Investigation of Age-Related Changes in Responsiveness of Mirror Neuron Systems using fMRI

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

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ABSTRACT

Observational learning, or the ability to learn a new skill by watching that same skill being performed by others, is one of the fundamental principles of motor learning. It is believed to be driven by a neural network known as the mirror neuron system (MNS), a group of brain regions that show a specialized response to both the observation and performance of motor activities. The MNS is traditionally thought to involve the inferior parietal lobule (IPL), ventral premotor cortex (vPMC), and inferior frontal gyrus (IFG), which are located in brain regions known to atrophy with age. It is not yet known if the responsiveness of the MNS declines or otherwise changes as a result of atrophy caused by natural aging. The current study used functional magnetic resonance imaging (fMRI) to observe the MNS in three increasing age groups with the purpose of determining whether there are any observable differences in MNS activity at different stages of aging.

Methods: Thirty-two participants, in three age groups (Group 1 = 18-40 years, Group 2 = 41-60 years, Group 3 = 61-80 years), were given an observational learning task while undergoing fMRI. fMRI data were analysed using general linear models (GLMs) on an individual and group level. Groups were compared using a two-way repeated measures ANOVA and a cluster threshold estimation with 1000 permutations to determine minimum cluster size to avoid false positives. A cluster threshold of 300 was set to find areas representing the greatest differences in signal change between groups.

Results: Group 2 showed significantly higher activation (percent signal change) than groups 1 and 3 in the IFG, precuneus, and insula, as well as lower activity in the putamen. Group 2 showed higher signal change than Group 1 in the IPL. Group 3 was higher than group 1 in the vPMC and postcentral gyrus.

Discussion: The MNS does not appear to be immune to effects of aging. The changes in IPL and IGF with age, in combination with the observation of more widespread and bilateral brain regions suggest that older participants not only work the motor circuits harder, but also recruit more cognitive brain regions in

order to complete the tasks at the same level of efficiency. Capitalizing on these cognitive compensatory networks may be beneficial in improving on video therapy techniques in the future.

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LIST OF ABBREVIATIONS

3T	3 Tesla
BA	Brodmann Area
BOLD	Blood Oxygen Level Dependent
EEG	Electroencephalography
fMRI	functional Magnetic Resonance Imaging
FOV	Field of View
FWHM	Full-Width Half-Maximum
GLM	General Linear Model
IFG	Inferior Frontal Gyrus
IPL	Inferior Parietal Lobe
MEG	Magnetoencephalography
MFG	Medial Frontal Gyrus
MNS	Mirror Neuron System
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
PMC	Premotor Cortex
ROI	Region of Interest
STG	Superior Temporal Gyrus
TMS	Transcranial Magnetic Stimulation
TR	Repetition Time
TE	Echo Time

INTRODUCTION

Background

As young children become mobile, one way in which they begin to learn basic motor skills, such as manipulating a favourite toy or using utensils on their own, is by watching the actions of others and imitating their movements. This mechanism of motor learning is called observational learning, and is thought to be driven by a neural network called the mirror neuron system (MNS).

While observational learning and the MNS have often been studied in young, healthy adults, it is not yet known if there is any change in the responsiveness of the MNS throughout natural aging. Because the MNS is present in the frontal and parietal lobes, which are known to degenerate with healthy aging (Long, Liao, Jiang, Liang, Qiu, & Zhang, 2012; Resnick, Dzung, Kraut, Zonderman, & Davatzikos, 2003) it is reasonable to expect that there may be changes in the MNS at different stages of the aging process.

Recent therapeutic rehabilitation techniques for stroke patients have reported increased success of rehabilitation when observational learning mechanisms are incorporated (Thieme, Mehrholz, Pohl, Behrens, & Dohle, 2012). This suggests that humans are still utilizing the MNS as older adults, and provides rationale for further investigation into this area of research. A better understanding of the natural degeneration of the MNS throughout the aging process is needed before we can be properly prepared to improve observational learning therapies to promote rehabilitation in aging stroke patients. My proposed research will utilize stimuli similar to those used in successful rehabilitation clinics, presented in a Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) environment to investigate changes in mirror neuron activity at different stages of the aging process.

Research Objectives

Objective: To determine changes in responsiveness of the brain regions involved in the mirror neuron system in participants of different ages using fMRI of the brain during observational learning tasks.

Hypothesis: Because age-related atrophy is common in the parietal lobe, it is hypothesized that a lower amount of activity in the MNS, characterized by a diminished BOLD response, will be observed in Group 3 (age range = 61-80 years) relative to Groups 1 (age range = 18-40 years) and 2 (age range = 41-60 years).

Relevance

By comparing participants of different age groups, the proposed research may contribute to the understanding of the age-related changes in neural responses underlying learning by visualizing alterations in the MNS. This knowledge will lay the ground work for both development and improvement of motor learning and rehabilitation techniques that use observational learning. The project will focus on the behaviour of these brain regions in different age groups, allowing for an understanding of how best to approach motor learning in the older population. This information is important, as older adults not only experience natural motor deficits, which may require learning to use new assistive devices, but they are also in the highest risk category for stroke, which nearly always affects motor abilities and requires extensive motor rehabilitation.

Chronic stroke patients are often unable to complete simple tasks, such as getting dressed or preparing meals, as a result of losing their fine motor function. This frequently requires them to leave their homes and enter residential care facilities. In many cases, stroke patients are also forced to give up hobbies or activities that they once enjoyed, but can no longer perform. This

leads to a decreased quality of life for these patients. Results from this research may lead to the development of new rehabilitation strategies that may aid stroke survivors in regaining their motor function and thus their independence.

LITERATURE REVIEW

Motor Control

In our daily lives we interact with hundreds of different objects and environments from the time we wake up until we go to bed. Most of these interactions are automatic- we don't have to think about using a toothbrush or grabbing a cup of coffee. These behaviours were learned long ago and seem simple. In reality, the neural networks recruited to perform these behaviours are complex and not yet fully understood. Studies of the posterior parietal cortex have shown this area to be involved in the control of motor movements, as it integrates sensory signals from the environment and transforms them into successful motor outputs for arm and hand movements (Batista, Buneo, Snyder & Anderson, 1999; Snyder, Batista & Anderson, 2000). The posterior parietal cortex is positioned in an ideal place to receive both visual and somatosensory input (Schnitzler, 2000) and to send motor information to the frontal cortex (Fogassi & Luppino, 2005). The essential role of the parietal lobe in these behaviours can be seen in some neurodegenerative diseases, such as Corticobasal Syndrome, during which parietal atrophy occurs and severe motor deficits result (Burrell, Hornberger, Vucic, Kiernan, & Hodges, 2014). As healthy adults go through the natural aging process, they also begin to experience some natural degeneration of the parietal lobes (Good, Johnsrude, Ashburner, Friston, & Frackowiack, 2001; Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003). This degeneration is thought to be associated with the general decline in the ability to perform basic motor tasks seen in older populations (Seidler et al., 2011).

Observational Learning and the Mirror Neuron System

Observational learning is one of the fundamental principles of motor learning, defined as the ability to learn a new skill by observing its performance by another person. This type of learning is believed to be driven by a neural network known as the mirror neuron system (MNS).

First discovered in 1992 by a group of researchers working with macaque monkeys (di Pelligrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992), the mirror neuron system is a mechanism by which individuals are able to learn movements by watching others perform those same movements and using the perceived visual information to inform their own motor behaviours. In order to say that a particular response represents mirror system activity, there must be selectivity of brain regions for particular actions and invariance in their responses across the observation and execution of actions (Chang, Cunnington, Williams, Kanwisher, & Mattingley, 2008). In humans, previous fMRI studies have reported involvement of the inferior parietal lobule (Brodmann's areas 39 and 40), the ventral premotor cortex (Brodmann's area 45) and the inferior frontal gyrus (Brodmann's area 44) in the MNS (Gazzola & Keysers, 2009; Iacoboni et al., 1999; Molenberghs, Cunnington, & Mattingley, 2012; Rizzolatti, Fogassi & Gallese, 2002). These brain regions are involved in visuomotor integration and spatial perception, self-awareness and coordination with the sensory system, and motor imagery of hand and arm movements, respectively. Together, these brain regions make up the parieto-frontal circuit, and they allow a person to understand the actions and intentions of others from a first-person perspective, which aids in learning new skills (Rizzolatti & Sinogaglia, 2010). Each of these areas in humans also has analogues in the brains of macaque monkeys, in regions where the mirror neurons were first discovered. In spite of the recent attention to the subject of the mirror neuron system in humans, there is a lack of understanding of the effects of aging on the

MNS. Understanding MNS changes in older adults is important. We do not know whether adults are still able to use this system with the same efficiency as younger populations, because most research has been conducted only in young adults. An additional concern is that certain areas of the brain have been shown to atrophy naturally with age, in particular the parietal lobe which is a part of the neural circuit thought to be involved in the MNS (Long et al., 2012; Pascolo, 2013). Investigation into whether age related degeneration of contributing structures is associated with a decline in our ability to utilize the MNS as we get older, or if the MNS remains robust in spite of these challenges, is still required.

Evidence for the Successful Use of the Mirror Neuron System in Aging Stroke Populations

Recent studies of stroke patients have shown the potential for observational learning and the recruitment of the MNS to improve results in rehabilitation. In 2007, Ertelt et al. were the first to conduct a pilot study of patients using a video therapy technique consisting of a series of video clips that they were instructed to imitate with their affected limb. The experimental group showed a clinically significant improvement compared to the control groups of stroke patients who had undergone the same prior physical therapy (Ertelt, Small, Solodkin, Dettmers, McNamara, Binkofski & Buccino, 2007). Shortly afterwards another study used Transcranial Magnetic Stimulation (TMS) to look at the formation of motor memories in order to confirm the advantage of video therapy, in congruence with physical training, for stroke patients (Celnik, Webster, Glasser, &Cohen, 2008). More recently, Franceschini et al. (2010) tested upper limb functionality in stroke patients who were exposed to rehabilitation treatment that involved observation and imitation of video clips of upper limb activities. They found significant improvement in functionality that remained at a two month post-treatment follow up (Franceschini et al, 2010), and at a 4 month post-treatment follow-up (Franceschini et al, 2012).

Other studies have looked at the success of a more commonly used observational learning technique called mirror therapy, wherein patients place the unaffected arm in front of a mirror and perform simple upper limb movements while envisioning the mirror reflection as their affected second arm. This method also recruits the MNS, and has been shown to be effective when used as a component of rehabilitation (Paik, Kim, Lee, Jeon, 2014; Selles et al., 2014; Thieme et al, 2013; Yun, Chun, Park, & Kim, 2011). It has also been shown to be more effective when designed using tasks of daily living versus simple movement tasks (Paik et al., 2013).

Functional Magnetic Resonance Imaging (fMRI)

Functional magnetic resonance imaging (fMRI) is a non-invasive and indirect method of detecting neural activity in the brain based on changes in blood flow. When a group of neurons are activated in a specific region, there is a resulting increase in metabolism and thus oxygen consumption. In response, the vascular system sends an abundance of oxygenated blood to the region; that is, more oxygenated blood, in fact, than is required. This results in a temporary, localized increase in the ratio of oxygenated to deoxygenated blood. Because oxygenated haemoglobin is diamagnetic and deoxygenated haemoglobin is paramagnetic, this creates a detectable fMRI signal change. This phenomenon is called the Blood Oxygenation Level Dependent (BOLD) effect (Ogawa, Lee, Nayak, & Glynn, 1990).

In order to visualize this signal in an fMRI scan, a series of data point sets are acquired from participants during alternating baseline and experimental conditions, usually while participating in a stimulus-based task. Each data point is representative of a single, three dimensional unit of space, called a voxel. The number and size of voxels that make up the image are decided by the researcher and determine the resolution of the resulting image. A higher number of voxels means the image has more spatial resolution. However, because fMRI

examines a change in signal over time, temporal resolution is also an important factor and a higher spatial resolution requires a longer acquisition time. Because each experiment will have unique requirements, researchers must find a balance between optimal spatial resolution and the acquisition time that will result in the best data for their needs.

Each set of data points makes up one full, three dimensional image of the brain, called a volume, and takes a few seconds to collect. That image will show the relative BOLD signal change at the point in time when it was collected. Each scan is made up of many of these images, collected constantly throughout periods of baseline and stimulus conditions (Huettel, Song, & McCarthy, 2004). By using statistical analysis to compare these images, we are able to visualize the change in blood oxygen levels throughout the scan, and compare them to the time course of the stimuli that were presented. This comparison results in images of the time course of neural activity throughout rest and activity in relevant brain areas (Huettel, Song, & McCarthy, 2004). These images, called activity maps, identify areas of the brain in which signal changes fluctuate with the same time course as the periods of rest and activity used in the experiment. In this way it is possible to see which brain areas respond to specific stimuli (Huettel, Song, & McCarthy, 2004).

Because the BOLD signal is dependent on the relationship between neural activity and cerebral blood flow, also known as neurovascular coupling, it is important to consider factors that may affect the vascular system. This is particularly important in older participants, as even those who are considered healthy may be experiencing clinically silent changes in vascular physiology. Previous studies have shown changes in BOLD responses resulting from age-related alterations in neurovascular coupling (D'Esposito, Deouell, & Gazzaley, 2003). These alterations may be due to changes observed in resting cerebral blood flow (Bentourkia et al., 2000),

atherosclerosis (Groschel et al., 2007), or vascular reactivity (Gauthier et al., 2013; Riecker et al., 2003). Each of these factors impacts neurovascular coupling and thus the BOLD response. Because these factors are so common to the natural aging process, and often go undetected, it would be nearly impossible to avoid including participants who experience these changes from studies. However, when comparing a population of young adults to older adults, these issues must be taken into consideration while viewing results.

Gaps in the Literature

Because the study of mirror neuron systems in humans is a fairly new area of research, there are gaps in the literature with regards to the effects of aging. In particular, there is a need for a study on healthy aging adults to show the functioning of the MNS and in different age groups. One previous study has used fMRI to investigate differences in MNS activity between groups of young and old participants (Nedelko, Hassa, Hamzei, Weiller, & Binkofski, 2010). The group did not find any significant differences in activation of brain regions thought to be involved with mirror neuron activity between the two groups. However, the researchers used only two age groups: a young group ranging from 19-35, and an older group ranging from 44-79. These age ranges, in particular with the older age group, may have been too wide to be sensitive to any difference in function between groups. It has been shown that there is a large age-related variance in structural and functional decline of motor systems (Carmeli, Patish & Coleman, 2003; Long et al., 2012; Sebastjan, Siwek, Koziel, Ignasiak, & Skrzek, 2014), as well as decline in different mechanisms of motor learning (Baugh & Marotta, 2009; Ren et al, 2015; Shea, Park, & Braden, 2013), between a 44 and 79 year old healthy adult. This particular methodology left some questions unanswered, and there is a need for a study which attempts to provide a more detailed insight as to what is happening to the MNS during the natural aging process.

I completed an fMRI study of observational learning in different age groups of healthy adults. The study used stimuli similar to those which are currently used in stroke rehabilitation, with the intention of providing solid evidence towards functionality of the brain regions in the MNS. The results of this study provide insight as to whether there is an age-related change in the responsiveness of the MNS, and which components of the MNS are affected by these changes. This information will contribute to helping future researchers and therapists improve existing patient care by adjusting current video therapy techniques and developing new observational learning-based rehabilitation tools for brain injured patients, based on the understanding of neural function during motor learning at different stages of the aging process.

METHODS

Ethics Approval

All procedures for this study were reviewed and approved by the Research Ethics Boards at the Thunder Bay Regional Health Sciences Centre (TBRHSC) and Lakehead University. See Appendix A for approvals.

Participants

Inclusion Criteria

Healthy, right-handed adults between the ages of 22 and 80, with normal or corrected to normal vision were recruited for this study.

Exclusion Criteria

This is a project based on visualization of brain activity during the learning of hand movements and requires participants to be able to see the projected videos, thus some exclusion criteria applied to participants. These were self-identified in a screening questionnaire conducted

prior to beginning the study. Exclusion criteria included individuals with neurological disease or injury, impairments that limit use of the right hand, any metal implanted within the body, medical conditions that can be worsened by stress, claustrophobia, vision deficits that cannot be corrected to normal, or the possibility of pregnancy. More details may be found in Appendix A.

Because this is an MRI project, additional exclusion criteria related to the risks of entering a magnetic field, were included to ensure the safety of participants. These criteria were self-identified in a screening questionnaire conducted by an MRI technologist prior to the beginning of the study, and include metal contained within the body and claustrophobia.

