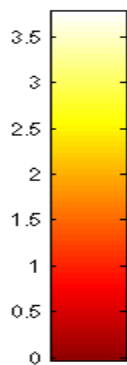
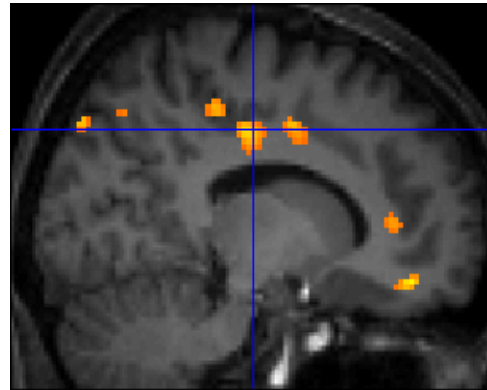
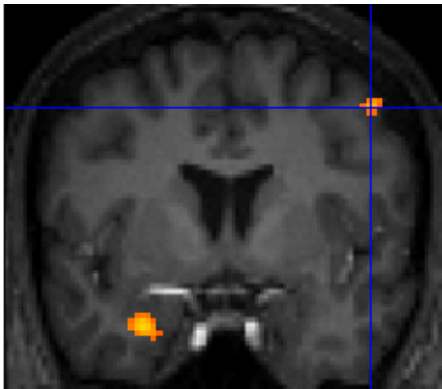


Figure 9: VBM Initial Analysis. Figure 9A shows the bilateral superior temporal sulcus activity in the initial time one VBM analysis. Figure 9B shows the intraparietal sulcus activity from the initial time one VBM analysis. Figure 9C demonstrates the activity in the precentral gyrus in the initial time one VBM analysis. In Figure 9D, several spots in the cingulate sulcus can be seen in the initial time one VBM analysis.

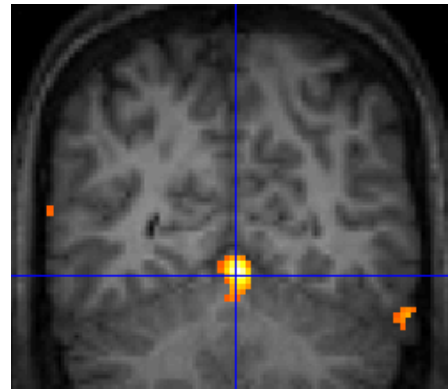
3.3.2 Group by Time Interaction

First, the interaction between group and time using the ANOVA described above was examined. Again, the same p values as the fMRI analysis were applied to the data ($p < .01$, uncorrected). Regions found in the initial analysis were removed by an exclusion mask. Remaining results showed effects in the right superior frontal gyrus, bilateral cerebellum, bilateral putamen, bilateral parahippocampal gyrus, bilateral calcarine sulcus, left lingual gyrus, right precentral gyrus, the left superior temporal gyrus, the left middle temporal gyrus, and the left inferior temporal gyrus (Figure 10).

10A



10B



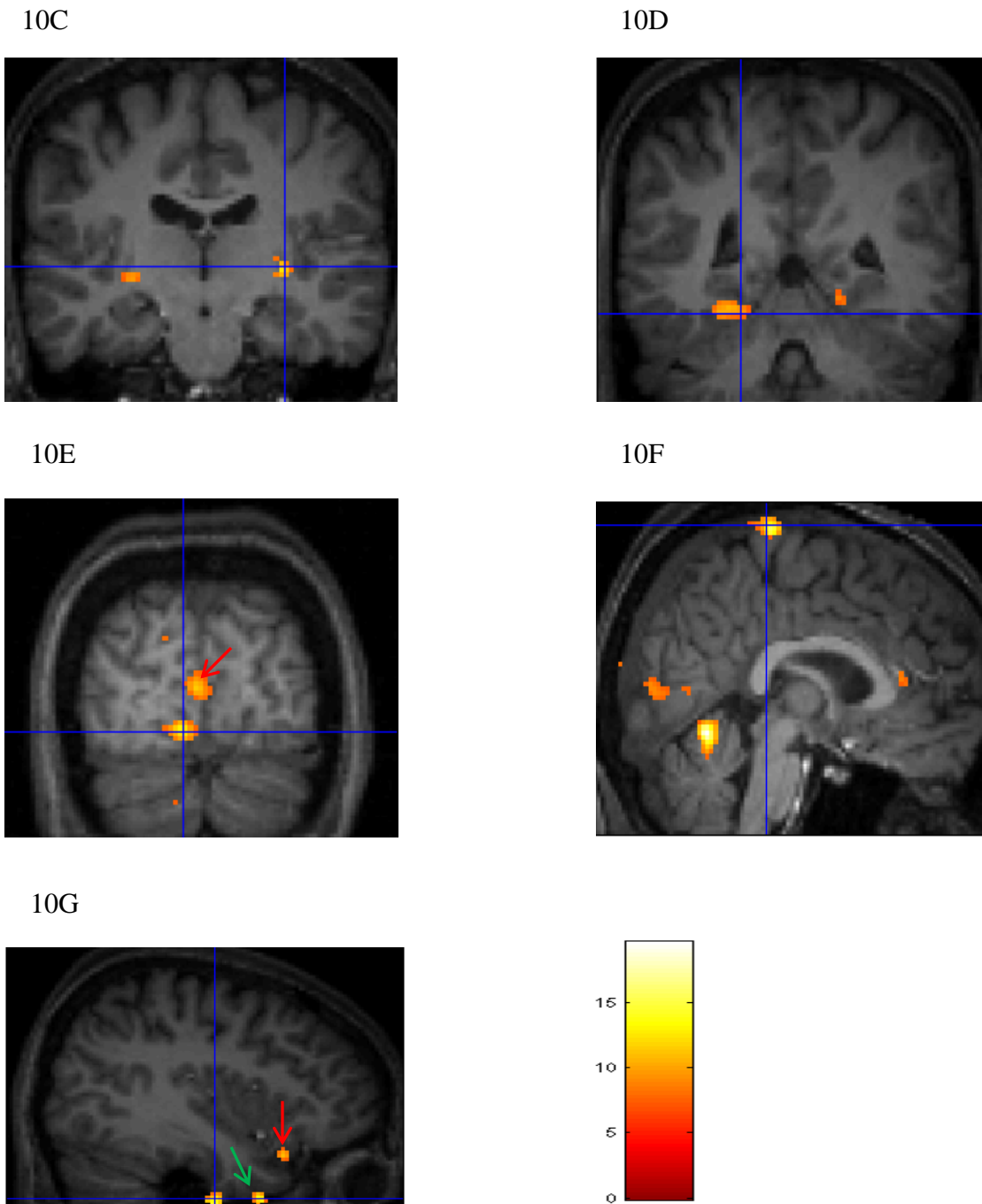


Figure 10: VBM Interaction of Group by Time Results. Figure 10A shows an increase in the grey matter in the group by time interaction of the modulated VBM data in the right superior frontal gyrus (Blue crosshairs). Figure 10B, 10C and 10D show grey matter changes bilaterally in the cerebellum, the putamen, and the parahippocampal gyrus, respectively, from the group by time interaction. Figure 10E shows bilateral grey matter changes in the calcarine sulcus (red arrow) as well as the left lingual gyrus (blue crosshairs) in the group by time interaction. Figure 10F shows blue crosshairs on the right precentral gyrus grey matter changes in the group by time interaction. Finally, Figure 10G shows the left superior (red arrow), middle (green arrow), and inferior (blue crosshairs) temporal gyrus grey matter changes in the group by time interaction.

3.3.3 *Group Main Effect*

Next, the main effect of group was investigated, again using the same p value ($p < .01$, uncorrected), as in the fMRI analysis. After using an exclusion mask to remove regions from the initial analysis, group main effects were apparent in the right precentral gyrus, bilateral superior frontal gyrus, left superior temporal gyrus, left superior frontal sulcus, and the left lateral orbital gyrus (Figure 11).

