5-7-2011

Deficits in Auditory, Cognitive, and Motor Processing Following Reversible MCAO in Mice: Understanding the Human Stroke Phenotype

Dongnhu Truong
dongnhu.truong@uconn.edu

Deficits in Auditory, Cognitive, and Motor Processing Following Reversible MCAO in Mice: Understanding the Human Stroke Phenotype

Dongnhu Thuy Truong

B.S., University of Connecticut, 2009

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Arts

at the

University of Connecticut

2011

APPROVAL PAGE

Master of Arts Thesis

Deficits in Auditory, Cognitive, and Motor Processing Following Reversible MCAO in Mice: Understanding the Human Stroke Phenotype

Presented by

Dongnhu Thuy Truong, B.S.

Major Advisor _____
R. Holly Fitch

Associate Advisor _____
Louise D. McCullough

Associate Advisor _____
Etan J. Markus

University of Connecticut

2011

ACKNOWLEDGMENTS

First of all, I would like to thank my Major Advisor, Dr. Holly Fitch, as well as my Masters advisory committee, Dr. Etan Markus and Dr. Louise McCullough, for their invaluable guidance and support through my graduate career thus far at the University of Connecticut. Thank you for helping me grow as a person and scientist, continuing to nurture and challenge me in my pursuit of understanding the intricacies of the brain. I am also grateful for your patience in scheduling (and rescheduling) my Masters Defense date; third time's a charm!

To Dr. Venu Venna -- thank you for taking time out of your busy schedule to conduct the surgeries for this study. It would not have been possible without your help!

To my colleagues, past and present: Dr. Steven Threlkeld, Courtney Hill, Caitlin Szalkowski, Michelle Alexander, and Amanda Smith; thank you all for not only your constant help and advice, but also your friendship -- offering some respite in this sometimes stressful environment.

I would also like to thank my family -- I would not be the person I am today without you. To my mother and father, Kimnga and Nang Truong -- you left your home and suffered many hardships just so that you could give me the greatest gift of all; the opportunity to follow and fulfill my dreams. I cannot thank you both enough for all that you have given me, and your determination will always be an inspiration to me. To my husband, Justin Grego – thank you for always being my "rock", giving me strength and stability whenever I need it. You are my constant in the many variables of life.

Finally, I would like to thank the University of Connecticut; most importantly the Psychology Department front office for making sure my life as a graduate student is in order, and the Bousfield OARS staff for taking wonderful care of my subjects!

This research was supported by funding from the University of Connecticut Research Advisory Council, UCHC/Storrs and Regional Campus Incentive Award to RHF and LM.

ABSTRACT

Stoke is characterized by a loss or alteration in neurological and/or bodily function resulting from a cerebral vascular accident (interruption of blood flow to the brain). Acute ischemic stroke is the third leading cause of death in the United States, and the leading cause of long-term functional disability in adults. Stroke-induced deficits may include various forms of aphasia (language loss), cognitive deficits (including attentional and memory impairments), and motor impairments. Through the use of animal models, researchers can experimentally induce "stroke-like" injuries comparable to those seen in clinical populations. Such models allow us to study and understand the neurophysiological, anatomical, and neurobehavioral consequences associated with ischemic insults to the brain. Middle cerebral artery occlusion (MCAO) can be induced in rodents, and is a widely used experimental technique to model focal ischemia in rodents. Various neurobehavioral tasks have been developed to assess the motor and cognitive dysfunctions associated with MCAO in rodents, and these studies have shown deficits related to impaired long-term sensorimotor function, as well as retention of spatial memory.