Recruitment

Participants were recruited from the community of Thunder Bay using snowball sampling procedures and recruitment posters located on the premises of the Thunder Bay Regional Health Sciences Foundation and Lakehead University, as well as an information booth set up in the Thunder Bay 55+ Centre and on the lab website. When a participant contacted the lab to express interest in participating in the project, he or she was provided with a study information package by email. If they expressed interest in person, they were given an information package at that time. Once the potential participant received the study package, they called the office and an appointment was booked for their participation. For a copy of the recruitment poster and study information package, see Appendix A.

Participant Numbers

Participants were recruited into 3 different age groups (18-40 years of age: N=15, 41-60 years of age: N=16, and 61-80 years of age: N=15), for a total of 46 participants. All participants were pre-screened for MRI compatibility and provided written informed consent before

participating. Each participant received $25 compensation following their provision of consent but before beginning the study.

Study Design

Participant Preparation

When a participant arrived for the study, the information package and consent forms were reviewed with them and any questions answered. The participant was then familiarized with the task instructions and equipment and had an opportunity to practise with a sample video and instructions. Each participant's MRI safety screening form was then reviewed by a certified MRI technologist and participants were asked to change into metal free clothing that they were instructed to bring with them, or into the provided hospital gowns. He or she was also offered the opportunity to use the restroom at this time and female participants were made aware of the availability of a pregnancy test should they be unsure of their pregnancy status. Prior to entering the MRI room the technologist checked to ensure that all metal was removed from the participant (e.g., objects such as retainers, hairclips, jewellery, etc.). The MRI Technologist then brought the participant into the scanner room, provided them with earplugs, and positioned them on the scanner bed with their head centred in the head coil. The participant was also informed of the importance of keeping the head still throughout the entire scan. The MRI technologist used memory foam padding to stabilize the participant's head and provided a sheet for warmth, a pillow beneath the legs for comfort, and an emergency squeeze ball, which was held in the participant's left hand. The participant was informed that he or she had a line of communication with the researchers and the MRI technologist through a two way intercom, and that if they needed to get the attention of the research team during a scan acquisition, they should squeeze the emergency ball. The study tasks were presented to the participant using a projection screen

which was viewed by the participant through a mirror box attached to the head coil. Participants followed the instructions on the screen for the duration of the study.

MRI Methods

Data were collected using a 3T Philips Achieva MRI scanner and associated 8-channel SENSE head coil (Philips, Andover, USA). First, localizer and reference scans were performed to locate and centre the brain in the field of view. Next, brain fMRI data were acquired during task performance using conventional BOLD imaging techniques. Whole brain echo-planar images were referenced and acquired along the anterior/posterior commissure to allow for localizing of the brain within the images. Gradient-echo planar images were acquired throughout stimulus presentation (Repetition Time (TR)/Echo Time (TE) = 2000/30 msec, α=90°). Each volume consisted of 30 contiguous slices, 4 mm slice thickness and a 64x64 matrix with 24cm FOV. This resulted in an in-plane resolution of 3.75mm. There were 222 volumes acquired resulting in a scan time of 7.5 minutes per task. Lastly, high resolution, 3-dimensional, T1-weighted gradient-echo anatomical images were acquired to use as the base over which to overlay the functional activity maps created from the functional MRI images. During these final high resolution anatomical scans, as well as the initial localizer scans, participants were verbally instructed to lie still and close their eyes.

fMRI Stimulus Task Design

For this study, two variations of the same experimental paradigm were created so that if there was an issue with compliance or motion in the first fMRI data set, there would be a backup one to ensure usable data could still be collected. Each of these study paradigms was 7.5 minutes long and consisted of three types of presented screens: a black screen with a fixation cross, a black screen with an instructional word, and a video stimulus presentation screen (Figure 1). All

stimuli were presented using Presentation stimulus delivery and experimental control software (Neurobehavioural Systems Inc., Berkeley, California).The stimuli for each paradigm consisted of 15 different everyday activities (Appendix B), such as the opening of a bottle cap or stirring with a spoon, represented by a 4-second silent video of the activity being performed. All study video stimuli were previously designed by our lab, and were created using a white background and containing only the right hand of the actor and the object being manipulated. This design allows minimal distractions to avoid stimulation of brain areas not associated with observational learning. Because no standardized set of stimuli exists for this type of experiment, an occupational therapist at TBRHSC was consulted to determine the types of activities currently being used in stroke rehabilitation. This is in keeping with the methodology of Nedelko et al., who also used stimuli similar to those used in rehabilitation therapy (Nedelko et al., 2010). All activities recorded for this study were simple enough to be carried out with one hand, which prevents complex movements within the constrained space of the MRI scanner.

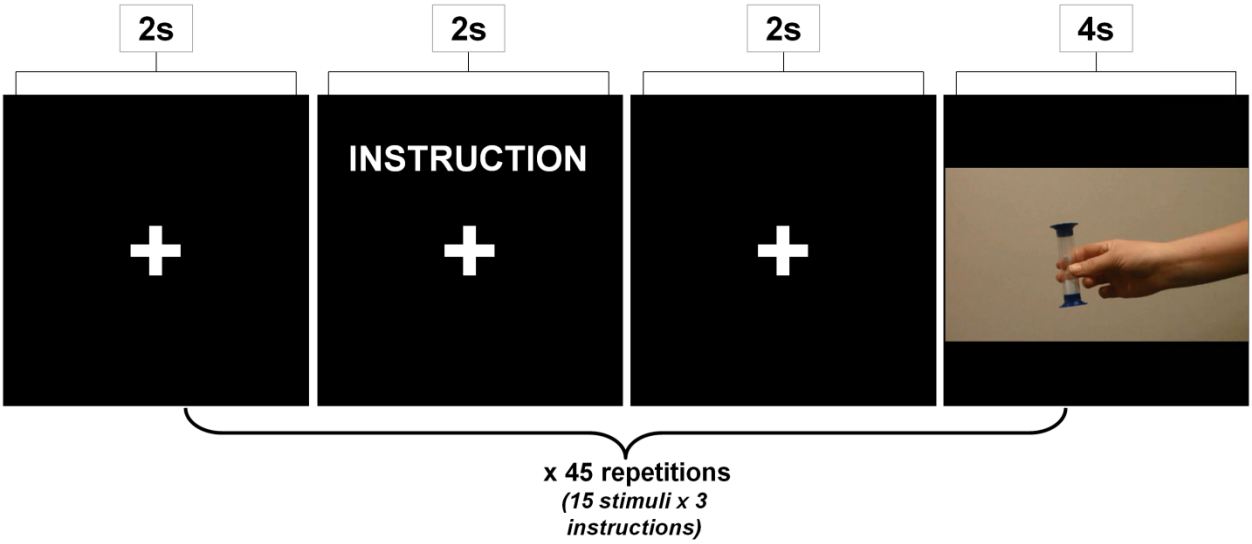


Figure 1. fMRI Paradigm Design. Four second video clips of 15 simple hand-specific activities were presented, each preceded by one of three instructional words: 'Watch', 'Imagine', or 'Do'. Participants interacted with the video clip according to instruction. This block was repeated 3 times for each stimulus - once with each instruction - for a total of 45 repetitions per task.

The background for the non-stimulus screens was dark to prevent contrast from the dark MRI environment, as the contrast may be hard on the eyes of the participant. The three types of screens were presented to the participant in a stimulus-related event design, as described below and as seen in Figure 1.

Two 7.5 minute long paradigms were shown to each participant. For each version, the participant first viewed a fixation screen, followed by an instruction screen with one of three instruction words with which the participant was familiarized before entering the scanner room. These words were "watch", which instructed participants to passively watch the upcoming stimuli, "do", which instructed participants to mimic the presented stimuli with their right hand, and "imagine" which instructed participants to imagine performing the activity in the presented stimuli with their right hand but to not perform the actual movement. After the instruction screen, another fixation was presented to allow the return of the hemodynamic response to

baseline before presenting the stimuli. Then the participants were presented with the video stimulus. This was repeated for the length of the paradigm. Each paradigm contained its own set of 15 stimuli, and the order in which the stimuli and the instructions were presented was randomized within each paradigm. Each paradigm consisted of 45 stimulus presentations- 3 instructional words for each stimulus. Because some participants showed excessive motion or non-compliance during one of the two paradigms, data from only one per participant were selected for final analysis. This was chosen based on compliance with the instructions, or in the case of full compliance for both paradigms, chosen at random.

After completion of the study, participants were asked to complete a form containing information about any comments or complaints they had regarding the study, and a question regarding hobbies or activities that may influence the activity of the mirror neuron system. They also indicated whether they were interested in receiving a summary of the results of the study, or if they would like to be removed from the contact list.

Analysis

Individual Analysis

Data from each participant were pre-processed using Brain Voyager QX Version 2.8.4 software (BrainInnovation, Maastricht, The Netherlands). For each fMRI data set, the first two volumes were discarded and pre-processing performed. Steps for pre-processing included slice scan time correction and high-pass temporal filtering (2 sines/cosines), in addition to 3D Motion correction. All data sets that showed more than 2mm, or half a voxel, of motion were discarded. T1 anatomical images were converted to standard radiological convention and transformed to standardized Talairach space (Talairach & Tournoux, 1988). Each participant's pre-processed fMRI data were co-registered to the corresponding Talairached T1 anatomical images. A design

matrix, describing the timing of stimuli during the fMRI acquisition was created for each data set (Figure 2). General Linear Models were performed for each individual. One data set from each participant was selected for the group analysis based on participant compliance in performing the task accurately, acceptable levels of head motion, and maintaining an approximately equal distribution of data sets between the two paradigms.

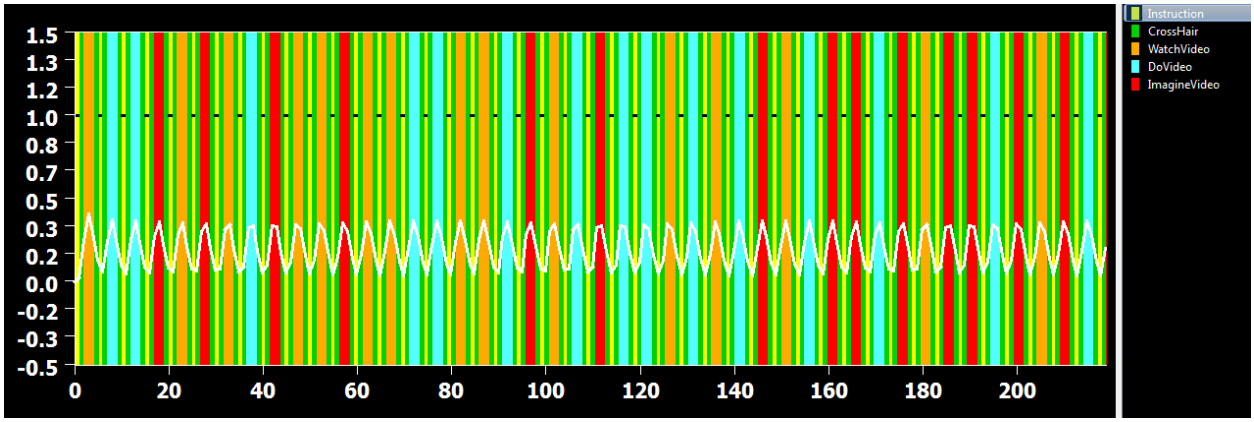


Figure 2. Sample design matrix for Paradigm 1. X-Axis shows the number of volumes collected, and the different coloured bars represent the screen being presented during the specified volume.

Group Analysis

A second level group Random Effects GLM with separate subject predictors was then performed for each of the three age groups. This allows for observation of the active regions within each group prior to carrying out between-group analysis. Next, a 2-Factor ANOVA with repeated measures was performed for each of three comparisons- Group 1 vs. Group 2, Group 1 vs. Group 3, and Group 2 vs. Group 3. A contrast was applied to the results to look only at the brain regions activated during all three stimulus conditions, with the crosshair presentation used as a control. A correction for multiple comparisons was performed at this time, using the Brain Voyager QX Plug-In 'Cluster-Level Statistical Threshold Estimator'. The estimator performs spatial smoothing at a Full Width Half-Maximum (FWHM) of 1.2 functional voxels, and Monte

Carlo simulation with 1000 iterations. This provided the minimum cluster size to be used to avoid false positives. For all three comparisons this number was between 75 and 125. In order to focus on major areas of activity, the cluster threshold was set to 300 voxels, with the statistical threshold at $p<0.01$.

RESULTS

Participants

Forty-six participants were recruited into three different age groups. Group 1 (18-40 years of age) had 15 participants, Group 2 (41-60 years of age), had 16 participants, and Group 3 (61-80 years of age), had 15 participants. Data from 14 participants were removed from the study as a result of technical issues with timing during the task display (n=3), voluntary withdrawal (n=2), head motion greater than 2mm (n=3) and non-compliance with the instructions (i.e. not completing the actions during 'Do' instruction) (n=6). In the end, 32 usable data sets (Group 1=9, Group 2=11, Group 3=12) were analysed. Further participant demographics may be seen in Table 1.

Table 1. Demographics information for participants of all age groups.

Group	**# of participants**	**# of excluded data sets**	**Mean Age of included participants (years)**	**Age (years) Range**	**# of males**
Group 1	15	6	30.55	22-39	5
Group 2	16	5	49.91	41-58	5
Group 3	15	3	70	64-80	6

All participants had normal or corrected to normal vision, reported no history of neurological disease or injury, and were determined to be right handed by a shortened version of the Edinburgh handedness test. All participants also filled out exit questionnaires regarding their hobbies or activities that may influence the activity of the MNS. Results may be found in Appendix C.

Within Groups Results

Group GLM's were run for each of the three age groups to determine whether the MNS was being recruited during the video tasks. The contrast between rest and stimulus conditions for Group 1 (Figure 3) showed activation in the classical MNS regions, including the IPL and PMC. In addition, the sTG and postcentral gyrus were also observed to be active. A list of clusters chosen according to the regions of interest (ROIs) for the MNS, along with their associated statistical significance, can be found in Table 2. A full list of all clusters can be found in Appendix D.

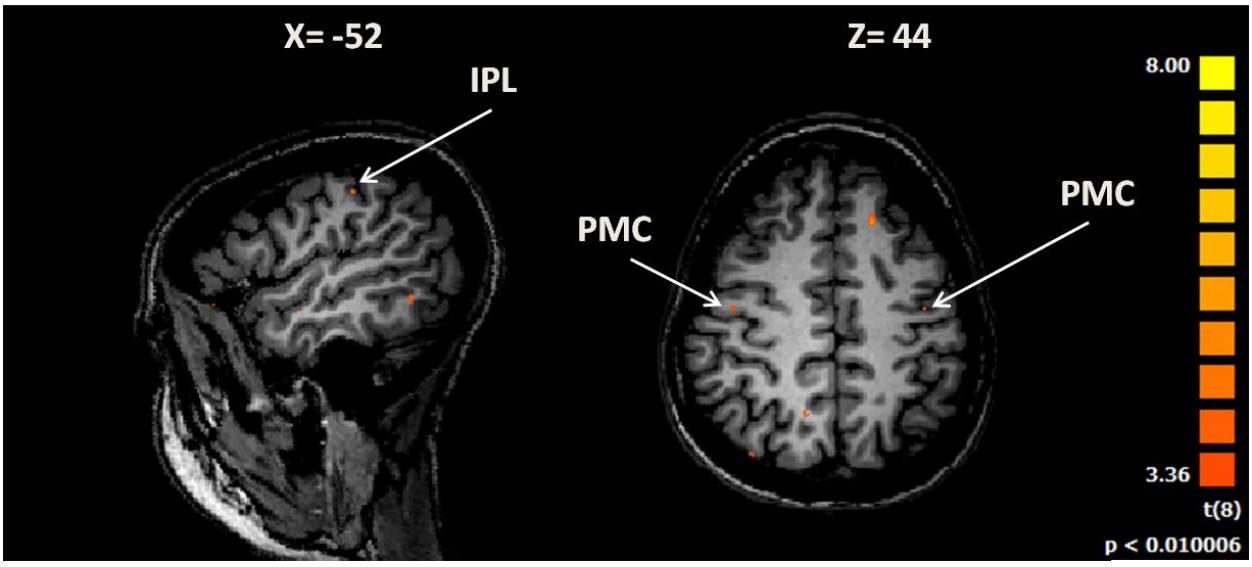


Figure 3. Sagittal (left) and transverse (right) slices showing areas of significant percent signal change in Group 1 at $p<0.01$. For the sagittal slices, negative x-coordinates represent left

hemisphere. Transverse images are displayed in radiological convention (left side of the image represents the right hemisphere of the brain).