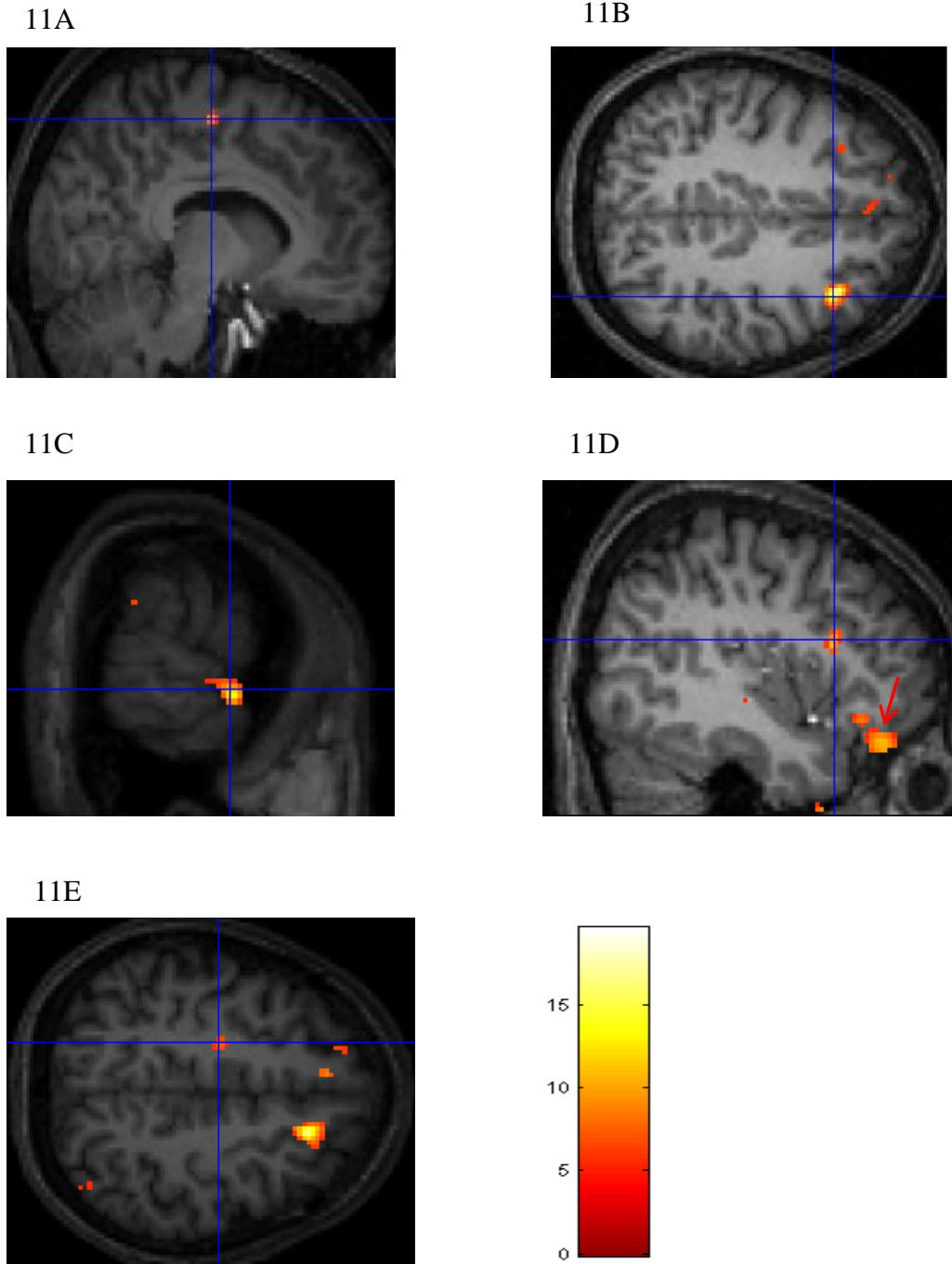
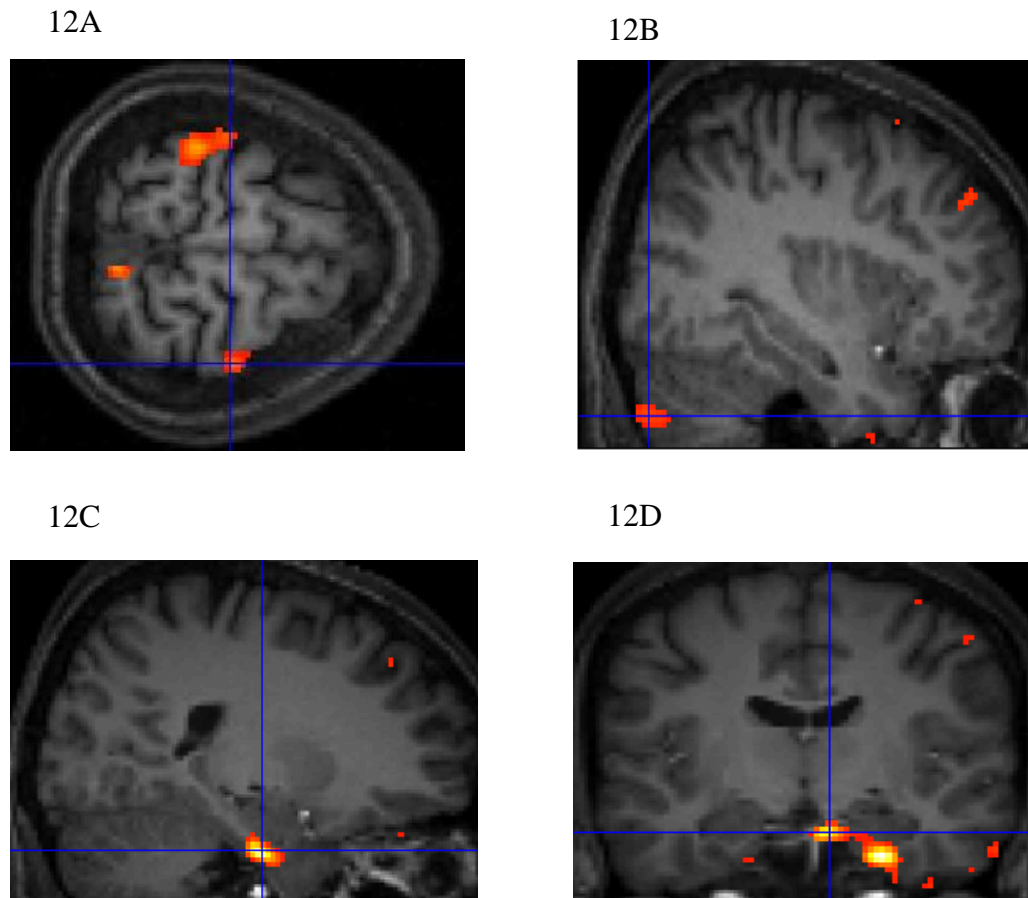


Figure 11: VBM Main Effect of Group Results. Figure 11A shows the grey matter increases in the group main effect in the right precentral gyrus. Figure 11B shows group main effects in the grey matter of the superior frontal gyrus, bilaterally. Figure 11C shows the left superior temporal gyrus grey matter changes in the group main effect. Figure 11D shows grey matter changes in the left superior frontal sulcus (blue crosshairs) and the left lateral orbital gyrus (red arrow) for the group main effect. In Figure 11E, blue crosshairs mark the position of the left precentral gyrus grey matter increases of the group main effect.

3.3.4 Time Main Effect

Finally, the main effect of time from the ANOVA was analyzed. Because group assignment is irrelevant in the time main effect, regions found in the initial analysis were not removed. The regions which showed a main effect of time ($p < .01$, uncorrected) were the right post central gyrus, right cerebellum, right parahippocampal gyrus, right head of hippocampus, right medial orbital gyrus, left cerebellum, left inferior temporal gyrus, left parahippocampal gyrus, and the left postcentral gyrus (Figure 12).