The current study was designed to develop a more comprehensive neurobehavioral profile associated with experimental focal cerebral ischemia induced by transient MCAO in adult C57Bl/6 mice. Using a modified pre-pulse inhibition auditory discrimination paradigm, and other tasks thought to tap language-related processing, mice subjected to 60 minute MCAO or Sham injury were assessed. These tasks were selected based on evidence that rapid auditory processing (RAP) skills are associated with language processing indices in clinical populations, as well as infant research showing

that early RAP scores are predictive of language development. Importantly, deficits in the encoding of temporal sound features have also been associated with deficits in speech perception in elderly listeners and aphasics. In addition, cognitive and sensorimotor ability was also evaluated using the Morris water maze, non-spatial water maze, and rotarod task. Combined behavioral results from post-MCAO mice provide evidence of a RAP deficit (suggesting "aphasia-like" deficits), and deficits in learning and memory, as well as sensorimotor function. Overall results support the ongoing use of MCAO mice as a valid model to study ischemic stroke in humans, and further suggest that language-related tasks can be used to model "aphasia-like" deficits in rodents.

1. Introduction

Ischemic stroke is defined by a loss or alteration of neurological function as a result of an interruption of blood flow to the brain. It is the third leading cause of death in the United States, and the leading cause of long-term functional disability (Writing Group Members *et al.*, 2008). Ischemic strokes account for 87% of all strokes, and typically arise from a blockage of the middle cerebral artery (MCA) -- which supplies blood and oxygen to the temporal, anterolateral frontal, and parietal lobes (Rordorf *et al.*, 1998; Writing Group Members *et al.*, 2008). Depending on the location of MCA occlusion, considerable variability exists in the localization and size of the resulting infarct (Rordorf *et al.*, 1998). Likewise, variability also exists in the neurobehavioral abnormalities associated with ischemic stroke. Stroke-induced deficits may include various impairments in cognition (including attention and/or memory) or motor function, but one of the most commonly developed post-stroke disorder is aphasia, with a post-stroke incidence of 21-38% (Ilvonen *et al.*, 2001; Påhlman *et al.* 2011; Stephens *et al.*, 2004).

1.1 Auditory Processing Deficits in Aphasia

Aphasia is defined as an acquired language disorder involving the disturbance of one or more previously functional modalities of language (Darley, 1982), and clinical research shows that aphasia may in turn be linked to disturbances in auditory processing and phonemic discrimination (Becker & Reinvang, 2007b; Hessler *et al.*, 2010; Ilvonen *et al.*, 2001; Ilvonen *et al.*, 2003; Ilvonen *et al.*, 2004; Jauhiainen & Nuutila, 1977; Miceli *et al.*, 1980; Tallal & Newcombe, 1978; Talvitie *et al.*, 2010; Varney, 1984). In particular, patients diagnosed with aphasia (secondary to left hemisphere lesions) show difficulties in performing tasks associated with auditory temporal processing of not only

verbal, but also nonverbal stimuli (Hessler *et al.*, 2010; Stefanatos *et al.*, 2007; Tallal & Newcombe, 1978; Talvitie *et al.*, 2010). For example, elongation of formant transitions between stop consonant-vowel sounds to 80 ms was found to facilitate auditory detection of different stop consonant-vowel pairings in aphasics, whereas formant transitions used in normal speech (~40 ms) led to poorer discrimination (Tallal & Newcombe, 1978). In a recent study utilizing nonverbal stimuli that mimicked formant transitions used in typical speech, Stefanatos *et al.* (2007) provides evidence that aphasics with left hemisphere lesions have reduced discrimination of rapidly presented silent gaps within narrowband white noise as compared to healthy controls. However, while these studies report behavioral evidence of auditory processing deficits among aphasics, such paradigms cannot dissociate perceptual changes from potential underlying deficits in pre-attentive auditory processes (neural processing) or attention (Hessler *et al.*, 2010; Jauhiainen & Nuutila, 1977; Miceli *et al.*, 1980; Stefanatos *et al.*, 2007; Tallal & Newcombe, 1978; Varney, 1984). Use of event related potentials (ERP) and mismatch negativity paradigms (MMN) has, however, allowed researchers to assess these pre-attentive neural components of sound processing and discrimination in aphasic patients.