Table 2. Brain areas showing activity in Group 1 during observational learning task

Anatomical Region	Hemisphere	x	y	z	t	p
Inferior Parietal Lobule	Left	-48	-31	31	4.657	0.00163
Inferior Frontal Gyrus	Left	-24	29	-2	4.583	0.00179
Premotor Cortex	Left	-18	-16	64	4.083	0.00352
Premotor Cortex	Right	45	-13	43	5.006	0.00104

The same contrast for Group 2 (Figure 4) also showed activation in the IPL, IFG, PMC, sTG and postcentral gyrus. In addition, it is shown that activity for this group is spreading not only ipsilaterally, but throughout both hemispheres. A list of clusters chosen according to the ROIs for the MNS, along with their associated statistical significance, can be found in Table 3. A full list of all clusters can be found in Appendix D.

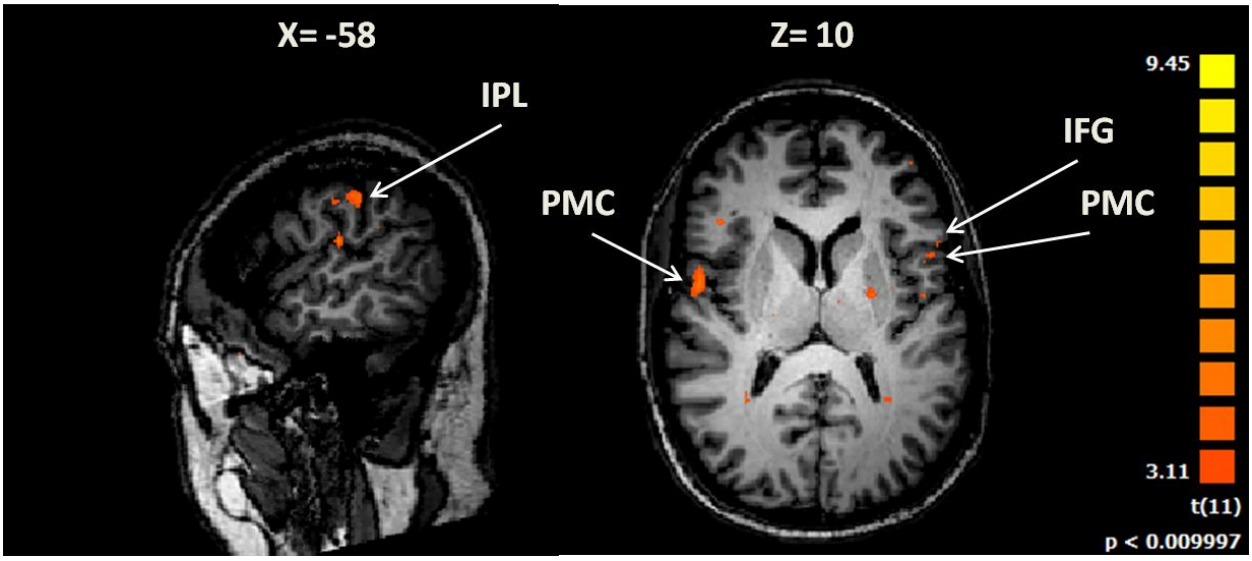


Figure 4. Sagittal (left) and transverse (right) slices showing areas of significant percent signal change in Group 2 at $p<0.01$. For the sagittal slices, negative x-coordinates represent left hemisphere. Transverse images are displayed in radiological convention (left side of the image represents the right hemisphere of the brain).

Table 3. Brain areas showing activity in Group 2 during observational learning task

Anatomical Region	Hemisphere	x	y	z	t	p
Inferior Parietal Lobule	Left	-48	-40	40	6.092	0.000078
Inferior Frontal Gyrus	Left	-51	11	10	3.953	0.002262
Inferior Frontal Gyrus	Right	39	29	1	4.092	0.001784
Premotor Cortex	Left	-33	2	28	4.089	0.001791
Premotor Cortex	Right	54	-1	10	5.220	0.000285

Finally, Group 3 expressed activation in the classical MNS regions of the IPL and PMC (Figure 5). Activity for this group is becoming less widespread. A list of clusters chosen according to the ROIs for the MNS, along with their associated statistical significance, can be found in Table 4. A full list of all clusters can be found in Appendix D

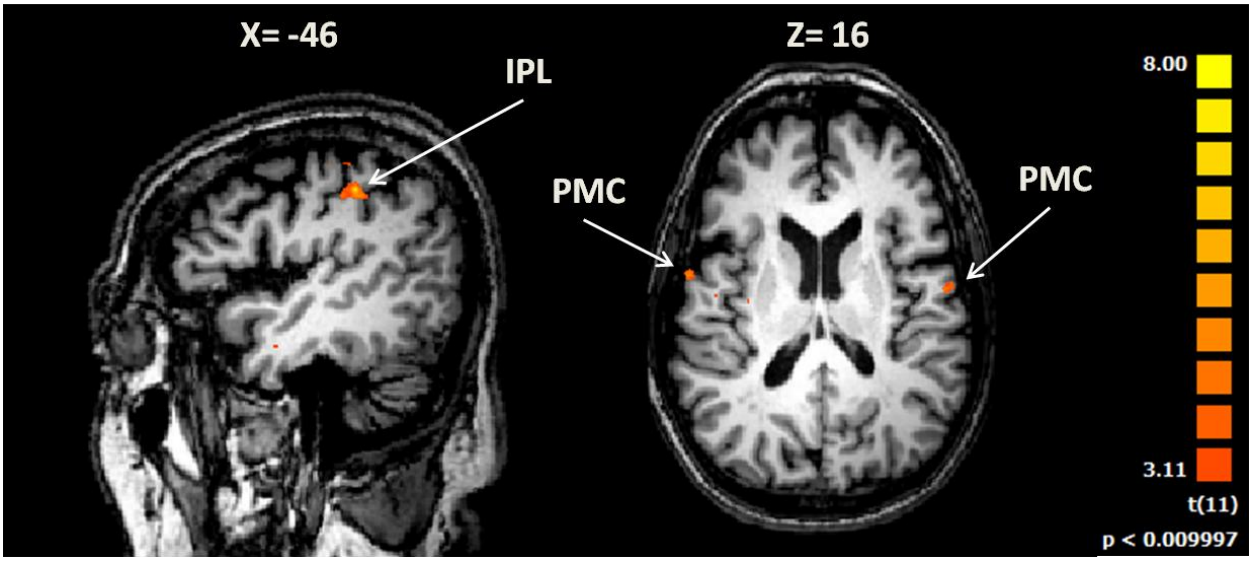


Figure 5. Sagittal (left) and transverse (right) slices showing areas of significant percent signal change in Group 3 at $p<0.01$. For the sagittal slices, negative x-coordinates represent left hemisphere. Transverse images are displayed in radiological convention (left side of the image represents the right hemisphere of the brain).

Table 4. Brain areas showing activity in Group 3 during observational learning task

Anatomical Region	Hemisphere	x	y	z	t	p
Inferior Parietal Lobule	Left	-48	-34	43	6.969	0.000024
Premotor Cortex	Left	-60	-1	13	5.024	0.000387
Premotor Cortex	Right	61	2	16	5.206	0.000292

Between Groups Results

A two-way (screen type, group) ANOVA comparison between Group 1 and Group 2 found several areas in which increased neural activity, determined by observing increases in BOLD signal change, was significantly larger for Group 2. These include the IFG, the IPL, and the STG. Two areas, the insula and putamen were identified as having higher activity than Group 1. Figure 6 shows the ANOVA results for the comparison between Group 1 and Group 2. A complete list of coordinates and associated statistical significance of the clusters displayed in Figure 6 are summarized in Table 2.

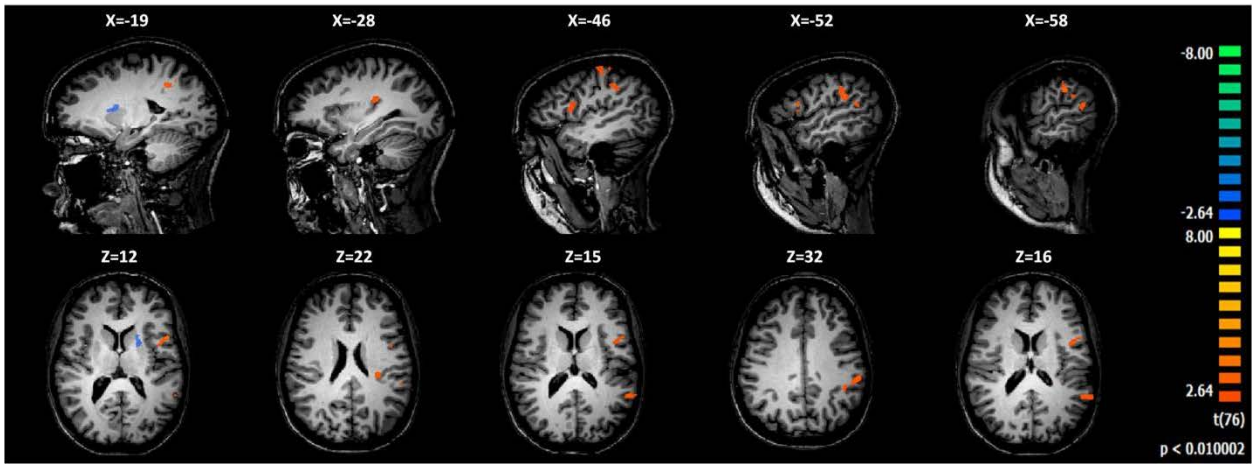


Figure 6. Sagittal (top) and transverse (bottom) slices showing areas differentially activated in Group 2 vs. Group 1 are shown using a two-factor (screen-type, group) repeated measures

ANOVA, with a cluster threshold of 300 at $p<0.01$. Orange colour denotes regions of higher activity for Group 2. For the sagittal slices, negative x-coordinates represent left hemisphere. Transverse images are displayed in radiological convention (left side of the image represents the right hemisphere of the brain).

Table 5. Brain areas differentially activated in Group 2 vs. Group 1. Areas previously associated with the MNS have been italicized.

Anatomical Region	**Hemisphere**	**x**	**y**	**z**	**t**	**p**
Group 1 > Group 2						
Insula	Right	33	8	16	-3.52966	0.00071
Putamen	Left	-21	5	13	-5.17531	0.000002
Group 2 > Group 1						
Precuneus	Left	-9	-58	43	3.972128	0.000161
Sub-gyral Parietal Lobe	Left	-24	-46	25	3.892387	0.000211
Insula (BA13)	Left	-30	-31	21	5.558250	<0.000001
Postcentral Gyrus	Left	-39	-22	40	4.353627	0.000041
Inferior Frontal Gyrus	Left	-51	11	10	5.473997	0.000001
Inferior Parietal Lobule	Left	-54	-40	25	4.952574	0.000004
Middle Occipital Gyrus	Left	-48	-79	7	3.798629	0.000291
Superior Temporal Gyrus	Left	-57	-52	16	3.920676	0.000192

The two-way (screen-type, group) ANOVA comparison between Group 2 and Group 3 revealed areas of higher activity for group 2 in both hemispheres, including the left IFG, bilateral cingulate and thalamic nuclei, and the right STG. The left putamen was shown to have higher activity for group 3, and there was no observable difference between groups for the IPL or premotor cortex. Figure 7 shows the ANOVA results for the comparison between Group 2 and Group 3. A complete list of coordinates and associated statistical significance of the clusters displayed in Figure 7 are summarized in Table 3.

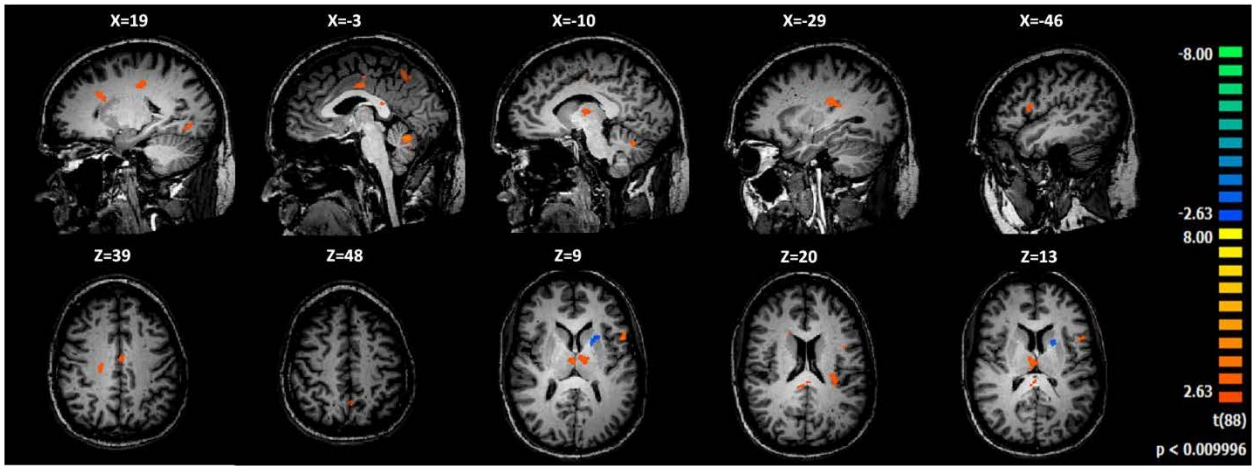


Figure 7. Sagittal (top) and transverse (bottom) slices showing areas differentially activated in Group 2 vs. Group 3 are shown using a two-factor repeated measures ANOVA, with a cluster threshold of 300 at $p<0.01$. Orange colour denotes regions of higher activity for Group 2. For the sagittal slices, negative x-coordinates represent left hemisphere. Transverse images are displayed in radiological convention (left side of the image represents the right hemisphere of the brain).

Table 6. Brain areas differentially activated in Group 2 vs. Group 3. Areas previously associated with the MNS have been italicized

Anatomical Region	**Hemisphere**	**x**	**y**	**z**	**t**	**p**
Group 2 > Group 3						
Superior Temporal Gyrus	Right	45	-22	7	4.14104	0.000079
Lateral ventricle	Right	31	-59	9	3.909853	0.000181
Lingual Gyrus (BA18)	Right	21	-73	-5	3.848353	0.000225
Caudate	Right	18	16	18	4.432560	0.000027
Cingulate Gyrus (BA31)	Right	18	-19	40	4.626436	0.000013
Posterior Cingulate	Right	9	-38	22	4.795129	0.000007
Medial Dorsal Thalamus	Right	3	-13	13	4.63685	0.000012
Precuneus (BA7)	Left	-3	-58	52	3.659664	0.000430
Cingulate Gyrus (BA24)	Left	-6	-7	37	4.56511	0.000016
Fastigium	Left	-3	-61	-20	5.053278	0.000002
Ventral Lateral Thalamus	Left	-6	-7	7	4.368678	0.000034
Inferior Frontal Gyrus (BA44)	Left	-51	11	10	5.372028	0.000001
Sub-gyral frontal lobe	Left	-30	-10	34	4.044993	0.000112
Insula (BA13)	Left	-29	-28	20	4.73855	0.000008
Group 3 > Group 2						
Putamen	Left	-21	9	9	-4.552413	0.000017

The two-way (screen-type, group) ANOVA comparison between Groups 1 and 3 revealed areas of higher activity for Group 1 in the left IFG and caudate, bilateral cingulate, and the right medial frontal gyrus (MFG). Group 3 was shown to have higher activity in the left motor cortex, which includes the Precentral gyrus. Figure 8 shows the ANOVA results for the comparison between Group 1 and Group 3. A complete list of coordinates and associated statistical significance of the clusters displayed in Figure 8 are summarized in Table 4.