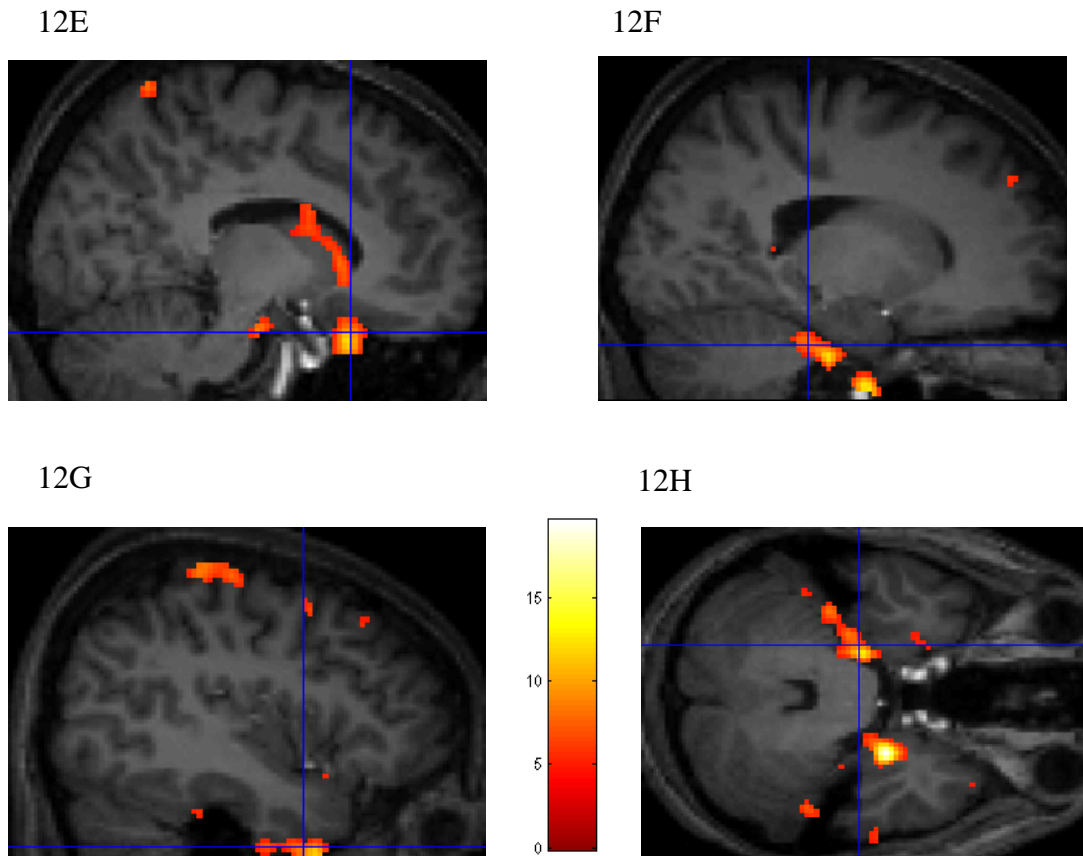


Figure 12: VBM Main Effect of Time Results. Figure 12A shows the increase in grey matter in the right and left postcentral gyrus for the main effect of time in the VBM analysis. Figure 12B shows the main effect of time in the right cerebellum. Figure 12C shows the grey matter changes for the main effect of time in the right parahippocampal gyrus. Figure 12D shows the increase from Figure 12C from a different view as well as the head of the hippocampus (blue crosshairs). Figure 12E shows grey matter increases in the right medial orbital gyrus (blue crosshairs) for the main effect of time. Figure 12F shows the cerebellum grey matter changes (blue crosshairs) from the main effect of time. Figure 12G shows the left inferior temporal gyrus in response to the main effect of time. Figure 12H shows the grey matter response to the main effect of time in the left parahippocampal gyrus (blue crosshairs), and another view of the activity in the right parahippocampal gyrus (Figure 12C).

3.4 Overlap Results

3.4.1 Overlap of the Group by Time Interaction

Using MarsBaR, significant SPM clusters were gathered from the analysis of group by time from both the fMRI analysis and the VBM analysis ($p < .01$, uncorrected). A separate binary mask was created for the fMRI active clusters and the VBM active clusters. The binary masks were overlapped in MarsBaR to determine if there were any shared regions. No voxel overlap resulted from this analysis for the group by time interaction.

3.4.2 Overlap of the Group Main Effect

Binary masks were created from images taken from the group interaction for both fMRI and VBM results ($p < .01$, uncorrected). These binary masks were overlapped in MarsBaR to determine if any overlap occurred. No voxel overlap resulted from this analysis of the main effect of group.

3.4.3 Overlap of the Main Effect of Time

3.4.3.1 ROI Formation

For the time main effect, binary masks were created for fMRI and VBM images ($p < .01$, uncorrected). MarsBaR was used to overlap the masks to determine if any voxels were significant in both the fMRI and VBM masks. Voxel overlap occurred in the

head of the hippocampus only. This overlap was made into an ROI for the subsequent mediation analysis (Figure 13). The ROI encompassed 32 voxels and was centered at coordinates 16, -13.5, -21.5.

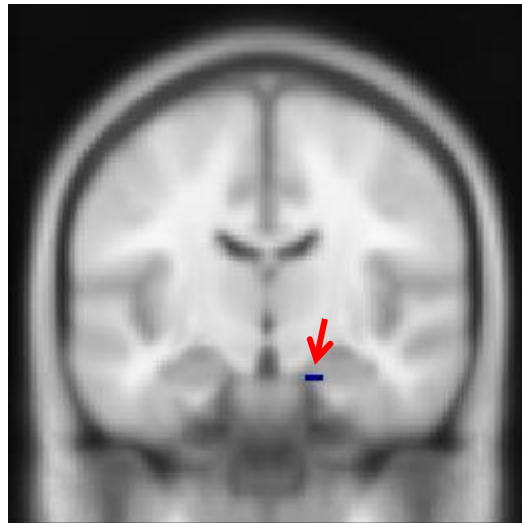


Figure 13: Overlap ROI. Figure 13 shows the ROI in the head of the hippocampus in the right hemisphere, derived from the overlap of the VBM and fMRI results. The red arrow points to the ROI.

3.4.3.2 Means of ROI

MarsBaR was used to extract the mean intensity values from the ROI for both fMRI and VBM images. Individual subject images were used, specifically the contrast images which show the contrast between moving images and static and blank images. The fMRI images resulted in intensity values for 20 subjects for time one ($M = -0.34$, $SD = 1.34$) and intensity values for 21 subjects for time three ($M = 0.80$, $SD = 1.18$). The subject number disparity was due to the location and nature of the ROI. In the ROI

analysis to obtain the means, it was discovered that the results for two subjects were being affected greatly by values known as Not-A-Number (NaN). MRI is susceptible to signal dropout around the sinuses in the medial temporal regions (Huettel, Song & McCarthy, 2004). When signal dropout occurs, there is no valid value in the voxel. As a result, the voxel cannot be used for analysis. To combat this, a nearest neighbor fix is generally applied. This entails using values from nearby voxels. In this case, a nearest neighbor fix would have resulted in voxels located outside of the ROI, and therefore the two subjects were dropped from the total analysis. The final fMRI results represent 20 subjects at time one ($M = -0.34$, $SD = 1.34$) and 20 subjects at time three ($M = 0.84$, $SD = 1.19$). The two subjects were also dropped from the VBM analysis, with the VBM analysis representing the same 20 subjects at time one ($M = 0.73$, $SD = 0.06$) and 20 subjects at time three ($M = 0.71$, $SD = 0.06$).

3.5 Mediation Results

In preparation for the mediation, a list of the individual mean intensities for each subject in the ROI was created in an excel document to be analyzed with SAS. Figure 14 depicts the group mean intensities in each time point.