In brief, MMN is observed electrophysiologically in the auditory cortex as a passive response to a deviant auditory stimulus within a repetitive acoustic stimulus, and MMN has been widely used in the clinical aphasic population to assess underlying neural processing abnormalities using various auditory stimuli (i.e., nonverbal tone deviants, phonemic discrimination, and auditory processing of rapid temporal sounds). Such studies have generally found either a diminished or nonexistent MMN component in aphasics with primary left hemisphere lesions (due to stroke) when compared to healthy

controls on auditory MMN paradigms. Tasks have included discrimination of variants in vowel sounds, syllable changes, and differences in the duration of acoustic information. Cumulative results show that aphasics are unable to effectively process auditory information at the neural level of the auditory cortex, regardless of whether stimuli are verbal or non-verbal in nature (Becker & Reinvang, 2007a; Becker & Reinvang, 2007b; Ilvonen *et al.*, 2001; Ilvonen *et al.*, 2003; Ilvonen *et al.*, 2004; Pettigrew *et al.*, 2005; Talvitie *et al.*, 2010). As one example, Ilvonen *et al.* (2001) examined passive and active discrimination requiring distinction of a 25 ms or 50 ms tone from a standard repeating 75 ms tone. Results showed that subjects with aphasia (secondary to left hemisphere stroke) not only presented with reduced MMN responses in the left hemisphere in comparison to controls when passively discriminating a 25 ms deviant tone from the standard, but also showed lower detection of the 25 ms tone when asked to actively respond to the deviant. For the 50 ms deviant condition, control subjects performed comparably to the aphasic subjects, with both groups showing minimal discrimination of the 50 ms deviant tone relative to the standard 75 ms tone. The results from this study suggest that for controls, a difference in duration between a 25 ms deviant and a 75 ms tone is large enough for significant passive (MMN) and active (correct response) detection of the deviant tone. However, for aphasic subjects, the difference may be insufficient to perceive the two stimulus durations as different under both passive and active conditions. The use of the MMN paradigm to supplement data from behavioral tasks clearly strengthens the notion that disrupted auditory processing associated with aphasia is not solely due to impairments in attention, and provides strong evidence of

specific rapid auditory temporal processing deficits among aphasic patients which may in turn contribute to language difficulties.

1.2 Middle Cerebral Artery Occlusion (MCAO): Rodent Models of Stroke

Transient occlusion of the middle cerebral artery in rodents is a widely used experimental technique to model human focal ischemia. Since stroke is implicated in various sensorimotor and cognitive deficits and represents one of the leading causes of long term disability in humans (Påhlman *et al.* 2011; Stephens *et al.*, 2004; Writing Group Members *et al.*, 2008), the applicability for neurobehavioral assessments to assess and better understand the motor and cognitive dysfunctions associated with MCAO in rodents are clear. In fact, MCAO models using rats and mice have revealed consistent evidence of sensorimotor deficits. (Bouët *et al.*, 2007; Ferrara *et al.*, 2009; Hunter *et al.*, 2000; Li *et al.* 2004). Cognitive deficits have also been found (albeit to a lesser extent) in rodent models of ischemic stroke, for example using passive avoidance tasks and Morris water maze tasks. However, such studies have shown considerable variability, which may be attributable in part to species and strain differences. In general, MCAO in rats leads to relatively consistent cognitive deficits as measured by both passive avoidance and Morris water maze tasks (DeVries *et al.*, 2001; Markgraf *et al.*, 1992; Modo *et al.*, 2000; Yamamoto *et al.*, 1988; Yonemori *et al.*, 1996). However, in the small body of literature assessing functional outcome in mice following MCAO, conflicting results have been found on passive avoidance and Morris water maze tasks (Bouët *et al.*, 2007; DeVries *et al.*, 2001; Gibson & Murphy, 2004; Gibson *et al.*, 2005; K. Klapdor & van der Staay, 1998; Li *et al.* 2004; Van der Staay *et al.*, 1992). Clearly, additional research on this important topic is warranted.