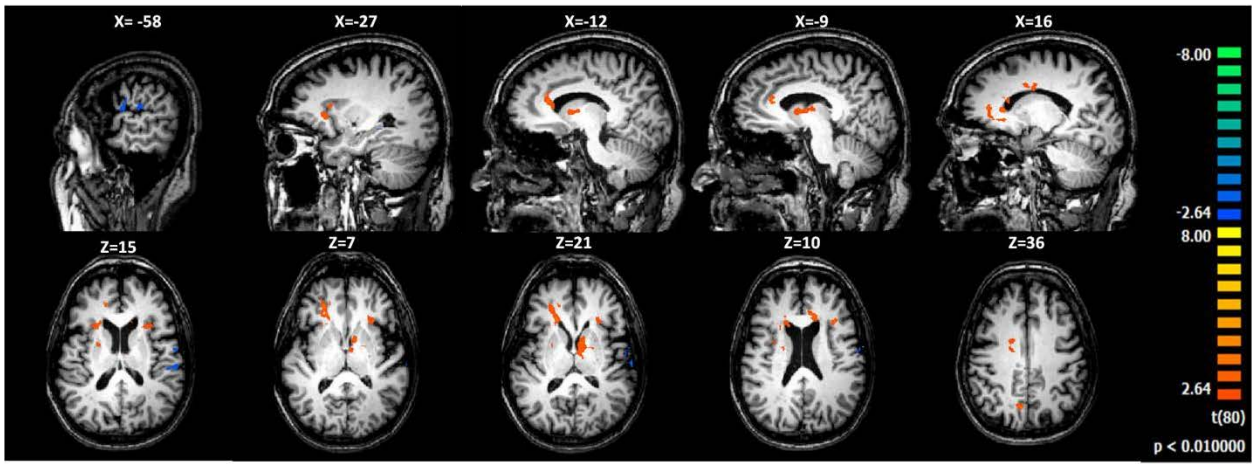


Figure 8. Sagittal (top) and transverse (bottom) slices showing areas differentially activated in Group 1 vs. Group 3 are shown using a two-factor repeated measures ANOVA, with a cluster threshold of 300 at $p<0.01$. Orange colour denotes regions of higher activity for Group 1. For the sagittal slices, negative x-coordinates represent left hemisphere. Transverse images are displayed in radiological convention (left side of the image represents the right hemisphere of the brain).

Table 7. Brain areas differentially activated in Group 1 vs. Group 3. Areas previously associated with the MNS have been italicized

Anatomical Region	Hemisphere	x	y	z	t	p
Group 1 > Group 3						
Putamen	Right	23	0	12	3.802456	0.000279
Medial Frontal Gyrus	Right	25	44	7	4.535239	0.000020
Medial Frontal Gyrus	Right	15	44	13	5.151133	0.000002
Cingulate Gyrus	Right	18	-4	34	4.86044	0.000006
Precuneus (BA7)	Right	6	-67	37	4.962618	0.000004
Caudate Body	Left	-9	5	10	4.813698	0.000007
Anterior Cingulate	Left	-12	32	22	5.848313	<0.000001
Sub-Gyral Frontal Lobe	Left	-33	23	19	4.788465	0.000008
Inferior Frontal Gyrus	Left	-30	25	5	5.420102	0.000001
Group 3 > Group 1						
Midbrain	Right	11	-26	-18	-5.047462	0.000003
Postcentral Gyrus	Left	-57	-22	19	-4.09543	0.0001
Precentral Gyrus (BA6)	Left	-60	-4	13	-5.05638	0.000003

DISCUSSION

This thesis was conducted with the purpose of determining whether there were any differences in responsiveness of the MNS in three age groups. The original hypothesis was that there would be lower neural activity of the MNS, characterized by a diminished BOLD response in Group 3 vs. Groups 1 and 2. Our results partially supported this hypothesis. While there was not a linear decline of activity over the age groups, there were differences seen between groups, with lower activity in Group 3 than Group 1. The results of this study have provided information that can be extrapolated to contribute to current and future observational-learning based rehabilitation tools for brain-injured patients.

Patterns of Activation

The first major finding was that there were no brain regions associated with the MNS that showed a consistent decline throughout all three age groups, although I had hypothesized that there would be. Instead, there was an increase in activity for many brain regions in Group 2, and a decrease into Group 3. This result is not, however, entirely unprecedented. While studies of brain structure throughout aging have shown that the parietal and frontal lobes show negative, approximately linear, changes in volume associated with age (Long et al, 2012; Resnick et al, 2003; Good et al, 2001), other papers looking at change in the BOLD response throughout aging have revealed a different pattern that some brain regions tend to follow. This pattern has been described as an inverted quadratic, or an 'inverted U', meaning that the BOLD response becomes stronger and more widespread with age until it reaches a peak, around 45 years of age (Cao et al, 2014). From there, the level of observable activity decreases. This phenomenon has been thought to occur as a result of compensation for natural atrophy (Heuninckx, Wenderoth, & Swinnen, 2008), as the aging brain experiences structural decline that affects the difficulty associated with performing a task. This results in an increase in both strength and spread of neural activity as cognitive strategies and excess sensory processing mechanisms are recruited to help to accomplish those goals, and an associated spread of the BOLD response to the prefrontal cortex, basal ganglia, and supplementary motor areas, which is observable with the various brain imaging techniques (Heuninckx et al, 2005; Naccarato, Calautti, Jones, Carpenter, & Baron, 2006, Seidler et al, 2010).

Behavioural evidence has also supported the idea of widespread recruitment for compensation, as older adults who perform poorly on motor tasks show activity levels similar to those of younger participants, whereas high-performing older adults show much more

widespread, even bilateral activation (Cabeza, Anderson, Locantore, & McIntosh, 2002; Heuninckx et al, 2008;). However, these regions that are recruited as compensation are also some of the most highly vulnerable to age-related atrophy (Long et al, 2012; Resnick et al, 2003). Therefore, there reaches a time when they can no longer be recruited, and strength and spread of activation begins to decrease once again, completing the inverted-U pattern. The IFG, vPMC, IPL, and precuneus are all brain regions associated with the MNS that have been shown to express this change in BOLD response previously (Cao et al, 2014). In looking at the within groups results, it can be seen that these brain regions follow the same pattern in the current study as well.

Our findings suggest that this phenomenon is relevant for brain regions involved in the MNS. One group of brain regions showed a change in responsiveness between age groups that followed this 'inverted U' pattern, meaning that activity was highest for Group 2, and declined into Group 3. The regions that followed this pattern in our study were the left IFG, the left precuneus, and the left insula, all areas which have been consistently shown to be involved in the MNS (Molenberghs et al, 2011). The IFG and precuneus have both been observed to follow this pattern previously, though the insula has not. In fact it was previously described as decreasing linearly in responsiveness (Cao et al, 2014) as well as structure (Long et al, 2012). The finding of this inverted-U pattern of activity change could explain why the previous study by Nedelko et al (2010) did not find any significant differences in these brain regions between their age groups. The division of participants into two age groups split the middle age group in half, and these changes would have been hidden in the group average.

There are also three brain regions in the left hemisphere that show an increase in activity from Group 1 to Group 2, but do not decline into Group 3: the postcentral gyrus, IPL, and

superior temporal gyrus (STG). The suspected cause for this is that the spread of activation that is seen beginning in Group 2 is still necessary for those in Group 3. This activation tends to occur in regions that are more involved in cognition (e.g., IPL, STG) and sensory processing (e.g., postcentral gyrus), which is what older adults are recruiting to compensate for a decline in motor abilities. There are also regions that begin to show activity in the right hemisphere with Group 3 especially. These include the STG and lingual gyrus, which play roles in cognition, and the caudate and thalamus, which are part of the system that is responsible for voluntary movement. Bilateral spread of activation for motor tasks has been documented in aging populations previously (Naccarato et al, 2006). This thesis provides further evidence to support the idea of the spread of neural activity in aging populations.

Behaviour of the Mirror Neuron System

Within group analyses for this study showed that there was activity in the MNS for each age group, which shows that the tasks used in this study were effective in recruiting the observational learning mechanism. The three classical MNS components are the IFG, the IPL and the vPMC. The IFG has already been discussed, and moving forward the behaviour of the vPMC and the IPL in the current study will be explored.

The vPMC is a part of the primary motor cortex, and is involved in motor planning (Schubotz & von Cramon, 2001) and execution (Stephan et al, 1995), as well as imagined movements (Stephan et al, 1995; Gerardin et al, 2000). In the current study, it was shown that there were no significant changes in the PMC between Group 1 and Group 2, or between Group 2 and Group 3. There was however, a significantly higher level of activity in Group 3 than Group 1. This suggests that while the change may occur slowly, it is indeed happening. The explanation for the fact that responsiveness is higher in the vPMC for Group 3 points to

compensation. It has previously been observed that activity in the motor cortex becomes more widespread (Seidler et al, 2010; Naccarato et al, 2006) and the percent signal change in the BOLD signal more significant (Heuninckx et al, 2008) as an increased difficulty in performing the task leads to compensatory mechanisms to accomplish the same goals.

The last classical MNS component is the IPL. The IPL only shows a significant change from Group 1 to Group 2, though interestingly there is no significant difference between Groups 1 and 3. Even when the minimum cluster threshold was lowered to 100 voxels, there were no significant differences revealed between Group 1 and 3, or Group 2 and 3. This suggests that this region may undergo the same 'inverted U' pattern of change in responsiveness as the IFG, insula and precuneus, albeit with less of a decline into older age. This would agree with Cao et al's (2014) findings that this pattern of BOLD response changed in the IPL with 126 participants. While this cannot be proven within the scope of this study, it is something to be considered when moving forward with future work. With a larger sample size it may be possible to examine this finding in more detail.

Vascular Changes with Age

The results from Group 3 especially need to be considered with a critical eye. Aging comes with a variety of side effects that can affect the way fMRI data are collected. More specifically, the effects of aging on the vascular system can cause a lower signal intensity in older adults during motor tasks (D'Esposito et al, 2003; Hesselmann et al, 2001), and can additionally create a small lag in the time to peak BOLD response, which could also affect the recorded signal intensities during the stimulus condition (Taoka et al, 1998, Kannurpatti, Motes, Rypma, & Biswal, 2010). If this were the case for Group 3 in this study, it may be suggested that perhaps the decrease in activity from Group 2 to Group 3 is not due to changes in functional

ability, but is rather a result of vascular differences. In this case, if we were able to control for these differences, Group 3 may have had activity levels similar to those of Group 2. This would fit with previous observations that older adults tend to show more activity when they are performing at a level equivalent to a younger group (Heuninckx et al, 2008, Naccarato et al, 2006). It may also explain the 'inverted U' pattern that is often seen in fMRI studies of aging, as these changes in vasculature were not considered in the studies showing this pattern.

Implications

The impact of these findings for future rehabilitation therapies is important. Not only are we seeing a more significant BOLD response, and thus more activity, in typical MNS locations, but also in more widespread cognitive systems. One possible explanation for this is that older adults may be recruiting cognitive strategies to compensate for the decline in functional motor abilities. These regions of the brain are working harder for those in Group 2, as well as Group 3, than those in Group 1. Thus, if video therapies can be designed to specifically target more cognitive processes involved with motor learning, it should be easier for older adults to strengthen the connections they need to complete the tasks. Having a strictly motor-based task is more problematic, as this is where older adults may experience problems. This may be why video therapy shows an increased improvement in rehabilitation over traditional therapies. It is possible that video therapy may by nature recruit more cognitive processes, as patients will be recognizing the tools, recalling what they are used for and how they have used them previously, incorporating information about the environment and using mental imagery as well as motor skills. Traditional movement therapy depends more on straightforward motor task completion, with tasks such as pouring water from one cup to another or building a stack of pennies. What can be taken from this thesis is that using video therapy to strengthen these cognitive networks

associated with motor learning can help the brain to be prepared for the carrying out of the learned task. Incorporating more cognitive components into the therapy for older patients may help them build up the necessary compensation to help make up for damage (i.e., due to aging or disease) to the motor areas. This is an important finding additionally because the region most commonly affected by stroke is the left parietal lobe. Many of the regions being recruited from the bilateral frontal and contralateral parietal lobes are also typically left uninjured following stroke. Thus these areas are likely to remain intact following stroke, and will be functional and accessible during observational learning therapies.

Strengths and Limitations

There are a number of strengths in the design of this study. Firstly, the use of fMRI in this study allows for the collection of information about the MNS that cannot be achieved using other imaging modalities. Using fMRI gives the ability to look at neural activity with very high spatial resolution, on the order of millimetres. In comparison, electroencephalography (EEG) and magnetoencephalography (MEG) detect brain activity on the order of centimetres (Huettel, Song, & McCarthy, 2004). In addition, fMRI has the capability to detect signals throughout the whole brain, and can record activity in deep brain structures that EEG and MEG cannot reach. While fMRI and PET are comparable in terms of spatial resolution, PET has a temporal resolution of tens of seconds or minutes, while fMRI has a temporal resolution of seconds (Huettel, Song, & McCarthy, 2004). In addition, fMRI is also a non-invasive mechanism of detecting neural activity, while PET makes use of exogenous radioactive detector molecules injected into the participant.

The level of detail provided by fMRI allows for good insight as to what is happening physiologically during observational learning in an aging population. Observational learning has

been observed in behavioural studies both with neurologically healthy and brain injured participants, but as of now, the involvement of the MNS is in these learning behaviours is only hypothetical. To be able to use fMRI to confirm involvement of the MNS in observational learning tasks that are effective in rehabilitation settings will be of great value in improving upon these existing techniques in a clinical setting. In addition, the current study design is comparable to the previous study by Nedelko et al (2010) of the same question and has addressed some of the limitations of that study by expanding upon the number of age groups in order to focus more specifically on different points in the aging process.

The recruitment of participants across the age range was a strength of the study. There was a very even distribution of age across each group, which resulted in within-group average ages very close to the middle of each age group. Therefore, participants were not skewed towards one end of the age range, which could have affected the results when comparing between the groups.

Lastly, the involvement of occupational therapists to provide insight as to the types of stimuli currently showing success in rehabilitation clinics allowed for the development of a study with real world application. Not only does this study show the ability of current stimuli to activate mechanisms of observational learning at different ages, it also provides a foundation for further studies testing these stimuli on brain-injured participants.

There are some limitations inherent to this study. In particular, the use of the cross-sectional design, while necessary for timing purposes, is not the preferred method of studying age-related changes. In the future, using a longitudinal study to follow one population throughout the aging process would be ideal. Similarly, it would be interesting to use a regression to look at age as a continuous variable. While fMRI analysis is typically done in groups to increase the

functional signal to noise ratio, future studies may be able to use a region of interest analysis to run a regression. This type of analysis would allow us to focus on one specific brain region at a time and see how activity levels within that one area change relative to our paradigm. This could offer a different perspective on the patterns of change. Additionally, an interesting future study could be designed to look at the change in MNS longitudinally within individual participants. This type of study would allow for control over intersubject variability. While this may be difficult to do within a singular lab, collaboration may help make this a feasible plan. A second potential limitation is the effect of vascular changes related to aging on fMRI data. As discussed earlier, alterations to neurovascular coupling can affect the BOLD signal and skew our results. An attempt was made to control for this by excluding participants who have uncontrolled high blood pressure or other vascular disease. However, vasculature changes are a natural part of aging, and thus had to be accepted as an inherent part of the data for older participants. It is not possible to tell at this time whether any of the current results were affected by vascular changes, and we rely on the literature to give suggestions as to what the data would look like if this was an issue. In the future, a breath-holding task, such as that used by Handwerker et al (2007) to reduce variability due to vascular changes (Handwerker, Gazzaley, Inglis, & D'Esposito, 2007), should be used to collect the necessary data to correct for this issue.

Compliance with task instructions was an issue for Group 1. The younger participants tended to claim that they were compliant and making very small movements with their hands. However, in looking at the fMRI data for some of the participants, it was clear that they were not doing as instructed. Because of the nature of the task, it would be expected to see activity in the primary motor cortex during, at least, the 'Do' condition. Inspection of the data for some participants showed this to not be the case. Non-compliance may have been a result of boredom

on the part of the participant. One way for us to be able to ensure that participants are compliant in the future is to have them practise the motions outside of the scanner before entering and beginning the task, so they know to make larger, more visible movements that can be monitored easily from outside of the scanner room. Another solution may be to move the MRI compatible video camera to a location where it can more clearly show the hand and its movements.

Although participants were recruited from the general population of healthy adults in Thunder Bay, the number of exclusion criteria involved in MRI studies causes the loss of a subset of the population who, while neurologically healthy, have had any kind of orthopaedic or cardiac surgery. Thus, while this study obtained data from a sample of the completely healthy population, the population remains skewed by excluding those participants who do not meet the criteria for MRI safety. Therefore these results may be generalized to the population of neurologically and physically healthy adults only.

Finally, it is important to address sample size. While significance is seen both within and between groups in areas that are in line with what is seen in the literature, and I am confident that what was found is relevant, it is possible that future expansion with more participants may be able to provide more detailed information. In particular, it was expected based on literature that there would be more activity in the IFG for all groups than was seen in the current study. The insula also behaved in a way that was unexpected based on literature. Perhaps with a higher sample size more details about the behaviour of these and other brain regions could be pulled out.