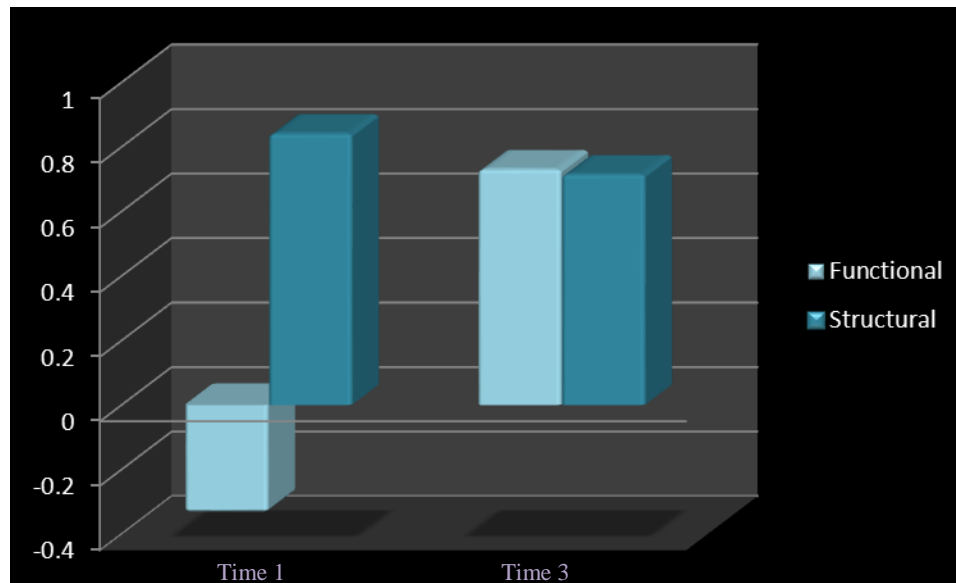


Figure 14: Mean Intensity Values. In Figure 14, both the functional and structural mean intensities are displayed for the main effect of time in the head of the hippocampus. The functional data show an increase in activity, indicating a greater response in action recognition as a result of training. The structural data decrease, indicating more streamlined processing by the neurons of the ROI. These data represent the first and third scans.

3.5.1 Baron and Kenny (1986) Equation One

In order for a mediation relationship to be established, there are four conditions that must be met. The first condition is that the relationship between the independent variable, in this case time, and the dependent variable, the functional changes, must be significant. In order to establish significance, a paired sample t test was conducted on the functional data. The relationship was found to be significant $t(19) = -2.39, p < 0.028$, upholding the first requirement of Baron and Kenny (1986).

3.5.2 Baron and Kenny (1986) Equation Two

The second requirement for establishing a mediation relationship is that the relationship between the independent variable, time, and the mediator, structural changes, must be significant. Significance was tested with a paired sample t test on the structural data. The relationship was found to be significant $t(19) = 3.02, p < 0.007$, upholding the second requirement of Baron and Kenny (1986).

3.5.3 Baron and Kenny (1986) Equation Three

The third requirement for establishing mediation is that the relationship between the mediator, structural changes, and the dependent variable, functional changes, must be significant. Significance was tested using an ANCOVA where both functional changes and structural changes were input as repeat measures; functional changes were set as a one factor variable and structural changes were set as a covariate. Structural changes were not significantly related to functional changes $F(1, 19) = 0.01, p < 0.94$. The requirement for mediation was not upheld, as this relationship was not significant $B = -0.310, p < 0.94$. Therefore, the results do not support the hypothesis that structural changes act as a mediator between the relationship of time and functional changes.

4. Discussion

This section discusses the implications of the results reported above.

4.1 Summary

Regions which show changes as a result of training in a one ball cascade juggling task were successfully determined in functional and structural activity. Functional changes represent an increase in action recognition based on the acquisition of expertise. Structural changes represent the underlying plasticity of the brain itself, based on training in a specific motor skill. These results provide confirmation that there are both functional and structural changes in the brain as a result of motor skill training. When the changes were overlapped, with the exception of the main effect of time, the functional and structural changes did not overlap in the same brain regions. This posed an issue for the hypothesis that structural changes mediate the relationship between time and functional changes. One region did show overlap - the head of the hippocampus. In this 32 voxel ROI, a mediation analysis was conducted. The mediation equation failed to meet Baron and Kenny's (1986) requirements, demonstrating that structural changes do not mediate the relationship between time and functional changes in the head of the hippocampus. The specific goal of this study was to address two challenges. The first was to show

action recognition changes as a result of training. The second was to perform a mediation analysis on the structural and functional data. Both of these goals were accomplished. Therefore, while the hypothesis was proven false, the aims of the study were successfully accomplished, and valuable information was obtained.

4.2 Behavioral Results

4.2.1 Motor Group Results

In order to draw any valid conclusions from the imaging data, the subjects must be considered experts at the task. Five weeks of juggling practice is not sufficient if subjects are still unable to successfully complete a cascade. The number of successful throws and catches within a cascade must also be greater upon completion of training than the initial training session. The behavioral results of this study show that training was significantly successful for the subjects of the nonvisual motor group. Without the input of vision, subjects were able to gain expertise in the one-ball cascade juggling task. While some subjects may have become more expert than others, all subjects were able to improve their performance.

4.2.2 Observational Group Results

The nonmotor visual group was also able to improve performance based on training. Randomized videos for each subject allow the conclusion that subjects learned the task and the improvement was not the result of variable difficulty for each video.

The observational group paradigm trained participants in recognizing a successful catch and counting successful catches in a cascade. The assumption behind the task is that participants would be closely observing actors performing the one-ball juggling cascade task. During observation, they would be learning to perform the task by visual means. As the imaging results show, observing the actors did affect action recognition, although not to the same extent as physically performing the action, similar to Calvo-Merino et al (2006). From this it is concluded that the nonmotor visual subjects did learn the juggling task.

An additional difference between the nonvisual motor and nonmotor visual groups lies in the skill level on any given day. In the motor group, the skill level of the day was dependent upon the previous amount of training. So a participant on day three in the five week training would be experiencing less expertise than on day twenty. For the observational group, videos were randomized. Randomization prevented confounds due to order of the videos. Videos which displayed the actors with greater skill were easier to count than unskilled actors. Some of the actors were also easier to count due to personal style. Therefore, randomization was essential to ensure that decreases in the number of errors made are actually due to an increase in skill for observational subjects. This means, however, that for observational subjects, the level of skill that they watched on any given day was independent of the skill level of the videos of the previous day. While the motor group had a gradual increase of skill, even within the three videos of each day the observational group could have a variety of skill levels. This does not negate the

results of the study. The observational group became more and more adept at recognizing what makes a throw successful over time, similar to the motor group. This aspect of the training is identical and conclusions can be based on the data with the assumption that both have acquired expertise.

4.2.3 Variance in Learning

In the behavioral data, there is considerable variance among the participant's performance. The difference between the first practice session and the final practice session was significant for the group as a whole. However, there is a broad spectrum of scores in these data. There was within group variance, as some participants became more "expert" than others. For both training groups, the subjects fell into two categories. One category of subjects showed a vast difference between their beginning and ending scores. The other showed barely any change at all. The subjects who showed minimal improvement were included in all analyses detailed above. It is possible that this inclusion lessened the effects in the functional and structural data. Although it was not formally tested, it is hypothesized that subjects who show a greater response in their training would have greater changes in the functional and structural data. There may have been an increase in overlap had the analysis been limited to subjects who showed greater increases in skill. However, Draganski et al. 2006 reported that there was no correlation between grey matter change and performance. Therefore, it is reasonable to perform all analyses with all subjects included.

Variance was also present between groups. The experimental design specifically had the nonvisual motor group and the nonmotor visual group train under different paradigms. This introduced a variance in the way that subjects learned. The aim of the experiment was to isolate visual and motor practice. In order to do this, the behavioral paradigms had to be different. The differences in paradigms may have caused subjects in the two groups to learn completely different tasks, rather than the same task in different ways. The motor skill required a great deal of spatial awareness which may not be evident to the visual only group. Therefore, quite a bit of variance is introduced here, as demonstrated in the structural and functional results. The group analysis of both the structural and functional results proves that different regions are affected by each group, rather than the motor group having a more robust response as would be expected by Calvo-Merino et al. (2006). Therefore, the results containing data from both groups may actually be looking at different effects, and consequently involve more regions in a less robust manner.