1.3 Current Study

Though rats have been commonly used to assess behavioral and functional outcome of MCAO as a model of ischemic stroke in humans, the emergent availability of transgenic and knockout mice -- and the application of these strains to the study of molecular mechanisms of stroke -- support the increasing use of the MCAO mouse model. Since only a small number of published studies have assessed the sensorimotor and cognitive outcome in mice with transient MCAO, the current study was designed to develop a more comprehensive neurobehavioral profile associated with experimental focal ischemia in C57Bl/6 mice. Specifically, the current study aimed to use tasks that were thought to tap language-related processing by: 1) assessing behavioral abnormalities that may result in "aphasia-like" deficits, using a modified pre-pulse inhibition paradigm to evaluate rapid auditory processing abilities; and 2) assessing cognitive and long term sensorimotor deficits using the Morris water maze, non-spatial water maze, and rotarod tasks to evaluate learning and memory as well as motor coordination. In the clinical literature, rapid auditory processing (RAP) ability is associated with language processing, and deficits in auditory temporal processing are found in both the elderly and aphasic patients as reviewed above (Hessler *et al.*, 2010; Ilvonen *et al.*, 2001; Talvitie *et al.*, 2010; Walton, 2010). In infants, RAP ability serves as a strong predictor of future language scores (Benasich & Tallal, 2002; Choudhury *et al.*, 2007), thus suggesting that the use of RAP may be able to model language-related cognitive loss associated with MCAO in rodents.

2. Methods

2.1 Subjects

A total of 22 male C57Bl/6 mice were ordered from Charles River Laboratories (Wilmington, MA) and arrived at the University of Connecticut (Bousfield vivarium), on postnatal day 37 (P37). Based on prior evidence that behavioral deficits and neural damage tend to be greater for males in rodent models of cortical disruption, coupled with evidence that stroke outcome in females varies depending on the estrous stage, only male subjects were used (Alkayed *et al.*, 1998; Fitch *et al.*, 1997; Herman *et al.*, 1997; Li *et al.*, 2011; Li *et al.* 2004; Peiffer *et al.*, 2002; Peiffer *et al.*, 2004). Upon arrival, subjects were single housed in standard lab cages (12 h light/dark cycle), with food and water available *ad lib*. Pre-MCAO behavioral testing began on P43 and continued through P54. Post-MCAO behavioral testing began on P60 and continued through P134. On P57 - P58, subjects were weighed in preparation for surgery (target weight range 20.3 – 25.3 g) and were assigned to receive either a middle cerebral artery occlusion (MCAO), sham surgery, or a sham surgery with external carotid artery cauterization. A preliminary auditory assessment was also performed to ensure that each group displayed comparable mean scores, and this was in fact found to be the case (no pre-surgical group differences). Following the completion of post-MCAO behavioral testing, all subjects were weighed, anesthetized, and transcardially perfused.

2.2 Surgical Procedures: Ischemic Model

Cerebral ischemia was induced via 60 minutes of reversible MCAO under isoflurane (1%) anesthesia, as described in detail in McCullough *et al.* (2004). Briefly, a ventral midline incision in the neck was made, and unilateral MCAO was induced by

inserting a 6.0 silicone coated monofilament into the right internal carotid artery via the external carotid artery stump. Following occlusion, the incision was sutured and mice were allowed to awake from anesthesia. Sixty minutes after ischemic onset (prior to reperfusion), mice were assessed for neurological deficits. Deficits were scored using the following rubric on a scale from 0-5: 0=no deficit, 1=forelimb weakness and torso turning to ipsilateral side when held by the tail, 2=circling to affected side, 3=unable to bear weight on affected side, 4=no spontaneous locomotor activity or barrel rolling, and 5=dead. Immediately after scoring for neurological deficits, MCAO mice were re-anesthetized for removal of the monofilament (reperfusion). A laser doppler blood flow monitor (Moor Instruments) was used to measure the relative blood flow within the middle cerebral artery prior to and during the occlusion to ensure a measurable reduction in blood flow.

Sham operated mice underwent the same surgical procedure as the MCAO mice, excluding the insertion of the suture up the right internal carotid artery into the middle cerebral artery. Sham operated mice with external carotid artery cauterization (sham ECA) received the same treatment as sham operated mice, except the external carotid artery was cauterized (as in MCAO mice, but without filament insertion). These two sham groups were compared to confirm that potential behavioral deficits found in MCAO mice were due to MCAO, and not cauterization of the external carotid artery alone.