REFERENCES

Batista, A., Buneo, C., Snyder, L., & Andersen, R. (1999). Reach plans in eye-centered coordinates. *Science, 285,* 257-260.

Baugh, L., & Marotta, J. (2009). When what's left is right: Visuomotor transformations in an aged population. *PLoSOne, 4(5),* 1-8.

Bentourkia, M., Bol, A., Ivanoiu, A., Labar, D., Sibomana, M., Coppens, A... De Volder, A. (2000). Comparison of regional cerebral blood flow and glucose metabolism in the normal brain: effect of aging. *Journal of the Neurological Sciences, 181(1-2),* 19-28.

Burrell, J., Hornberger, M., Vucic, S., Kiernan, M., & Hodges, J. (2014). Apraxia and motor dysfunction in Corticobasal Syndrome. *PLoS ONE 9(3)*, 1-12.

Cabeza, R., Anderson, N., Locantore, J., & McIntosh, A. (2002). Aging gracefully: Compensatory brain activity in high-performing older adults. *NeuroImage, 17(3)*, 1394-1402.

Cao, M., Wang, J., Dai, Z., Cao, X., Jiang, L., Fan, F., ... He, Y. (2014). Topological organization of the human brain functional connectome across lifespan. *Developmental Cognitive Neuroscience, 7,* 76-93.

Carmeli, E., Patish, H., & Coleman, R. (2003). The aging hand. *Journal of Gerontology, 58(2),* 146-152.

Celnik, P., Webster, B., Glasser, D., & Cohen, L. (2008). Effects of action observation on physical training after stroke. *Stroke, 39(6),* 1814-1820

Chang, T., Cunnington, R., Williams, M., Kanwisher, N., & Mattingley, J. (2008). fMRI adaptation reveals mirror neurons in human inferior parietal cortex. *Current Biology, 18,* 1576–1580.

D'Esposito, M., Deouell, L., & Gazzaley, A. (2003). Alterations in the BOLD fMRI signal with aging and disease: A challenge for neuroimaging. *Nature Reviews*, *4*, 863-872.

di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., & Rizzolatti, G. (1992). Understanding motor events: a neurophysiological study. *Experimental Brain Research, 91(1),* 176-180.

Ertelt, D., Buccino, G., Small, S., Solodkin, A., McNamara, A., & Binkofski, F. (2007). Movement observation has a positive impact on rehabilitation of motor deficits after stroke. *NeuroImage, 36(Suppl 2),* 164-73.

Fogassi, L., & Luppino, G. (2005). Motor functions of the parietal lobe. *Current Opinions in Neurobiology, 15*, 626-631.

Franceschini, M., Agosti, M., Cantagallo, A., Sale, P., Mancuso, M., & Buccino, G. (2010). Mirror neurons: action observation as a tool in stroke rehabilitation. *Journal of Physical and Rehabilitation Medicine, 46,* 517-523.

Franceschini, M., Ceravolo, M. G., Agosti, M., Cavallini, P., Bonassi, S., Dall'Armi, V., ... Sale, P. (2012). Clinical relevance of action observation in upper-limb stroke rehabilitation: A possible role in recovery of functional dexterity. A randomized clinical trial. *Neurorehabilitation & Neural Repair, 26(5),* 456-462.

Gazzola, V, & Keysers, C. (2009). The observation and execution of actions share motor and somatosensory voxels in all tested subjects: Single subject analyses of unsmoothed fMRI data. *Cerebral Cortex 19,* 1239-1255.

Gauthier, C., Madjar, C., Desjardins-Crepeau, L., Bellec, P., Bherer, L., & Hoge, R. (2013). Age dependence of hemodynamic response characteristics in human functional magnetic resonance imaging. *Neurobiology of Aging, 34,* 1469-1485.

Gerardin, E., Sirigu, A., Lehéricy, S., Poline, J., Gaymard, B., Marsault, C., Agid, Y., & Bihan, D. (2000). Partially overlapping neural networks for real and imagined hand movements. *Cerebral Cortex, 10(11),* 1093-1104.

Good, C., Johnsrude, I., Ashburner, J., Friston, K., & Frackowiak, R. (2001). A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage 14,* 21-36.

Groschel, K., Terborg, C., Schnaudigel, S., Ringer, T., Riecker, A., Witte, W., & Kastrup, A. (2007). Effects of physiological aging and cerebral risk factors on the hemodynamic response to brain activation: A functional transcranial Doppler study. *European Journal of Neurology, 14,* 125-131.

Handwerker, D., Gazzaley, A., Inglis, B., & D'Esposito, M. (2007) Reducing vascular variability of fMRI data across aging populations using a breathholding task. *Human Brain Mapping, 28(9)*, 846-859.

Hesselmann, V., Weber, O., Wedekind, C.,Krings, T., Schulte, O., Kugel, H., … Lackner, K. (2001). Age-related signal decrease in functional magnetic resonance imaging during motor stimulation in humans. *Neuroscience Letters, 308(3),* 141-144.

Heuninckx, S., Wenderoth, N, & Swinnen, S. (2008). Systems neuroplasticity in the aging brain: Recruiting additional neural resources for successful motor performance in elderly persons. *The Journal of Neuroscience, 28(1),* 91-99.

Heuninckx, S., Wenderoth, N., Debaere, F., Peeters, R., & Swinnen, S. (2005). Neural basis of aging: The penetration of cognition in action control. *Journal of Neuroscience 25(29),* 6787-6796.

Huettel, S., Song, A., & McCarthy, G. (2009). *Functional magnetic resonance imaging (2 ed.)*, Boston: Sinauer.

Iacoboni, M., Woods, R.P., Brass, M., Bekkering, H., Mazziotta, J.C., et al. (1999). Cortical mechanisms of human imitation. *Science, 286*, 2526-2528.

Kannurpatti, S., Motes, M., Rypma, B., & Biswal, B. (2010). Neural and vascular variability and the fMRI-BOLD response in normal aging. Magnetic *Resonance Imaging, 28(4),* 466-476.

Long, X., Liao, W., Jiang, C., Liang, D., Qiu, B., & Zhang, L. (2012). Healthy aging: An automatic analysis of global and regional morphological alterations of human brain. *Academic Radiology, 19(7)*, 785-793.

Molenberghs, P., Cunnington, R., & Mattingley, J. (2012). Brain regions with mirror properties: A meta-analysis of 125 human fMRI studies. *Neuroscience Behavioural Reviews, 36(1),* 341-349.

Naccarato, M., Calautti, C., Jones, P., Carpenter, T., & Baron, J. (2006). Does healthy aging affect the hemispheric activation balance during paced index-to-thumb opposition task? An fMRI study. *NeuroImage, 32(3),* 1250-1256.

Nedelko,V., Hassa, T., Hamzei, F., Weiller, C., & Binkofski, F. (2010). Age-independent activation in areas of the mirror neuron system during action observation and action imagery. An fMRI study. *Restorative Neurology and Neuroscience, 28,* 737-747.

Ogawa, S., Lee, T.M., Nayak, A.S., & Glynn, P. (1990). Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magnetic Resonance in Medicine, 14*, 68-78.

Paik, Y., Kim, S., Lee, J., & Jeon, B. (2014). Simple and task-oriented mirror therapy for upper extremity function in stroke patients: A pilot study. *Hong Kong Journal of Occupational Therapy, 24(1),* 6-12.

Pascolo, P.B. (2013). The mirror neuron system simply. An hypothesis? *Biomedical Sciences Instrumentation, 49,* 251-258.

Ren, J., Huang, S., Zhang, J., Zhu, Q., Wilson, A. D., Snapp-Childs, W., & Bingham, G. P. (2015). The 50s cliff: A decline in perceptuo-motor learning, not a deficit in visual motion perception. *Plos ONE, 10(4),* 1-17.

Resnick, S., Dzung, P., Kraut, M., Zonderman, A., & Davatzikos, C. (2003). Longitudinal magnetic resonance imaging studies of older adults: A shrinking brain. *The Journal of Neuroscience, 23(8)* 3295-3301.

Riecker, A., Grodd, W., Klose, U., Schulz, J., Groschel, K., Erb, M., … Kastrup, A. (2003). Relation between regional functional MRI activation and vascular reactivity to carbon dioxide during normal aging. *Journal of Cerebral Blood Flow & Metabolism, 23,* 565-573.

Rizzolatti, G., Fogassi, L., & Gallese, V. (2002). Motor and cognitive functions of the ventral premotor cortex. *Current Opinion in Neurobiology, 12,* 149–154.

Rizzolatti, G., & Sinigaglia, C. (2010). The functional role of the parieto-frontal mirror circuit: Interpretations and misinterpretations. *Nature Reviews, 11 (4),* 264-74.

Schnitzler, A. (2000). Neurophysiology and functional neuroimaging of the somatosensory system. *Journal of Clinical Neurophysiology, 17,* 537-538.

Schubotz, R., & von Cramon, Y. (2001). Functional organization of the lateral premotor cortex: fMRI reveals different regions activated by anticipation of object properties, location and speed. *Cognitive Brain Research, 11(1)*, 97-112.

Sebastjan, A., Siwek, K., Koziel, S., Ignasiak, Z., & Skrzek, A. (2014). Age and sex variation in the results of the 2Hand Test in an adult population. *Human Movement, 15(1),* 21-24.

Seidler, R., Bernard, J., Burutolu, T., Fling, B., Gordon, M., Gwin., … Lipps, D. (2010). Motor control and aging: Links to age-related brain structural, functional, and biochemical effects. *Neuroscience and Biobehavioural Reviews,* 34(5), 721-733.

Selles, R., Michielsen, M., Bussmann, J., Stam, H., Hurkmans, H., Heijnen, I., ... Ribbers, G. (2014). Effects of a mirror induced visual illusion on a reaching task in stroke patients: Implications for mirror therapy training. *Neurorehabilitation and Neural Repair, 28(7),* 652-659.

Shea, C., Park, J., & Braden, H. (2013). Age-related effects in sequential motor learning. *Journal of the American Physical Therapy Association, 86(4),* 478-488.

Snyder, L., Batista, A., & Andersen, R. (2000). Intention-related activity in the posterior parietal cortex: A review. *Vision Research, 40(10-12)*, 1433-1441.

Stephan, K., Fink, G., Passingham, R., Silbersweig, D., Ceballos-Baumann, A., Frith, C., & Frackowiak, R. (1995). Functional anatomy of the mental representation of upper extremity movements in healthy subjects. *Journal of Neurophysiology, 73(1),* 373-386.

Talairach, J., & Tournoux, P. (1988). *Co-planar stereotaxic atlas of the human brain.* New York: Thieme.

Taoka, T., Iwasaki, S., Uchida, H., Fukusumi, A., Nakagawa, H., Kichikawa, K., ... Ohishi, H. (1998). Age correlation of the time lag in signal change on EPI-fMRI. *Journal of Computer Assisted Tomography, 22(4)*, 514-517.

Thieme, H., Mehrholz, J., Pohl, M., Behrens, J., & Dohle, C. (2013). Mirror therapy for improving motor function after stroke. *Stroke*, 44(1), e1-2.

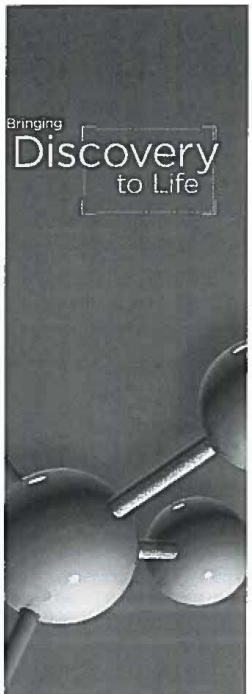
Yun, G., Chun, M., Park, J., & Kim, B. (2011). The synergic effects of mirror therapy and neuromuscular electrical stimulation for hand function in stroke patients. *Annals of Rehabilitation Medicine*, 35(3), 316-321.

Appendix A. Study Documentation

i. Participant information package and pre-screening forms

Appendix 3 – MRI Study Information Package

Date



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Dear Prospective Participant:

You are receiving this information package because you have contacted our lab at the Thunder Bay Regional Research Institute and are invited to participate in our study called **Investigation of the neural mechanisms underlying changes in motor learning in healthy aging**. This study will be performed at the Thunder Bay Regional Health Sciences Centre using the research dedicated MRI system.

Please find the following documents enclosed:

- Study Information Sheet
- MRI Information Sheet
- Prescreening Form
- Consent Form
- Map with directions to the study location

Please:

1. Read the enclosed material. It contains information that we hope will answer any questions you may have about this study. Please ask me to explain anything that you do not clearly understand. If you have further questions, please feel free to contact me.

2. Carefully review the list of medical conditions that might exclude you from this study. This is for your safety. If you have any of the exclusion criteria, you should not enter the study. If you have any questions or concerns, please contact me.

3. Please allow yourself at least 24 hours after reading the information in this package before scheduling an appointment. When you wish to participate in this study, **please call me to arrange a date and time.** At that time I will review a pre screening form to confirm you are eligible for the study.

Thank you for your interest in our research study.

Sincerely,

Jane Lawrence Dewar, PhD
Scientist, Thunder Bay Regional Research Institute

Phone: 684-7289
Email: dewarja@tbh.net
Website: lawrencedewarlab.com

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Appendix 3 – MRI Study Information Package

RESEARCH SUMMARY AND INFORMED CONSENT

Title of Research Project:
Investigation of the neural mechanisms underlying changes in motor learning in healthy aging

Researcher involved:

Jane Lawrence-Dewar, Principal Investigator (807) 684-7289

This document, reviewed and approved by the Thunder Bay Regional Health Sciences Centre Research Ethics Board, contains information regarding the purpose of the study, the methods involved, and the risks and benefits of participating. If you find any of the provided information unclear or have further questions, or after taking time to review this information you wish to make an appointment, please contact Jane Lawrence-Dewar at (807) 684-7289. If you have any concerns regarding your rights as a research participant, or wish to speak to someone other than a research team member about this research project, you are welcome to contact the:

Chair, Research Ethics Board
Thunder Bay Regional Health Sciences Centre
980 Oliver Road, Thunder Bay, Ontario P7B 6V4
phone: 807-684-6422 fax: 807- 684-5904
email: ResearchEthics_Chair@tbh.net

This study will also serve as the graduate research of Lakehead University students who are part of the research team. Therefore, this study has also been reviewed and approved by the Lakehead University Research Ethics Board. If you would like to speak to someone outside of the research team at Lakehead University, please contact:

Sue Wright, Research Ethics Board
Lakehead University
Phone: 807-343-8283
Email: research@lakeheadu.ca

WHAT IS THE RESEARCH ABOUT?

If we are learning how to do a new skill, we may learn by observation or watching someone else do it. We may also learn by changing or adapting our movements until we are successful. Our lab is interested in the areas of the brain that are needed for this type of learning and how brain activity in these areas change in natural aging. To observe brain activity we use a type of Magnetic Resonance Imaging (MRI) called functional MRI (fMRI). Enclosed in the package you received is a sheet with more information about this technique.

In the future, we hope that the results of this study will help us understand how injury to these brain regions affect the abilities of stoke patients to learn how to regain hand function and use this knowledge to improve rehabilitative care.

AM I ELIGIBLE TO PARTICIPATE?

All participants who participate in this research study must be right-handed, healthy individuals, between 20 and 85 years of age and must pass a prescreening check to be completed by the investigator prior to scheduling the study appointment as well as by the MR technologist at the study appointment. This study involves entering a high magnetic field which may not be safe for all individuals. An MRI information sheet is included in your participant package to help answer any questions you may have.

You may not participate in the study if you:

1. Have a history of neurological injury or disease
2. Have a physical impairment or affliction that limits use of your right hand
3. Have metal implants inside your body
4. Have a medical condition that could be made worse by stress
5. Are claustrophobic
6. Are or may be pregnant
7. Weigh more than 350 pounds

This study is purely voluntary. You may decide not to participate in this study or you withdraw from the study at any time.

WHAT WILL I HAVE TO DO?

When you call to make an appointment, you will be asked a series of questions to confirm you are eligible. These will include several questions to make sure that there are no metal objects in your body so that it is safe for you to have an MRI done. A copy the detailed prescreening form with the questions that Jane Lawrence-Dewar will ask you when you make an appointment is enclosed for you to review.

You will make one visit to the Thunder Bay Regional Health Sciences Centre where you will have a MRI of your brain using the research dedicated MRI system. The scan itself will take about 60 minutes, but allow a total of 2 hours for the visit.