4.3 fMRI Results

4.3.1 Initial Analysis

In order to infer any implications or conclusions from the data, it is necessary to be able to dismiss any potential confounds. The initial analysis at time point one showed significant differences prior to experimental manipulation. These initial differences were located in the right precentral gyrus, right cerebellum, right fusiform gyrus, and bilateral

parieto-occipital fissure. The location of the initial differences is a red flag for the analysis, as these regions are part of the motor system (Tyc, Boyadigan & Devanne, 2005; Ungerleider, Doyon & Karni, 2002). The subjects were randomly assigned to a group using a reliable randomizer so these differences are most likely either due to error or organic differences in the individual brains. However, the results, especially the precentral gyrus and the cerebellum, are regions which would be expected to show change as a result of training. Motor regions are preferentially affected by motor skill training. Since there were group differences initially, subsequent analysis of group data would be compromised. The analysis was performed several times and every step was checked for error. It was concluded that the data are accurate, and that the differences shown between groups are inherent to the data, not the product of error from analysis. Using an exclusion mask, the initial analysis data were removed from later analyses. These initial differences were removed from all analyses that did not merge the data from the two groups.

4.3.2 General fMRI Change effects

In current literature, changes in fMRI activity have been shown to be based on learning. The more time spent on learning, the more the activity changes. The results of this study show similar patterns in activity. As subjects gained expertise in the one ball juggling cascade task, they increased activity in motor related regions over time. The results from the time one scans and the time three scans were chosen for analysis due to the fact that the differences were more robust than between the time one and time two

scans or the time two and time three scans. More time and training had passed and the results indicated a greater chance of overlap with the VBM results.

The fMRI response is derived from the progress through the learning stages (Buitrago, Schulz, Dichgans & Luft, 2004; Doyon & Benali, 2005; Karni, 1995; Kleim et al., 2004; Ungerleider, Doyon & Karni, 2002). As each subject progresses through the stages of learning, the fMRI signal changes based on the current stage (Buitrago, Schulz, Dichgans & Luft, 2004; Doyon & Benali, 2005; Karni, 1995). Learning goes through an initial rapid phase of acquisition, followed by a slower phase of learning (Karni, 1995; Ungerleider, Doyon & Karni, 2002). The slower phase seems to show more reorganization of the metabolic needs (Buitrago, Schulz, Dichgans & Luft, 2004; Doyon & Benali, 2005; Karni, 1995; Kleim et al., 2004; Ungerleider, Doyon & Karni, 2002). It is thought that synaptogenesis occurs in the slower stage, causing an increase in the metabolic needs of the cell, or recruitment of the cell. Since the BOLD signal is the metabolic usage of the neurons, as time passes, we see more of a BOLD signal from expertise acquisition. This holds true when the changes in functional activity indicate a more robust response, rather than an increase in efficiency. The results support this theory, as the signal from time three has greatly increased from the signal of time one. Therefore, in the head of the hippocampus, as training and expertise increase, there is an increased demand of the neuron's metabolic needs. This signifies increased activity.

In non-human research, it appears as though motor skill learning is dependent upon LTP (Rioul-Pedotti et al. 1998). In the human literature, plasticity is measured by fMRI results. However, minimal fMRI literature focuses on skill acquisition over an extended period of time. This study focused on a five week period of time. During those five weeks, sufficient expertise was gained to express a change in action recognition in the fMRI signal. It is assumed that this change is due to the LTP, occurring as expertise is acquired.

It has been found that training in a specific skill results in an enlarged map area in the primary motor cortex (Tyc, Boyadijan & Devanne, 2005; Ungerleider, Doyon & Karni, 2002). The map area can be demonstrated with the mirror neuron system. Showing a subject a task that they are familiar with should mimic the activity of a subject actually performing the task. Action observation is used to see the mirror neuron system in this study. The mirror neuron system includes the primary motor cortex in the classical classification (Rizzolatti & Luppino, 2001). As action recognition changes, it is reflected in the mirror neuron system. The changes in recognizing an action are shown through the functional changes in the brain of a subject. As subjects gained expertise in the task, they presented a more robust functional response. These changes in the primary motor cortex are thought to be the slower response which evolves after several sessions or weeks of practice (Karni, 1995; Ungerleider, Doyon & Karni, 2002).

In the rapid learning phase, the cerebellum, striatum, and other motor-related areas of the frontal lobe show a rapid response (Ungerleider, Doyon & Karni, 2002). This rapid response can occur even within a single practice session (Ungerleider, Doyon & Karni, 2002). Over the course of practice, however, this rapid activity decreases to the point where it is not detectable using imaging methods (Ungerleider, Doyon & Karni, 2002). To capture this rapid activity, this study would have to look at the second time point, following a week of practice. In order to focus on potential mediation, the focus of this study was on the first and third scans. Comparing the first and third scans ignores the rapid activity in favor of the long term changes. Once enough practice has occurred, the plasticity becomes stabilized, and appears resistant to interference from outside sources. The plasticity that is going to occur for that skill has been completed. This promotes the idea that training drives long term cortical plasticity.

The current results indicate the involvement of motor related regions. In the group by time interaction, as well as the main effects of group and time, the cerebellum seems particularly active in the right hemisphere. As discussed above, the cerebellum is part of the rapid activity which generally decreases with time (Ungerleider, Doyon & Karni, 2002). However, the exact schedule of the degradation of signal is unknown. The main effects of group and time show a response around M1. Since M1 is thought to be one of the major regions involved in the long term changes (Tyc, Boyadijan & Devanne, 2005; Ungerleider, Doyon & Karni, 2002), it is reasonable to assume that if there is M1 activity then the initial effects of long term plasticity are displayed in the brain. It can be

concluded that five weeks is sufficient to at least start to show long term effects, even if the maximum effect has not yet been reached. The minimum practice length to show maximum effects has not been determined. The current five week paradigm follows a similar time table to Ungerleider et al. (2002). The presence of activity in the cerebellum indicates that there may be other more appropriate timetables not yet described since the cerebellum activity should have degraded at this point based on current theory. It is possible that the signal in the cerebellum remains longer than anticipated by Ungerleider et al. (2002). Additionally, juggling may cause a more initial robust signal in the cerebellum than finger motion. This signal may take longer to degrade than the signal derived from finger practice. Since juggling requires constant feedback of motion and space, it is also possible that the cerebellum is being constantly stimulated, and that even given a longer period of time, the activity would remain constant.

Existing literature on long term skill acquisition shows that there is enhanced synaptic plasticity, and suggests that it is due to an increase in synaptogenesis (Rosenkranz, Kacar, & Rothwell, 2007). Other studies have also suggested that initial improvement is due to an increase in the efficacy of existing synaptic connections through the horizontal circuitry, but as time progresses, the additional practice leads to synaptogenesis (Kleim et al., 2002; Kleim, 2004; Nudo, 1997; Rosenkranz, Kacar, & Rothwell, 2007; Sanes & Donoghue, 2000). Animal research supports this idea as well, demonstrating significantly more synapses per neuron in layer V in rats that were trained for a motor skill (Kleim et al., 2002; Kleim et al., 2004). This supports the current study,

where the hemodynamic response increased with training. The increased number of synapses requires more metabolic resources, which would increase blood flow to the region, and increase the BOLD signal. However, these studies contrast with more recent literature which suggests that motor skill learning is associated with an increase in efficiency, and therefore a decrease in activity (Reithler, Mier & Goebel, 2010). However, the Reithler et al. (2010) study was performed during a shorter period of time, and so synaptogenesis would not have occurred based on the timetable described above.

The fMRI changes were thought to be based on the mirror neuron system. The results of this experiment, however, do not completely agree with the typical regions of the mirror neuron system. Typically, the mirror neuron system encompasses the primary motor cortex, the premotor cortex, the supplementary motor region, Broca's area, the superior temporal sulcus, and the inferior parietal cortex (Rizzolatti & Luppino, 2001). Nishitani et al. (2000) found frontal activations (from viewing an action) that were similar to the action execution. However, they were preceded by occipital activation when visually stimulated. Generally, the mirror neuron system seems to be reacting as if the subject was performing the action in visual areas with motor related regions as well (Rizzolatti & Craighero, 2004). The current results do not stay within the framework of the mirror neuron system, instead expanding beyond the scope of the traditionally classified mirror neuron regions. This may be due to the fact that seeing someone perform the one ball cascade juggling task is not the same as physically performing it in the absence of vision. The task is also different than watching the action with the intent

of counting the cascades. Therefore, the extent to which the mirror system is present in this study may be modulated by the experimental design.

4.3.3 Group by Time Interaction

These data prove a group by time interaction showing changes in the fMRI results. The regions involved in these changes included the right cerebellum, right fusiform gyrus, right superior temporal sulcus, right parieto-occipital fissure, and the left collateral sulcus. These data represent the group by time interaction after the data from the initial analysis was removed by means of an exclusion mask. The independent variables interact with each other rather than act as separate entities. Both group and time rely on each other's levels to an extent. Therefore, the effects of time are dependent upon which group the participant was in, even after the initial group differences were removed. This follows the idea in Calvo-Merino et al. (2006) that actively training in a task (the nonvisual motor group) has a different effect than merely observing a task (the nonmotor visual group).

The regions involved in the group by time interaction are regions which are recruited for the motor network. As discussed above, the cerebellum is part of the rapid skill acquisition response. The superior temporal sulcus is often associated with biological motion, and as subjects were involved in the action recognition of a biological motion, an increase in activity in this region would be expected. Following expectations of structural changes, visual regions are incorporated into training effects (Draganski et

al., 2004). In the group by time interaction, visual areas were also incorporated. While the observational group (nonmotor visual group) did have vision, the motor group (nonvisual motor group) had a blindfold on to prevent vision. In an interaction that is dependent upon group, visual regions play a logical role.

4.3.4 Group Main Effect

The group main effect showed changes between groups in the right precentral gyrus, right cerebellum, right fusiform gyrus, bilateral calcarine sulcus, bilateral cingulate, left anterior cingulate sulcus, left cingulate sulcus, marginal segment, and the left angular gyrus. Similarly to the group by time analysis, the initial differences between the groups were removed, as these initial differences were inherent in the participants and not due to experimental manipulation. The strong difference between groups backs up the findings of Calvo-Merino et al. (2006). Calvo-Merino et al. (2006) demonstrated greater motor region activity when participants were well versed in an action rather than learning by observational means. While both observational and motor learning result in functional changes, the motor group's response is more robust in all group effect analyses. However, the results show a more expansive difference than reported in Calvo-Merino et al. (2006).

The group differences lie not only in the robustness of the response, but in the regions themselves. The data reported are based off of an F test which shows that differences exist between regions of the two groups. Further analysis is necessary to

determine exactly which regions are affected in each group. The analysis shows clearly that while some of the regions are the same, some differences do exist between the two training groups. This is potentially due to the differences in training. As previously discussed, the training for the two groups was not identical, essential to the experimental design. This introduced an additional source of variance into the data, which in turn caused different regions of the brain to be affected by group.

These findings also back up previous literature, described above, displaying expertise-related changes in motor regions. Activity in the cingulate is apparent in the main effect analysis. The cingulate motor areas (CMA) have been associated with learning a sequence of movements (Doyon et al. 1996; Grafton et al., 1998). This may be displayed in the group analysis due to the fact that one group is physically performing the sequence for juggling cascade (nonvisual motor group), while the other is merely observing (nonmotor visual group). Learning to perform physically has a greater response as described above.

4.3.5 Time Main Effect

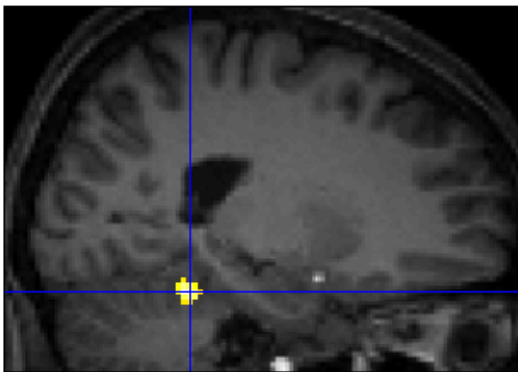
In the main effect of time, affected regions included the right central sulcus, right circular insular sulcus, right supplementary motor region, right cingulate sulcus, right cerebellum, right head of the hippocampus, right parahippocampal gyrus, and the left cerebellum. Because the groups were not separated in this analysis, the initial differences had no effect on the analysis and were not excluded.

It was expected that training would induce fMRI changes in the brain, regardless of what group participants were in. However, the results of the main effect of time differ from previously described research. There are several possible reasons for this to occur. The first reason is that here the two groups are merged. Based on the conclusions of the interaction effect and the group main effect, the observational group and the motor group do not have identical responses to viewing the novel one ball juggling cascade task. Previous research did not isolate the modalities in this manner and therefore making comparisons is difficult. Since the groups were different, combining them would provide additional regions of activity that would not exist in previous studies. Along a similar vein, a second issue with making comparisons between this study and previous literature lies in the style of training. This study isolated modality types into strictly motor and strictly visual. Therefore, influences of the other modality did not occur in a paradigm that was purely visual or purely motor. All other studies allowed participants vision while training, which may have influenced the results into visual systems or other regions across the brain.

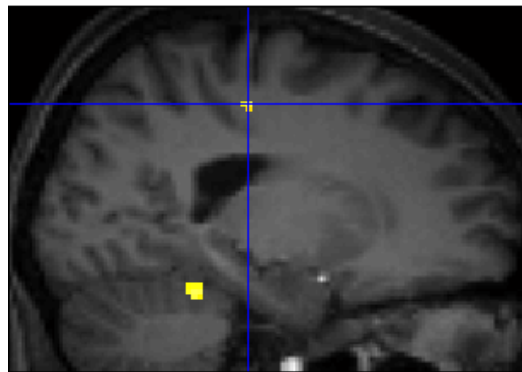
Despite these issues with comparisons, this study does provide some evidence to substantiate previous literature. The cerebellum seems to be a recurring region in the literature and shows up as having a response here based on time. Activity found in the cerebellum can be seen in Figure 15A below. The cerebellum is very active in motor control, providing a relay station for all motion. Consequently, one would expect the region to show a reaction to motor stimulation. Additionally, while literature describes

M1 as responding, the current data show a response in M1, as seen in Figure 15B below, a sign of agreement with current literature. The activity in the supplementary motor region (Figure 15C) also is in agreement that these motor regions play a large role in the acquisition of expertise in a motor task.

15A



15B



15C

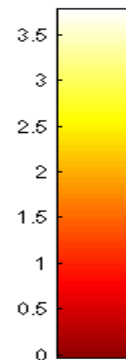
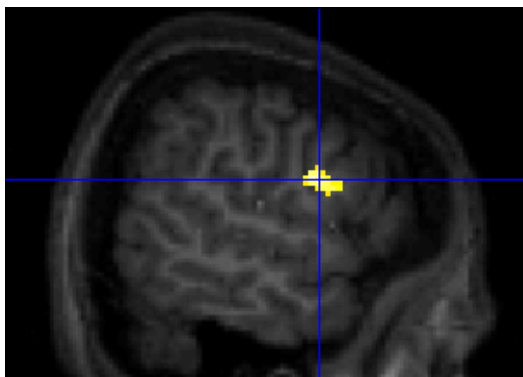


Figure 15: Main Effect of Time Comparisons to Current Literature. Figure 15A shows the cerebellum activity in the main effect of time for fMRI results. Figure 15B highlights activity in M1 as a result of the main effect of time for fMRI results. Finally, in Figure 15C, fMRI activity in the supplementary motor region for the main effect of time.