At your appointment for the Research MRI, an MR technologist will go through the MR Safety Screening Form with you. You will be offered the option of a pregnancy test if you think there is a chance you could be pregnant. The investigator will explain all of the details of the experiment and review this consent form with you as well as answer any questions you may have. You should make sure that all your questions are answered and you agree to participate in the study before signing the consent form.

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Appendix 3 – MRI Study Information Package

Before entering the magnet room, you will be asked to change into clothing which does not contain metal. You have a choice of wearing your own clothing, if it is metal-free (e.g., jogging suit) or the hospital gowns that we can provide. Before you enter the magnet room, we will ask you to remove all metal objects, such as keys, coins, since they could be attracted to the MRI scanner with great force.

For the MRI scan, you will be positioned comfortably on your back and provided with soft earplugs to reduce the noise from the MRI scanner (the sound it produces is a loud knocking noise). A special receiver will be placed around your head. You will then be slid into the large, tunnel-shaped scanner until your head is at the centre of the magnet. The tunnel approximately 2 feet wide and is open at both ends.

We may monitor your pulse rate using a small sensor on your finger, or your breathing using a strap placed over your abdomen. These monitors are used to improve the quality of images for the study.

During the scan, the MR operator will talk with you regularly through a two-way intercom to let you know what to do. During the study, you will be asked to make a series of arm movements to target objects placed in the magnet. For example, on some trials, you will be asked to reach out and pick up a target object. On others, you will be asked to look at target objects or visual images. You may also be given a trackball to control a cursor during a computer based task.

After the scan has been completed and you have left the magnet room, we will ask you to fill out a questionnaire about how the study went for you. We will also ask you if you would like remain on our potential participant list for future studies you may be eligible for of if you would like us to erase your contact information from our database. You can ask to have your name and information removed from our list at any time.

IS THE STUDY CONFIDENTIAL?

Normally, only people directly involved with the research procedure are allowed in the area while a study is being conducted.

Information gathered in this research may be published or presented in public forums; however your name will not be used or revealed. Records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act. All data obtained during your scan will be stored with an alpha-numerical code instead of your name. Only your file, which is kept securely in the Principal Investigator's office, will have information which relates your name to the code. Identifying information will be kept for 7 years. Anonymous data may be kept indefinitely.

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It is possible that our records will be audited by the Research Ethics Board. We have formatted our forms to seal personal information to facilitate inspection of our forms without revealing personal information.

WHAT ARE THE POSSIBLE HARMS OR BENEFITS?

Metal objects can be attracted to the scanner with great force. If a metal object hit anyone in the way, it could cause serious injury. It is for this reason that we are cautious in our procedures and ask that you change into metal free clothing and remove jewelry and items from your pockets.

Metal can also be located inside your body if you have had a surgery or implant. Some metal objects may move or heat up. We will screen you to make sure that it is safe for you to participate. You must tell us if you have had surgery, as metal may be left in your body after certain types of surgery. Please consider if you have any of the following:

- Previous Surgery involving metal, such as: clips, rods, screws, pins, wires.
- Heart pacemaker
- Implanted electrodes, pumps or electrical devices
- Cochlear (inner ear) implants
- Intraocular lens (eye) implants
- Any metallic foreign body, shrapnel or bullet (Have you have ever been a grinder, metal worker, welder, wounded during military service, etc.?)
- Intrauterine contraceptive device (IUD) or contraceptive diaphragm
- Dental work held in place by magnets
- Non-removable dental braces and retainers
- Metal dental work, unless it is composed predominantly of precious or semiprecious alloy or amalgam (Please discuss with the researcher)
- Tattooed eyeliner
- Some tattoos (if you have tattoos, please discuss with the researcher)
- Non-removable metal jewellery (body piercing)

MRI is completely painless, but some people have felt minor, transient discomforts during MRI scans (e.g. dizziness, lightheadedness or a feeling of continued motion after being moved into the magnetic field) which usually subside within a few minutes. In rare cases, the dizziness progressed to the point of nausea, but subsided quickly outside the magnetic field. Some people may have a feeling of claustrophobia while they are in the scanner. Please let us know immediately if you experience claustrophobia or any other discomforts, and we will stop the study. Participation in the study is voluntary and you are free to withdraw at any time without penalty.

No long-term adverse effects of MRI have been reported. We would contact you if any new risks are discovered. Please contact us or ask your physician to contact us if you experience any effects that you feel may be a result of your participation in the study.

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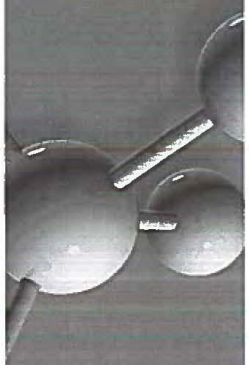

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This is a research study so you will not personally benefit by participating in this study. Eventually, the results of this study may benefit future stroke patients.

WHAT ELSE SHOULD I KNOW?

You have the right to withdraw from the research study at any time and for any reason. The investigators reserve the right to end your participation for any reason. If the study is ended before completion, any information you have already provided will be retained.

This is not a diagnostic scan and the investigator is not clinically trained. We cannot provide you with any medical information regarding your scan. The images obtained are for research purposes only and the methods used for are different than those used in the clinic. That being said, it is possible that your scan could reveal something unexpected.,If the investigator notices an irregularity in a scan, the scan will be forwarded to a radiologist. In addition, scans from one in every ten participants will be randomly selected and forwarded to the radiologist. If the radiologist identifies something that should be followed up further they will contact the physician you identify with a report. If you have a general practitioner or family physician, you are invited to provide their contact information. If you do not have a physician, a report will be sent to neurologist Dr. Ayman Hassan.

We will give you $25 to cover any expenses you incur to participate in this research study. You may also request a copy of some of the images.

The goal of this study is to better understand what brain areas become active during a motor learning task. We do not anticipate that the results of this study will directly lead to commercialization.

Please contact us if you would like any more information about the study. Please let us know if you would like copies of any published scientific reports about the research project.

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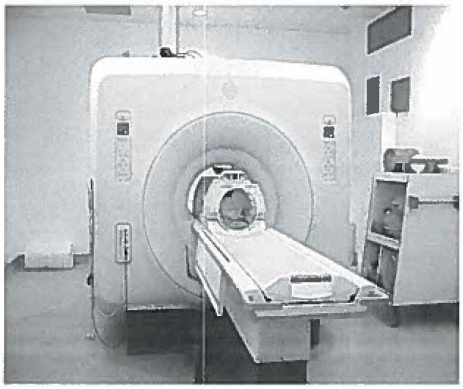
ADDITIONAL INFORMATION SHEET: Magnetic Resonance Imaging

What is magnetic resonance imaging (MRI)?



Magnetic resonance imaging (MRI) is an imaging method based on a large magnetic field. By entering the hole or "bore" of the MRI, you are entering a magnetic field much larger than that experienced by the earth's magnetic field. In hospitals or research centres, the most common strength of magnetic field is 1.5 or 3 Tesla (T). The research dedicated MRI scanner housed on the first floor of the thunder Bay Regional Health Sciences Centre is a 3 T system.

MRI uses the water in your body as a source of signal. By applying a radiofrequency (RF) pulse, we are able to disturb the "spins" away from aligning with the magnetic field. When we remove the RF pulse, the spins realign themselves with the field but how quickly this occurs depends on the type of imaging being performed and the type of tissue that the spins are in. This is how we are able to obtain contrast between bone, fat, muscle, fluid and tissues such as the brain.

What is Functional magnetic resonance imaging (fMRI)?

Functional magnetic resonance imaging (fMRI) is a method of detecting areas of activity in the brain and spinal cord. When an area of the brain or spinal cord becomes "active" it needs more oxygen. To compensate for this, there is a much greater increase in blood flow and therefore oxygen to the area. Oxygenated and deoxygenated blood have different magnetic susceptibilities therefore, there is a localized change in signal that can be detected in an area of neuronal activity.

During a fMRI scan there will be periods where we will ask you to do nothing or "rest". During other times you may be asked to do a task such as move your hand or look at pictures. By comparing the signal in your brain during the times that you are "resting" and those when you are doing the task, we can identify what areas of the brain or spinal cord are active.

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STUDY PRESCREENING FORM

You do not need to complete this form. If you choose to participate in this study, prior to booking an appointment, Dr. Lawrence-Dewar will review over the following criteria to confirm your eligibility for the study. Some of these criteria are important for the design of our experiment but most are because of safety concerns related to entering a MRI. It is important that you answer them to the best of your knowledge.

Handedness

Which hand do you use for the following tasks:

	Right	Left		Right	Left
Comb your hair	☐	☐	Brush teeth	☐	☐
Hammer a nail	☐	☐	Eat soup	☐	☐
Swing a hockey stick	☐	☐	Throw ball	☐	☐
Swing a tennis racket	☐	☐	Write name	☐	☐

Medical History

Do you have a history of:	Yes	No
Neurological disease (Parkinson's, Alzheimer's, etc)	☐	☐
Brain injury (Stroke, Traumatic Brain injury)	☐	☐
Diagnosed brain tumor	☐	☐
Seizures/Epilepsy	☐	☐
Arthritis (primarily affecting your right arm/hand)	☐	☐
Headache/Migraine	☐	☐

What medications are you currently taking?

_____

_____

Demographic information:

Sex (circle): Male Female Age: _____years

Computer usage(circle): 1 2 3 4 5
Low High

Participant number:_____

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STUDY PRESCREENING FORM (CONTINUED)

MR Safety

Do you have the following:	Yes	No
Stents, wire mesh, or metal implants	☐	☐
Cardiac pacemaker	☐	☐
Aneurysm clips in head	☐	☐
Neuro/bio stimulator device	☐	☐
Implanted insuluin pump	☐	☐
Hearing aid	☐	☐
Cochlear/ear implant	☐	☐
Shrapnel	☐	☐
Piercings	☐	☐
Dental implants (non-removable dentures, bridges, crowns)	☐	☐
Artificial limb or joint	☐	☐
Metal rods, screws, plates, nails	☐	☐
IUD	☐	☐
Tattoos	☐	☐
Have you ever been a metal worker (welder)?	☐	☐
Are you or could you be pregnant?	☐	☐
Have you previously had surgery?	☐	☐

If yes what and when?

To the best of your knowledge is any metal left behind? ☐ ☐

Thunder Bay Regional Health Sciences Centre
OCT 23 2014
Research Ethics Board

Appendix 3 – MRI Study Information Package

CONSENT FORM FOR MRI STUDY

By signing this consent form you acknowledge and agree that:

- I have received a copy of and I have read the Research Summary and accompanying information sheets.
- I understand the nature of the study, including the potential risks and benefits.
- I have had adequate time to consider the information.
- I have talked to Dr. Jane Lawrence Dewar. All my questions about the study have been answered.
- If I have any more questions, I may call Dr Jane Lawrence Dewar at the Thunder Bay Regional Research Institute at 684-7289.
- I realize that by signing this document I am not waiving any legal rights.
- I understand that information regarding my personal identity will be kept confidential with the following exceptions:
- I give permission to disclose information to a radiologist, and if further follow up is needed, the identified physician below (check one):

☐ I have named Dr. _____ at _____ as the physician to be contacted for follow-up purposes.

☐ I agree to have neurologist, Dr. Ayman Hassan, as the physician to be contacted for follow-up purposes. Dr. Hassan can contact me with the information I provide below.

I hereby agree to participate in the research protocol, "Investigation of the neural mechanisms underlying changes in motor learning in healthy aging" and I understand that I can end my participation at any time and for any reason. My consent has been given freely.

Seal line (all information below this line is confidential and will be sealed)

_____

Participant information:

Name (Print): Contact information (for follow up)

_____ _____

Signature: _____

_____ _____

Investigator information:

Name (Print):

_____

Signature: Date:

_____ _____

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Translational Research Office:
Room #2162
980 Oliver Road,
Thunder Bay, Ontario
P7B 6V4

Pre-Clinical Research Office:
290 Munro Street,
Thunder Bay, Ontario
P7A 7T1

www.tbrri.com

Bringing Discovery to Life

Thunder Bay Regional Research Institute

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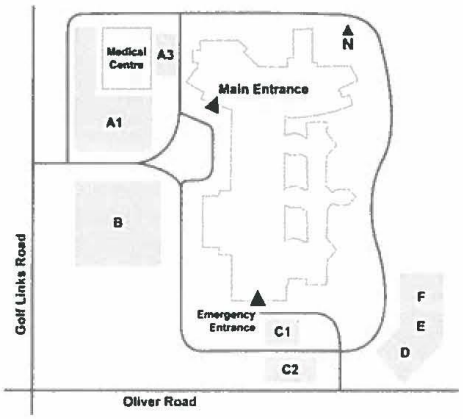
DIRECTIONS TO THE RESEARCH STUDY

** PLEASE BRING THIS PAPER WITH YOU TO THE STUDY**

After you have reviewed all of the provided information, if you have any questions or wish to volunteer for this study, please contact Dr. Jane Lawrence-Dewar at (807)684-7289. She will book an appointment for the study if you wish to do so. Participation is completely voluntary and you can withdraw from the study at any time.

Studies will take place at the Thunder Bay Regional Health Sciences Centre. If you require parking, the closest lots to the main entrance are A1 and B.



When you enter the main entrance of the hospital you will see an information booth. Turn right and proceed towards the main staircase. At the bottom of the staircase is a hall on the right. Proceed down the hall watching for the sign for Diagnostic Imaging. Once you find the Diagnostic Imaging reception let them know you are here for a research study on the research MRI and provide them with this piece of paper.

TO DIAGNOSTIC IMAGING RECEPTION:

PLEASE CALL THE RESEARCH 3T CONSOLE ROOM (x 6569) TO NOTIFY DR. JANE LAWRENCE-DEWAR THAT HER RESEARCH PARTICIPANT HAS ARRIVED. SHE WILL COME GET THEM. PLEASE DO NOT ENTER THEIR INFORMATION INTO THE CLINICAL COMPUTER SYSTEM.

ii. Research study exit questionnaire

Research Study Exit Questionnaire

Your responses are confidential and voluntary. This questionnaire is meant to provide us with feedback about your experience only.

All studies:
What did you notice during the study?

Did you notice anything change during the study?

Did you find anything particularly challenging?

Please circle the correct response:

Based on your experience, would you participate in a study like this again?	Yes	No
Based on your experience, Would you recommend this study to a friend?	Yes	No

Participant number: _____

MRI Studies ONLY:

Did you experience any of the following while in the MRI:

	Yes	No
Nausea	☐	☐
Headache	☐	☐
Dizziness	☐	☐
Sleepiness	☐	☐
Claustrophobia	☐	☐
Anxiety	☐	☐
Heat	☐	☐
Cold	☐	☐
Tingling	☐	☐
Discomfort	☐	☐

Other:

Participant number: _____

All Participants:

Do you participate in any activities that require you to observe and repeat behaviours of others (e.g. fitness classes, or skill-specific training)? If so, please describe below.

Exit Questionnaire – Permission for further contact

When you first contacted the lab, your contact information was collected in a secure, password protected database. This information is not shared with anyone. With your permission we will keep this information indefinitely, to contact you if you become eligible for another study of Dr. Jane Lawrence-Dewar's in the future. Your information will otherwise be erased. You make ask to be removed from the list at any time.

- ☐ I give Dr. Jane Lawrence Dewar permission to retain my contact information with the purpose of contacting me if I become eligible for another research study in her lab. I understand that this information will not be shared with anyone.

  I would prefer to be contacted by (please circle) : e-mail Phone

- ☐ I do not wish to be contacted. Please erase my contact information.

As this page contains your signature, it will be attached to your consent form and kept separate from all data.

Seal Line (Identifying information below this line will be sealed).

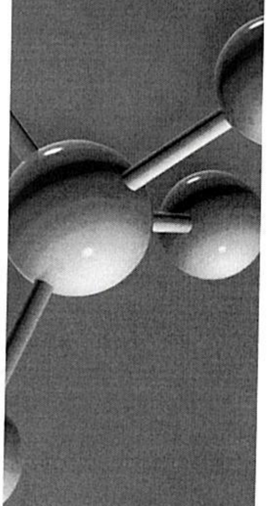
I understand that I can request that Dr. Jane Lawrence-Dewar remove my name and contact information from this list at any time and if I do so it will be deleted immediately.