4.4 Voxel-Based Morphometry Results

4.4.1 Initial Analysis

Due to initial differences found in the fMRI analysis, an initial analysis on the VBM results was performed. Again, this was to ensure that any results that were found from group differences were a result of experimental manipulation. If the groups show significant differences before the experiment began, then any results are flawed unless these differences are accounted for. In the VBM results, there were also differences between the groups at time point one found in the initial analysis. These differences were located in the right parahippocampal gyrus, bilateral superior temporal sulcus, bilateral cingulate sulcus, left intraparietal sulcus, and the left precentral gyrus.

Similarly to the fMRI results, the VBM initial differences by group were located in regions which are susceptible to the manipulations of this experiment. Every step of the analysis was checked in minute detail, as with the fMRI analysis. Every step was proven accurate and again it was concluded that these differences were inherent in the groups prior to any training. Therefore, these regions were taken out of subsequent analyses that dealt with the data at a group level.

4.4.2 General Voxel-Based Morphometry Plasticity

VBM results indicate that there are structural changes following motor skill training. This is consistent with motor literature investigating structural effects.

Literature shows transient and highly selective expansion in the grey matter. Specifically, the changes in the grey matter are found bilaterally in the mid-temporal area (MT/V5) (Boyke, Driemeyer, Gaser, Buchel & May, 2008; Driemeyer, Boyke, Gaser, Buchel & May, 2008; Draganski et al., 2004) and in the left posterior intraparietal sulcus (Draganski et al., 2004). From these results, Draganski et al. (2004) conclude that motor skill learning is a greater stimulus for plasticity in visual areas, compared to motor regions. Here, more motor region involvement was found in all of the analyses. The group by time interaction, and the main effects of group and time all seem to be centered in motor regions.

The Draganski et al. (2004) results contrast with previously described fMRI results which indicate that motor regions are more susceptible to change under similar circumstances. Literature also agrees with results showing more motor region involvement. For example, Boyke et al. (2008) also discovered an increase in the left hippocampus and bilateral nucleus accumbens. Further, Cannonieri et al. (2007) found that professional typists had an enlarged grey matter volume bilaterally in the medial inferior cerebellar hemisphere, right medial orbital region, right paracentral lobule, and the right temporal pole. This extends previous literature, but the participants in this study were professionals, working in their field for an average of 11 years, a considerably longer practice period than in the experiments described in the above mentioned literature. Interestingly, these regions are involved in the motor system rather than the visual system. This may indicate that motor region plasticity occurs on a long term basis,

longer than the several month studies listed above. In this study, however, the number of motor related regions outnumbers the visual regions after a period of only five weeks. It is postulated that this is due to the fact that participants in the nonvisual motor group had no visual input during training and therefore the motor system overcompensated. The nonmotor visual group may account for the minimal visual regions seen in the results. However, the exact contributions cannot be determined from current results.

Draganski et al. (2006) showed bilateral increases in the parietal cortex, specifically the posterior and inferior parietal lobule, as well as an increase in the right hippocampus. The study, however, was looking at a more abstract type of learning, studying for an exam. The results from this study do follow the timing long term pattern of the fMRI learning studies more closely. The regions do not show expansion (and subsequent contraction following a long term period of rest) happening in all regions concurrently. Different regions respond and change structure at different points in the learning process. The current results only take into account two time points, therefore the timing cannot be determined. As with the functional data, it is postulated that five weeks is sufficient to achieve long term effects, but short enough to have some lingering rapid response to learning effects present.

Recently, the groundwork for determining the structural basis of the grey matter changes has been laid. Quallo et al. (2009) trained macaques to use tools. As a result of this training, significant increases in the structure were found in the right superior

temporal sulcus, right second somatosensory area, and the right intraparietal sulcus. Less significant effects were found in the left hemisphere. Two out of the three monkeys showed increases after a week of intensive training and peak increases appeared when training performance plateaued. The third monkey, who was slower to learn the task, did not show increases. This may be because he did not reach an expertise level required to facilitate grey matter changes. If animals can be successfully trained and demonstrate similar regional changes to humans as above, then it is possible to take a closer look at the structure to determine the physical cause of expansion in the grey matter, which cannot be determined in humans at the present time.

4.4.3 Group by Time Interaction

In the VBM results, the group by time interaction yielded significant results. The initial differences in group had to be removed by an exclusion mask, but the leftover results were significant. There was a group by time interaction in the right superior frontal gyrus, right precentral gyrus, bilateral cerebellum, bilateral putamen, bilateral parahippocampal gyrus, bilateral calcarine sulcus, left lingual gyrus, left superior temporal gyrus, left middle temporal gyrus, and the left inferior temporal gyrus. As with the fMRI results, group assignment determined the effects of time. The majority of these regions are involved in motor control. This contrasts with the Draganski et al. (2004) results which suggest that visual regions are more affected by training. However, in the current experiment, the subject's training occurred either without visual input (nonvisual motor group) or with only visual input (nonmotor visual group). Therefore, the visual

system responds differently than in Draganski et al. (2004), where subjects had both motor practice and visual influences.

4.4.4 Group Main Effect

The group main effect also needed the initial differences to be removed by an exclusion mask before the results could be properly analyzed. Following this, there was still a significant effect in the right precentral gyrus, bilateral superior frontal gyrus, left superior temporal gyrus, left superior frontal sulcus, left lateral orbital gyrus, and the left subcentral gyrus. Some of these regions seem to mimic the fMRI results, however, the exact spatial location is skewed and they do not overlap. Still, the presence of motor regions is evident. Again, this seems to oppose the Draganski et al. (2004) conclusions that the visual system is primarily affected. Modality of training seems to be sufficient to change affected regions from mostly visual (Draganski et al., 2004) to the current results which show a mostly motor response.

As discussed in the fMRI section above, the group differences were robust. This was due to differences in the extent to which the region responded to each group as well as regional differences between groups. As previously discussed, the training paradigm for each group was very different. While it is thought that the similarities outweigh the differences for the sake of analysis, the fact remains that the two groups had differences in training. This caused different regions to be affected for each group in the VBM and

fMRI paradigms. Overlap of the groups still occurred, but training paradigm had an effect on the regions which were recruited.

4.4.5 Time Main Effect

The main effect of time also had significant effects. Since this analysis combines the groups together, it was not necessary to exclude the initial group differences. Therefore, the unedited effect includes the right precentral gyrus, right post central gyrus, right cerebellum, right parahippocampal gyrus, right head of hippocampus, right medial orbital gyrus, left cerebellum, left inferior temporal gyrus, left parahippocampal gyrus, and the left post central gyrus. This activity seems to be widespread throughout the motor related regions. These results support previously described research as well as add additional brain regions to the equation. Again, modality seems to play a larger role here. Observational learning studies have not focused on VBM analyses. Therefore, the role in observational learning is not well understood in terms of structural changes. In the absence of visual input, purely motor learning methods may involve more recruitment of motor resources to compensate. It is potentially worthwhile to investigate the use of motor imagery and other techniques which may have been used by the motor participants during training. These techniques may have recruited a widespread motor response, which would explain the greater response of the motor system found here.

It should be noted that while the phrase greater response is used to describe the motor system, the mean intensity values of the ROI show that the grey matter in the head

of the hippocampus is actually decreasing. It is thought that this is a streamlining effect, whereby the synapses increase in efficacy. This does not agree with the fMRI results, however, as the fMRI mean intensities have drastically increased. This issue may be resolved by looking at the time two scans, which were ignored in this analysis. The given information is not sufficient to draw any exact conclusions as to why the fMRI results increased while the VBM results decreased. It is postulated that the stage of learning may play a role. The head of the hippocampus may have had an initial burst of activity which is then being streamlined. The data from time point three may be at a junction where the structure has streamlined but the function still remains high. This would indicate structure driving the functional response, though this theory was disproven in the mediation equation. At present, however, exact conclusions cannot be drawn.

4.5 Overlap Analysis

4.5.1 General Overlap Results

These data show that there is very little overlap between the fMRI and VBM results. Kleim et al. (2002) successfully proved that in rats, structural changes are located in the same spatial location as functional changes. Further, they indicate that the structural changes contribute to the slower learning phase of a motor skill. Given the general overlap of animal and human literature, it is reasonable to expect the same effects in humans. Specifically, it is expected that training induced change should spatially overlap between the fMRI and VBM results, and that the VBM results should have a

contributing role to the fMRI changes. The binary masks for the fMRI interaction effect and the VBM interaction effect were overlaid. There were no regions that overlapped in the interaction effect for both fMRI and VBM. The same lack of overlap was also evident when the same procedure was performed on the group main effect results. Therefore these analyses could not be used further in a mediation equation.

The basic premise behind BOLD fMRI is that it represents the metabolic activity of the neurons where the fMRI activity occurs. Changes in fMRI signal represent a change in the metabolic activity in the brain. Through research, it has been shown that this generally corresponds to brain activity (i.e. an increase in fMRI signal represents an increase in the metabolic activity of the brain and by association an increase in neuronal activity, though it does not directly measure neuronal activity). Therefore, any changes that are seen due to the experimental training will represent changes in metabolic demands on the neurons themselves. If the structural changes are the mediating force behind functional changes, it is reasonable to believe that changes in the structure may be responsible for the change in metabolic demand. As a result, the location of changes is very important in this study. fMRI and VBM results must match spatially in order for any kind of conclusions to be drawn.

The largest challenge facing this experiment is the lack of overlap between fMRI and VBM analyses. Interestingly, while both the fMRI and VBM analyses overlap with the results in current literature, both also seem to be expanding upon the reported regions,

providing a large area with which to overlap. Statistically speaking, the greater the regions available for an overlap analyses, the greater chance that some overlap will be found. However, only the head of the hippocampus provided a section of overlap. As discussed in both the fMRI and VBM sections, the results here may be affected by the modalities of training. It may be that the structurally affected regions are involved with spatially anticipating the ball, rather than merely the action of throwing it. However, it is also possible that the presence of vision also requires a subject to anticipate the ball, as in Draganski et al. (2004). Further study is necessary to extract the exact mechanisms behind these actions.

The fMRI task is testing for action recognition, not spatial knowledge. So if the training is giving subjects a more spatially based plastic response, then the fMRI task would not necessarily overlap. Action recognition is displayed in the mirror neuron system when observing an action. Any activity seen during action observation should be the same as if the participant was performing the action. Since participants cannot juggle in an MRI scanner, action observation is a useful tool. This does not necessarily indicate that the spatial knowledge and anticipation would be the same as physically performing the task. While performing the task, nonvisual motor subjects are paying attention to how hard they threw the ball, the arc at which the ball was thrown, and speed at which the cascade is progressing. While nonmotor visual subjects are observing an actor, the action is performed by the actor without vision. They are undergoing to same sensation as the nonvisual motor subjects. The technique used may not be apparent to the

nonmotor visual subjects, and it may be difficult to conclude that the activity from action observation is the same mechanism. The nonmotor visual group's task is much closer to the action recognition task, and as a result the action recognition task would show all effects of training as much as the observation allows.

In this study, the results for the group by time analysis and the group main effect analysis do not allow for the mediation to be tested. The results do not overlap in the same regions. Therefore, it is highly probable that there are separate forces driving the changes in fMRI and the changes in VBM activity, as described in the previous sections.

4.5.2 Overlap of Time Main Effect

4.5.2.1 Summary

Upon overlapping the fMRI and VBM changes from the main effect of time, it was found that there was a small section of overlap in the head of the hippocampus. It is a small region, extending for only 32 voxels. Despite the size of the ROI, this overlap was enough to successfully run a mediation analysis.

The hippocampus is often associated with spatial tasks. When developing the study paradigm, the experimenters juggled blindfolded on several occasions. The key to successfully completing a cascade involves not only skill acquisition, but an awareness of the arc of the ball. Verbal discussion with subject and research assistants confirmed that

everyone felt that the arc of the ball was essential to a successful catch. Recruitment of the hippocampus is essential for successful cascades. It stands to reason that the hippocampus would show both structural and functional changes in response to training.

4.5.2.2 ROI Formation

In the head of the hippocampus, all overlap was captured and used as an ROI. This resulted in a 32 voxel region. For most participants, the means of the ROI were easy to capture using MarsBaR. In the case of two subjects, there was an issue of NaN's. Upon visual inspection, it was discovered that the artifact caused by scanner signal interacting with the sinuses caused the signal to dropout at the area of the ROI. Since a nearest neighbor fix would have resulted in taking data far from the ROI, subjects lacking this data were removed from the analysis.

4.5.3 Mediation Results

The data in this study do not support the idea of a mediation relationship between time and functional changes with structural changes as a mediator. In prior research, it was shown that the BOLD response can act as a mediator between grey matter volume and thought disorder ratings (Weinstein, Woodward & Ngan, 2007). In this case, as the functional data changed, the repeated demands caused LTP which then caused a physical change in the volume of the grey matter. However, the Weinstein et al. (2007) article was not looking at learning, but at the relationship between grey matter and thought

disorder ratings. A further argument in favor of functional priority stems from Thomas et al. (2009). This study determined that functional changes occur as a result of learning, but that VBM results were artifact in analysis, and not significant results. If this is the case, then functional changes cannot possibly be derived from structural changes, breaking one of the Baron and Kenny (1986) requirements. The results above show clear significant VBM changes in the structural data and so this can be discounted in favor of alternative theories.

In this study, it was anticipated that structural changes would drive functional changes. This mediation setup was based on the theories that formed from Cajal's application of the Neuron Doctrine to explain the relationship between brain plasticity and mental processes from a structural perspective (DeFelipe, 2006). Additionally, the work by Kleim et al. (2002) suggests that in rats, the structural changes play a role in driving the functional changes. The animal model provides a strong argument in favor of testing the possibility of structural changes as a mediator. In this study, it was shown that structural changes do not act as a mediator in the relationship between time and functional changes. A possibly useful follow-up to this study would be to run the analysis with functional changes as the mediator and structural changes as the dependent variable. The main restrictive measure of this study, however, remains the lack of overlap between the regions of fMRI and VBM changes.

After all the analyses were done, there was no mediation. In the head of the hippocampus, structural changes do not mediate the relationship between time and functional changes. In practical terms, this means that as a function of time, both structural changes and functional changes do occur. However, the functional changes are not reliant upon the structural changes. This lack of a relationship can only be established for the head of the hippocampus. Further study of the brain is necessary, including controlling for the learning modalities, in order to be able to truly draw conclusions.

4.5.4 Concluding Remarks

Action recognition is an important part of human life. Learning to perform a novel one ball cascade juggling task, by either purely visual or motor means, changes activity in the mirror neuron system. The mirror neuron system responds to action observation with regions that would be recruited to perform the action. The fact that the VBM results do not overlap with the fMRI results suggests that different regions may be recruited to perform the task based on the lack of visual input (nonvisual motor group) or the differences in learning through observation (nonmotor visual group).

The one section of the brain that did show an overlap was the head of the hippocampus. The data do not support the idea that structural changes mediate the relationship between time and functional changes in the head of the hippocampus. This

does not preclude the idea that structural changes act a mediator elsewhere in the brain. A different type of modality training for the skill acquisition may result in greater overlap of the fMRI and VBM results. If this is the case, then there is the possibility of a mediation relationship in the areas of the brain that are specifically related to the motor system. Following the work of Draganski et al. (2004), visual regions may also be involved. This study provides a look at action observation and the effects on functional data. Additionally, this study provides a continuation of the groundwork for establishing the relationship between functional and structural changes as a result of motor skill acquisition. Future work may lead to greater understanding of the basis of these changes and how they are expressed.

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CURRICULUM VITAE

Shira L. Levy graduated from Nova High School, Davie, Florida, in 2002. She received her Bachelor of Science from Columbia University in 2007, and her Bachelor of Arts from the Jewish Theological Seminary in 2007. She received her Master of Arts in Psychology from George Mason University in 2010.