Signature:

Date:

iii. Recruitment poster

Bringing Discovery to Life



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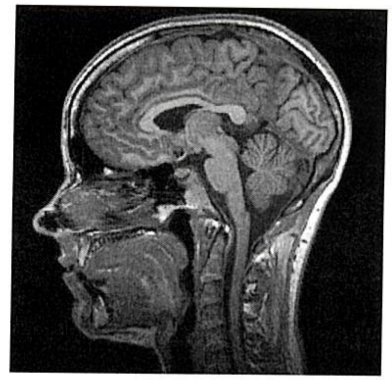
VOLUNTEERS NEEDED FOR RESEARCH STUDY

Researchers as the Thunder Bay Regional Research Institute (TBRRI) are conducting brain imaging studies to investigate changes in motor learning in natural aging.



During the study we use a type of Magnetic Resonance Imaging (MRI) to take images of the brain. During some scans you will be asked to do a task such as look at images or reach for objects.

To be eligible for this study you must:

- Be between the ages of 20 and 85 years old
- Be right handed
- Have normal or corrected to normal vision (wear corrective lenses)
- Have no history of brain injury or disease
- Have no physical or psychological condition that would prevent you from entering a magnet field (for example but not limited to: pace maker, metal implants, anxiety, claustrophia)

The study takes place at the Thunder Bay Regional Health Sciences Centre. The whole study will take 2 hours but you will only be in the MRI for 1 hour. You will be compensated $25 for your time and costs incurred for participating. You may also request an image of your brain.

To request more information about this study please contact:

Investigator: Dr. Jane Lawrence-Dewar (807) 684-7289.

For more information about your rights as a research participant, contact the Thunder Bay Regional Health Sciences Centre Research Ethics Office at 684-6422.

Brain Imaging Study Contact:
Dr. Jane Lawrence-Dewar
(807) 684-7289

Brain Imaging Study Contact:
Dr. Jane Lawrence-Dewar
(807) 684-7289

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Dr. Jane Lawrence-Dewar
(807) 684-7289

iv. University Ethics Approval

Lakehead UNIVERSITY

Research Ethics Board
t: (807) 343-8283
research@lakeheadu.ca

October 21, 2014

Principal Investigator: Dr. Jane Lawrence-Dewar
Student Investigators: Shayna Parker, Andrea Hantjis, Andrea Pepe
Health Sciences
Lakehead University
Thunder Bay Regional Research Institute
Room 3115, 980 Oliver Road
Thunder Bay, ON P7B 6V4

Dear Dr. Jane Lawrence-Dewar:

Re: REB Project #: 072 14-15 / Romeo File No: 1464121
Granting Agency: Thunder Bay Regional Research Institute
Granting Agency Project #: 1463991

On behalf of the Research Ethics Board, I am pleased to grant ethical approval to your research project titled, "Investigation of the neural mechanisms underlying changes in healthy aging".

Ethics approval is valid until October 21st, 2015. Please submit a Request for Renewal form to the Office of Research Services by September 21st, 2015 if your research involving human subjects will continue for longer than one year. A Final Report must be submitted promptly upon completion of the project. Research Ethics Board forms are available at:https://www.lakeheadu.ca/research-and-innovation/forms. During the course of the study, any modifications to the protocol or forms must not be initiated without prior written approval from the REB. You must promptly notify the REB of any adverse events that may occur.

Completed reports and correspondence may be directed to:

Research Ethics Board
c/o Office of Research Services
Lakehead University
955 Oliver Road
Thunder Bay, ON P7B 5E1
Fax: (807) 346-7749

Best wishes for continued success with your research project.

Sincerely,

Dr. Lori Chambers
Chair, Research Ethics Board

v. Thunder Bay Regional Health Sciences Centre Ethics Approval

RESEARCH ETHICS BOARD
Re-Approval Application

Please complete, sign and submit this form to the Research Ethics Office
If you require any assistance, please contact: REO@tbh.net

TBRHSC Research Ethics Office Use Only

Re-approval Granted on: June 18, 2014

Starting on: June 18, 2014 Expiring on: June 18, 2015

~~REB meeting date:~~ to be approved: Sept 22, 2014

Signature of Chair: [signature]

Date: 18 June 2014

Thunder Bay Regional Health Sciences Centre
MAY 09 2014
Research Ethics Board

TBRHSC REB # 2012107 Current expiry date: May 28, 2014

Principal Investigator: Dr. Jane Lawrence Dewar

Full Study Title: Investigation of the neural mechanisms underlying changes in motor learning in healthy aging

1.	Study Status	Yes	No
	Enrollment Closed/Completed	☐	☒
	All Assessments/Intervention Completed	☐	☒
	Follow-up Completed	☐	☒
	Data verification/Data analyses Completed	☐	☒

FOR STUDIES INVOLVING CHART REVIEWS		
Study Status	Yes	No
Review of all charts completed	☐	☒

2.	**Number of local study participants** (since study initiation)		
	Enrolled in Study	A	11
	In active intervention phase of study	B	0
	In follow-up phase of study	C	0
	Completed study	D	11
	Withdrew from study	E	0

NOTE:

All participants need to be accounted for

A = B + C + D + E + F

Comments, if needed:

	Deceased, lost-to-follow up, transferred	F	0	

3.	Reports/Updates of Research Study	Yes	No	Check if attachment
a.	Has an interim data analysis been done? → If Yes, attach a summary.	☐	☒	☐
b.	Have articles been published or presentations given using results of the study? → If Yes, submit a copy of the abstract(s) or a list of references	☐	☒	☐
c.	Have all serious adverse events been reported? [√] not applicable → If No, include with this report.	☐	☐	☐
d.	Has new literature changed your assessment of risk/benefits for participants?	☐	☒	☐
	→ If Yes, have participants been informed? → If No, attach an explanation of how & when participants will be informed	☐	☐	☐
e.	Have there been any changes in investigators since the last approval?	☒	☐	☐
	→ If Yes, has the REB been notified? → If No, submit an amendment with this application	☐	☒	☐
f.	Is there new evidence from other studies that impact your study? → If Yes, attached summary	☐	☒	☐
g.	Have there been any changes to the local study protocol or consent form? → If Yes, submit an amendment form with this application.	☒	☐	☐
h.	Are study results available? → If Yes, attach a brief summary of study results to date.	☐	☒	☐

Principal Investigator's Signature:
(sign final hard copy after printing) Jane Lawrence Dewar

Print Name: Jane Lawrence Dewar

Date: [month day, year] May 8 2014

Progress to date

Since the last re-approval, nine participants have been recruited for the behavioral component. Recruitment for this part of the study is ongoing.

To date, two participants have completed the fMRI component. Recruitment for the fMRI component had been halted when the radiologist currently listed in the protocol for reading incidental findings left TBRHSC. An amendment is attached to change the radiologists who have agreed to read for incidental findings.

The progress of this research has also been impacted by the absence of Dr. Jane-Lawrence, who was on a maternity leave from July 2, 2013 to April 1, 2014.

Appendix B. Example Stimulus Images

Images used in Task 1.

Using a stapler.

Stirring a spoon.

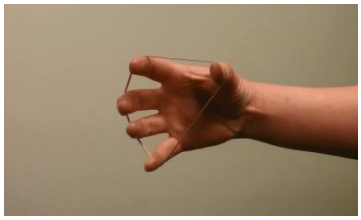
Opening a carabiner.

Pressing a perfume bottle.

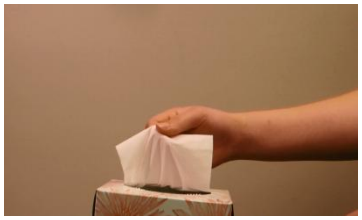
Turning a doorknob.

Using a knife.

Using a fork.



Stretching an elastic band.

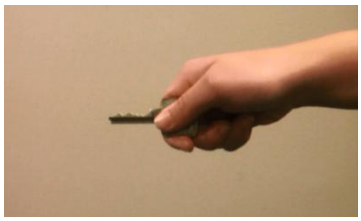


Picking up tissues.

Turning a knob on a toaster.

Twisting a lid.

Flipping a lid.

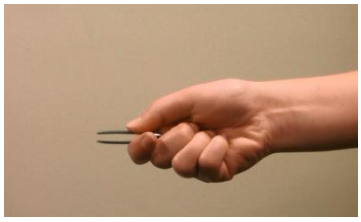


Turning a key.

Squeezing a spray bottle.

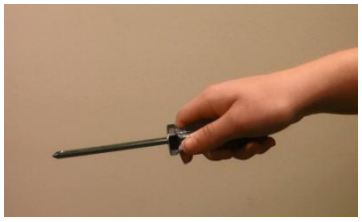
Flipping an hourglass.

Images used in Task 2.

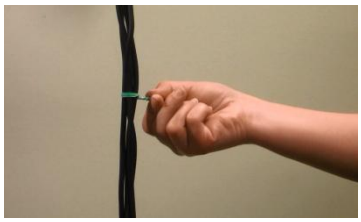


Pinching tweezers.

Squeezing a hole-punch.

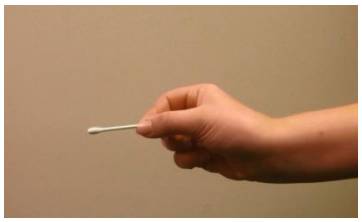


Twisting a screwdriver.



Twisting a twist-tie.

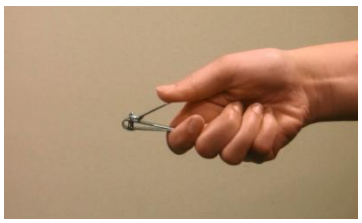
Sliding a card.



Spinning a Q-tip.

Pressing remote buttons.

Squeezing a sponge.



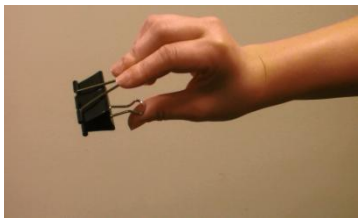
Squeezing nail clippers.

Pouring a pitcher.

Clicking a pen.

Using a wrench.

Turing a light bulb.



Squeezing a clip.

Cutting with scissors.

Appendix C. Responses from Questionnaires

Please note that **BOLDED writing indicates participant withdrawal, *ITALICS* indicates technical difficulties**

Participant Number	Sex	Age	Task Compliance	Participation in Activities Requiring Observational Learning	Medications Present Within Group
Group 1					
01	*M*	*29*	*N/A*	*N/A*	- Metformin hydrochloride 500mg, Ramipril 5mg
02	*F*	*23*	*N/A*	*N/A*	
03	*M*	*26*	*N/A*	*N/A*	
04	M	29	NO	N/A	
05	M	26	NO	N/A	
06	F	24	YES	N/A	
07	M	25	YES	N/A	
08	M	26	NO	N/A	
09	M	36	YES	N/A	
14	F	22	YES	None	
11	M	35	YES	N/A	
12	M	25	YES	Playing games and had to repeat behaviours to get better.	
18	F	34	YES	None	
46	M	35	YES	None	
27	M	39	YES	Coaching, teaching, swimming, and cross country skiing. Learned from repetitive behaviour.	
Group 2					
13	F	44	YES	Dance classes, various mechanical tasks which require watching and learning to perform.	- Advil, Tylenol on occasion. - Effexor, Zopiclone - Birth control
15	*F*	*55*	**N/A**	**N/A**	
16	F	46	NO	Fitness classes, online courses.	
10	M	47	YES	N/A	
17	F	46	YES	None	
19	M	41	NO	None	
20	F	53	YES	Yoga, skiing lessons, often learns by watching.	
21	F	46	YES	None	
22	F	41	YES	Warm-ups before running and Pilates.	
24	F	55	YES	None	
26	F	58	YES	None	
42	M	58	YES	None	
28	M	43	YES	Previously guitar lessons.	
29	M	58	YES	Operating equipment, scissor lifts.	
30	M	53	NO	Curling.	
31	M	52	YES	None	
Group 3					
32	F	64	YES	Watching aquarobics instructor.	- Blood pressure

33	F	66	YES	"How-to" videos on how to fix things.	medication - Blood thinners - Spironolactone, rosuvastatin, ezetrol, hydrochlorothiazid, candesartan, ASA 81 mg, insulin - Drops for glaucoma - Parkinson's medication for restless leg syndrome. - Warfarin
34	F	67	YES	Yoga	
35	M	73	YES	Taijiquan (Tai-Chi), Feldenkrais, body-mind awareness in movement.	
36	M	70	YES	Fitness, dark room photography training.	
37	F	68	YES	None	
38	F	67	YES	Painting classes.	
39	M	73	YES	Singing in choruses.	
40	F	71	NO	Aquarobic classes.	
41	*M*	*66*	**N/A**	**N/A**	
43	F	76	YES	Exercise classes, cake decorating	
44	F	67	YES	None	
45	M	80	YES	Feldenkrais	
23	M	69	YES	None	
25	F	65	NO	Yoga	

Appendix D. List of regions of significant activity during observational learning tasks for within groups analyses

i. Areas of significant activity for Group 1 during observational learning task.

Anatomical Region	**Hemisphere**	**x**	**y**	**z**	**t**	**p**
Superior Temporal Gyrus, Brodmann area 22	Right	58	5	1	4.711918	0.001518
Inferior Frontal Gyrus, Brodmann area 44	Right	51	-1	19	5.457417	0.000603
Precentral Gyrus, Brodmann area 4	Right	45	-13	43	5.006011	0.001045
Middle Frontal Gyrus, Brodmann area 11	Right	45	35	-17	3.903165	0.004524
Precentral Gyrus, Brodmann area 44	Right	45	5	7	4.050819	0.00368
Inferior Frontal Gyrus, Brodmann area 46	Right	39	35	10	4.851346	0.00127
Inferior Frontal Gyrus, Brodmann area 47	Right	42	29	-2	4.002093	0.003938
Inferior Frontal Gyrus, Brodmann area 13	Right	39	26	13	4.102093	0.003428
Insula, Brodmann area 13	Right	39	11	13	4.459836	0.002112
Posterior Lobe, Cerebellar Tonsil,	Right	39	-55	-41	4.383624	0.002337
Fusiform Gyrus, Brodmann area 20	Right	36	-10	-23	4.622996	0.001704
Posterior Lobe, Cerebellar Tonsil,	Right	33	-55	-41	3.5233	0.007808
Precentral Gyrus, Brodmann area 6	Right	30	-1	34	4.984812	0.001073
Middle Frontal Gyrus, Brodmann area 9	Right	30	32	25	4.137284	0.003266
Claustrum,	Right	24	23	16	5.685431	0.000462
Lentiform Nucleus, Putamen	Right	27	-4	4	3.982959	0.004045
Limbic Lobe, Anterior Cingulate, Brodmann area 32	Right	24	38	7	6.427223	0.000203
Inferior Frontal Gyrus, Brodmann area 47	Right	18	29	-2	5.357485	0.00068
Medial Frontal Gyrus, Brodmann area 9	Right	21	41	25	3.685529	0.00617
Cingulate Gyrus, Brodmann area 24	Right	18	5	40	4.119302	0.003347
Lentiform Nucleus, Lateral Globus Pallidus	Right	18	-1	7	3.938772	0.004303
Cingulate Gyrus, Brodmann area 24	Right	18	-4	40	4.242725	0.002827
Anterior Cingulate, Brodmann area 32	Right	18	23	22	4.564257	0.00184
Anterior Cingulate, Brodmann area 32	Right	18	32	10	4.649714	0.001645
Precentral Gyrus, Brodmann area 4	Right	18	-25	55	3.607262	0.006909
Caudate, Caudate Body	Right	15	-1	13	4.17136	0.003116
Posterior Lobe, Pyramis	Right	15	-64	-29	4.17253	0.003111
Precuneus, Brodmann area 7	Right	12	-61	46	5.280542	0.000746
Uncus, Brodmann area 34	Right	12	-1	-23	4.282369	0.002679
Parietal Lobe, Precuneus, Brodmann area 7	Right	9	-70	37	3.926297	0.004379

Limbic Lobe, Cingulate Gyrus, Brodmann area 24	Right	6	5	28	4.3529	0.002436
Medial Frontal Gyrus, Brodmann area 9	Right	3	50	34	5.587759	0.000518
Limbic Lobe, Anterior Cingulate,	Right	3	29	4	4.017111	0.003857
Medial Frontal Gyrus, Brodmann area	Left	-3	-16	61	4.90286	0.001189
Medial Frontal Gyrus, Brodmann area 6	Left	-3	-10	64	4.0864	0.003503
Thalamus	Left	-3	-7	10	3.830228	0.005016
Superior Frontal Gyrus, Brodmann area 9	Left	-9	59	28	4.708097	0.001525
Thalamus, Pulvinar	Left	-9	-31	13	4.375803	0.002362
Caudate, Caudate Body	Left	-9	26	7	3.577101	0.007219
Medial Frontal Gyrus, Brodmann area 6	Left	-12	-1	55	4.30934	0.002583
Superior Frontal Gyrus, Brodmann area 8	Left	-15	35	40	4.630155	0.001688
Cingulate Gyrus, Brodmann area 24	Left	-12	2	34	3.807612	0.00518
Superior Frontal Gyrus, Brodmann area 9	Left	-12	53	22	3.826606	0.005042
Anterior Cingulate, Brodmann area 24	Left	-12	17	22	3.89816	0.004556
Medial Frontal Gyrus, Brodmann area 6	Left	-15	-19	55	3.980998	0.004056
Cingulate Gyrus, Brodmann area 24	Left	-15	-16	40	5.781771	0.000414
Superior Frontal Gyrus, Brodmann area 8	Left	-18	26	46	5.45215	0.000607
Precentral Gyrus, Brodmann area 6	Left	-18	-16	64	4.083347	0.003518
Lentiform Nucleus, Putamen	Left	-18	-1	10	4.67667	0.001589
Anterior Cingulate, Brodmann area 32	Left	-21	29	22	4.897923	0.001197
Caudate, Caudate Body	Left	-21	5	22	8.225547	0.000036
Posterior Lobe, Declive,	Left	-21	-70	-14	4.817452	0.001326
Superior Frontal Gyrus, Brodmann area 8	Left	-21	17	49	4.044981	0.00371
Inferior Frontal Gyrus, Brodmann area 47	Left	-24	29	-2	4.582865	0.001795
Inferior Frontal Gyrus, Brodmann area 47	Left	-24	17	-14	4.134144	0.00328
Lentiform Nucleus, Putamen	Left	-24	-1	-5	3.999767	0.003951
Middle Frontal Gyrus, Brodmann area 9	Left	-27	32	31	5.370453	0.000669
Middle Frontal Gyrus, Brodmann area 9	Left	-27	17	31	4.012335	0.003882
Insula, Brodmann area 13	Left	-27	-34	16	3.721917	0.005855
Insula, Brodmann area 13	Left	-30	-19	22	6.333496	0.000225
Inferior Frontal Gyrus, Brodmann area 47	Left	-30	32	-8	4.86665	0.001245
Claustrum	Left	-36	-7	-8	8.117435	0.000039
Middle Frontal Gyrus, Brodmann area 10	Left	-36	41	25	8.835148	0.000021
Insula, Brodmann area 13	Left	-36	-19	19	3.937429	0.004311

Middle Frontal Gyrus, Brodmann area 11	Left	-39	44	-12	3.771231	0.005456
Insula, Brodmann area 13	Left	-42	-7	13	4.816184	0.001328
Inferior Frontal Gyrus, Brodmann area 47	Left	-45	26	-11	4.090248	0.003484
Inferior Parietal Lobule, Brodmann area 40	Left	-48	-31	31	4.657128	0.00163
Inferior Temporal Gyrus, Brodmann area 37	Left	-54	-58	-5	4.155752	0.003184
Postcentral Gyrus, Brodmann area	Left	-60	-13	25	5.196527	0.000826
Superior Temporal Gyrus, Brodmann area 22	Left	-63	-34	13	5.510149	0.000567

ii. Areas of significant activity for Group 2 during observational learning task.

Anatomical Region	**Hemisphere**	**x**	**y**	**z**	**t**	**p**
Middle Temporal Gyrus, Brodmann area 21	Right	63	-19	-5	4.100615	0.001758
Precentral Gyrus, Brodmann area 6	Right	54	-1	10	5.220074	0.000285
Middle Temporal Gyrus, Brodmann area 21	Right	54	-22	-5	4.170434	0.001562
Superior Temporal Gyrus, Brodmann area 22	Right	48	-7	1	4.060563	0.001881
Precentral Gyrus, Brodmann area 6	Right	51	-1	31	3.702366	0.003487
Inferior Frontal Gyrus, Brodmann area 9	Right	48	5	28	5.080504	0.000355
Superior Temporal Gyrus, Brodmann area 22	Right	48	-1	-5	3.255815	0.007656
Inferior Frontal Gyrus, Brodmann area 1	Right	45	26	10	4.684933	0.000666
Insula, Brodmann area 13	Right	45	5	1	3.395641	0.005975
Precentral Gyrus, Brodmann area 4	Right	39	-16	55	3.710283	0.003439
Inferior Frontal Gyrus, Brodmann area 47	Right	39	29	1	4.091821	0.001784
Precentral Gyrus, Brodmann area 4	Right	36	-19	49	4.316838	0.001221
Fusiform Gyrus, Brodmann area 20	Right	39	-37	-14	3.617697	0.004043
Middle Temporal Gyrus, Brodmann area 21	Right	36	-7	-23	3.715404	0.003409
Precentral Gyrus, Brodmann area 6	Right	36	-7	37	3.570472	0.004391
Claustrum	Right	33	-4	4	4.652637	0.000702
Caudate, Caudate Tail	Right	33	-25	-5	4.331348	0.001192
Lingual Gyrus, Brodmann area 19	Right	33	-55	7	3.99858	0.002091
Lentiform Nucleus, Putamen	Right	24	-4	-2	4.983468	0.000413

Medial Frontal Gyrus, Brodmann area 9	Right	27	29	19	3.354217	0.006429
Precentral Gyrus, Brodmann area 6	Right	21	-13	61	4.296021	0.001264
Superior Frontal Gyrus, Brodmann area 8	Right	21	17	40	3.371544	0.006235
Anterior Lobe, Culmen	Right	21	-52	-20	4.168849	0.001566
Posterior Lobe, Cerebellar Tonsil	Right	21	-49	-38	4.319876	0.001215
Lentiform Nucleus, Putamen	Right	21	5	16	3.585138	0.00428
Cingulate Gyrus, Brodmann area 24	Right	18	-1	43	4.481199	0.00093
Midbrain, Substania Nigra	Right	18	-22	-5	3.543536	0.004604
Posterior Lobe, Pyramis	Right	18	-70	-29	4.200562	0.001484
Cingulate Gyrus, Brodmann area 32	Right	18	20	25	3.525964	0.004748
Thalamus, Medial Geniculum Body	Right	15	-25	-5	3.874055	0.00259
Posterior Lobe, Declive	Right	9	-61	-17	4.976448	0.000418
Thalamus, Ventral Posterior Medial Nucleus	Right	15	-22	1	3.43019	0.005621
Cingulate Gyrus, Brodmann area 31	Right	9	-34	37	3.64918	0.003826
Cingulate Gyrus, Brodmann area 24	Right	6	-1	46	3.908463	0.002441
Posterior Lobe, Cerebellar Tonsil	Right	6	-55	-41	3.720531	0.003379
Precuneus, Brodmann area 7	Right	6	-64	52	3.793133	0.002978
Cingulate Gyrus, Brodmann area 24	Right	6	8	34	4.10563	0.001743
Medial Frontal Gyrus, Brodmann area 6	Right	0	-13	55	4.436597	0.001001
Superior Frontal Gyrus, Brodmann area 6	Right	3	8	61	3.659177	0.00376
Medial Frontal Gyrus, Brodmann area 6	Right	0	-1	52	3.894697	0.002499
Cingulate Gyrus, Brodmann area 24	Right	3	2	34	3.585607	0.004276
Anterior Lobe, Culmen	Right	3	-31	-23	3.817933	0.002853
Posterior Lobe, Pyramis	Right	0	-67	-26	4.705446	0.000644
Posterior Lobe, Inferior Semi-Lunar Lobule	Right	0	-64	-35	4.993467	0.000407
Limbic Lobe, Cingulate Gyrus, Brodmann area 24	Left	-6	-7	40	8.445928	0.000004
Paracentral Lobule, Brodmann area 6	Left	-6	-28	49	4.256393	0.001351
Anterior Lobe, Culmen	Left	-3	-58	-17	3.656857	0.003775
Medial Frontal Gyrus, Brodmann area 6	Left	-6	5	49	4.23394	0.001403
Posterior Lobe, Declive	Left	-6	-64	-20	3.882453	0.002553
Cingulate Gyrus, Brodmann area 24	Left	-6	8	34	4.975858	0.000418
Inferior Semi-Lunar Lobule	Left	-9	-67	-41	3.45683	0.005363
Precentral Gyrus, Brodmann area 6	Left	-12	-16	61	5.696031	0.000139
Cingulate Gyrus, Brodmann area 31	Left	-12	-25	37	4.222836	0.00143
Medial Frontal Gyrus, Brodmann area 6	Left	-15	8	52	3.991335	0.002117
Cingulate Gyrus, Brodmann area 31	Left	-15	-19	37	3.461809	0.005316
Superior Frontal Gyrus, Brodmann area 6	Left	-18	-13	67	5.961023	0.000094

Precentral Gyrus, Brodmann area 4	Left	-18	-25	58	4.071436	0.001847
Caudate, Caudate Head	Left	-15	23	-8	3.419921	0.005724
Lentiform Nucleus, Putamen	Left	-18	11	-5	3.823537	0.002826
Precentral Gyrus, Brodmann area 4	Left	-27	-22	67	4.625954	0.000733
Precuneus, Brodmann area 7	Left	-21	-49	40	4.041699	0.001943
Cingulate Gyrus, Brodmann area 24	Left	-21	-13	37	4.72835	0.000621
Sub-lobar, Lentiform Nucleus, Putamen	Left	-27	-7	4	5.075052	0.000358
Superior Parietal Lobule, Brodmann area 7	Left	-24	-52	43	3.237869	0.007904
Insula, Brodmann area 13	Left	-27	-34	25	6.429827	0.000049
Postcentral Gyrus, Brodmann area 3	Left	-27	-31	70	3.721426	0.003373
Precentral Gyrus, Brodmann area 4	Left	-33	-13	49	3.745301	0.003236
Precentral Gyrus, Brodmann area 6	Left	-33	2	28	4.089646	0.001791
Sub-lobar, Insula, Brodmann area 13	Left	-30	-40	22	3.782075	0.003036
Parahippocampal Gyrus, Brodmann area 30	Left	-30	-55	10	3.380987	0.006132
Postcentral Gyrus, Brodmann area 3	Left	-36	-31	55	4.502599	0.000897
Middle Occipital Gyrus, Brodmann area 37	Left	-39	-64	-2	3.533342	0.004687
Precentral Gyrus, Brodmann area 4	Left	-39	-16	52	3.427692	0.005646
Insula, Brodmann area 13	Left	-39	-13	-2	5.06813	0.000362
Precentral Gyrus, Brodmann area 6	Left	-42	-16	61	4.064249	0.00187
Inferior Parietal Lobule, Brodmann area 40	Left	-42	-37	52	5.68399	0.000142
Insula, Brodmann area 13	Left	-42	5	13	4.972892	0.00042
Middle Frontal Gyrus, Brodmann area 9	Left	-45	29	37	4.143653	0.001634
Superior Temporal Gyrus, Brodmann area 13	Left	-45	-1	-8	5.237357	0.000278
Inferior Parietal Lobule, Brodmann area 40	Left	-48	-40	40	6.092073	0.000078
Supramarginal Gyrus, Brodmann area 40	Left	-45	-43	31	3.888943	0.002524
Insula, Brodmann area 13	Left	-45	14	1	4.113978	0.001718
Inferior Frontal Gyrus, Brodmann area 9	Left	-48	-1	22	3.453355	0.005396
Postcentral Gyrus, Brodmann area 3	Left	-48	-16	52	4.433087	0.001006
Precentral Gyrus, Brodmann area 6	Left	-48	-7	10	4.017962	0.002023
Inferior Frontal Gyrus, Brodmann area 44	Left	-51	11	10	3.952751	0.002262
Superior Temporal Gyrus, Brodmann area 21	Left	-51	-22	-2	3.532078	0.004698
Inferior Parietal Lobule, Brodmann area 40	Left	-57	-31	43	4.92343	0.000454
Postcentral Gyrus, Brodmann area 40	Left	-60	-25	19	5.221154	0.000285
Inferior Parietal Lobule, Brodmann area	Left	-60	-43	25	3.251065	0.007721

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Postcentral Gyrus, Brodmann area 2	Left	-60	-22	37	3.46136	0.00532
Inferior Parietal Lobule, Brodmann area 40	Left	-63	-25	28	3.564973	0.004434

iii. Areas of significant activity for Group 3 during observational learning task.

Anatomical Region	**Hemisphere**	**x**	**y**	**z**	**t**	**p**
Precentral Gyrus, Brodmann area 6	Right	61	2	16	5.205784	0.000292
Middle Temporal Gyrus, Brodmann area 21	Right	57	-22	-11	3.715343	0.003409
Superior Temporal Gyrus, Brodmann area 22	Right	48	-22	-8	5.780913	0.000123
Claustrum	Right	36	-4	10	5.015853	0.000393
Parahippocampal Gyrus, Brodmann area 19	Right	36	-40	-5	4.288088	0.001281
Lentiform Nucleus, Putamen	Right	30	-10	-5	4.001236	0.002082
Lentiform Nucleus, Putamen	Right	21	2	7	4.666585	0.000686
Middle Frontal Gyrus, Brodmann area 6	Right	18	-4	58	4.014972	0.002033
Caudate, Caudate Tail	Right	18	-37	19	4.62945	0.000729
Lentiform Nucleus, Putamen	Right	18	5	-2	4.277915	0.001303
Posterior Lobe, Cerebellar Tonsil	Right	18	-49	-35	3.538806	0.004642
Medial Frontal Gyrus, Brodmann area 6	Right	3	-10	49	5.248375	0.000273
Cingulate Gyrus, Brodmann area 24	Left	-3	5	25	3.712007	0.003429
Medial Frontal Gyrus, Brodmann area 6	Left	-6	-10	61	3.738347	0.003276
Medial Frontal Gyrus, Brodmann area 6	Left	-6	-25	58	4.835759	0.000523
Medial Frontal Gyrus, Brodmann area 6	Left	-9	-16	52	5.766501	0.000125
Paracentral Lobule, Brodmann area 6	Left	-9	-25	46	3.674494	0.003661
Cingulate Gyrus, Brodmann area 24	Left	-12	-10	40	4.855781	0.000506
Cingulate Gyrus, Brodmann area 24	Left	-15	-4	37	4.461946	0.00096
Lingual Gyrus	Left	-15	-52	1	5.618487	0.000156
Lentiform Nucleus, Putamen	Left	-21	17	-5	4.073942	0.001839
Lentiform Nucleus, Putamen	Left	-21	8	7	3.435442	0.005569
Lentiform Nucleus, Putamen	Left	-27	2	-2	4.283342	0.001291
Middle Frontal Gyrus, Brodmann area 47	Left	-30	35	-5	4.564365	0.000811
Middle Frontal Gyrus, Brodmann area 11	Left	-30	44	-12	3.543374	0.004605
Middle Frontal Gyrus, Brodmann area 11	Left	-33	47	-8	5.571414	0.000167
Postcentral Gyrus, Brodmann area 3	Left	-33	-28	67	3.442466	0.005501
Postcentral Gyrus, Brodmann area 3	Left	-39	-25	52	5.055696	0.000369

Insula, Brodmann area 13	Left	-39	-10	7	5.237342	0.000278
Middle Frontal Gyrus, Brodmann area 47	Left	-39	38	-11	4.390845	0.001079
Inferior Parietal Lobule, Brodmann area 40	Left	-48	-34	43	6.969985	0.000024
Precentral Gyrus, Brodmann area 6	Left	-42	-10	28	3.810901	0.002888
Inferior Parietal Lobule, Brodmann area 40	Left	-48	-31	55	3.459546	0.005337
Insula, Brodmann area 13	Left	-45	-1	4	3.758689	0.003162
Middle Temporal Gyrus, Brodmann area 21	Left	-45	-1	-26	3.816202	0.002862
Postcentral Gyrus, Brodmann area 43	Left	-51	-10	22	3.654589	0.00379
Superior Temporal Gyrus, Brodmann area 22	Left	-54	-25	4	3.442427	0.005501
Middle Temporal Gyrus, Brodmann area 21	Left	-54	5	-11	3.695316	0.00353
Precentral Gyrus, Brodmann area 6	Left	-60	-1	13	5.024421	0.000387
Supramarginal Gyrus, Brodmann area 40	Left	-60	-43	28	4.075996	0.001833
Inferior Parietal Lobule, Brodmann area 40	Left	-66	-25	34	3.753267	0.003192