Following recovery (7 days post surgery with 50% MCAO mortality; weight: Sham=20.97 g, SEM ± 0.55; Sham ECA=20.63 g, SEM ± 0.55; MCAO=17.70 g, SEM ± 0.82; [$F(2,13)=7.35$, $p<0.01$]), the total remaining n was as follows: n=6 Sham; n=4 Sham ECA; and n=6 MCAO.

2.3 Auditory Testing

Startle Reduction Paradigm

The startle reduction paradigm measures the acoustic startle reflex (ASR) -- a large amplitude involuntary response for each subject following the presentation of a startle eliciting stimulus (SES). When a subject is able to detect a pre-pulse cue presented just prior to the SES, the ASR response is attenuated (pre-pulse inhibition). Thus an uncued SES (i.e., one that does not follow a pre-pulse cue) should evoke a greater ASR response in relation to a cued SES. Based on this expected ratio, acoustic discrimination was assessed by comparing cued and uncued SESs on each task, for each subject. Attenuation was further measured by an "attenuation score" (attenuation score = [cued trial/uncued trial]*100), with an attenuation score of 100% indicating a chance response (no difference in the startle reflex for cued and uncued trials). A score below 100% indicates a reduction in startle response during cued trials, and thus detection/discrimination of the cue. In this study, the SES was a 105 dB, 50 ms white noise burst.

Equipment

Subjects were placed on individual load-cell platforms (Med Associates, Georgia, VT). The output from each platform was amplified (linear amp PHM-250-60 Med Associates) into a Biopac MP100WS Acquisition system connected to a Macintosh computer that recorded the amplitude of the startle reflex for each trial. Specifically, the amplitude of each subject's ASR was recorded in mV after the presentation of the SES by extracting the maximum peak value within the 200 ms signal period following the onset

of the SES. These values were coded for cued and uncued trials, and provided a mean absolute response amplitude for cued vs. uncued trials. Auditory stimuli were produced using a Dell Pentium IV PC with custom programmed software and a Tucker Davis Technologies real time processor. Sound files were created and played using a custom program (RPvdsEx), and delivered via powered Cambridge Sound Works speakers located approximately 53 cm above each platform.

Normal single tone

Data collected from this task provided an attenuation baseline score for each subject, thus demonstrating whether subjects had intact (normal) hearing and normal pre-pulse inhibition. This normal single tone task was comprised of 104 cued/uncued trials presented pseudorandomly through the session, and a variable inter-trial interval (ITI) ranging from 16-22 seconds in length was used. Uncued trials were comprised of a silent background with a SES, and cued trials were defined by the presentation of a 50 ms, 75dB, 2300 Hz, tone pip, 50-100 ms prior to the SES. Pre-surgery testing was performed on P43, and post-surgery testing took place on P63 and P81 (5 and 23 days post-MCAO, respectively). All subjects were found to be able to detect the pre-pulse cue -- both pre and post-surgery (i.e., successfully hear and attenuate their startle response) before and after surgery -- and thus all subjects were included in all analyses (Figure 2).

Silent Gap Procedure

The silent gap task consisted of a broadband white noise background, with embedded silent gaps (cues) of variable duration presented 100 ms prior to the SES. On uncued trials, silent gap duration was equal to 0 ms (i.e., no gaps were used). ITI for the

silent gap task varied from 16-24 seconds throughout the 300 trial session, and two different variations of the silent gap test were used -- long gap duration and short gap duration. The long gap task consisted of trials containing silent gap cues ranging from 50 to 300 ms in length, while the short gap task consisted of silent gap cues ranging from 2 to 100 ms in duration. Pre-surgery testing was conducted for the long-gap task only (3 days) and began on P52-54 (3-5 days pre-MCAO). Post-surgery testing for the long-gap (5 days) followed by the short-gap task (8 days) began on P64 and continued on through P80 (6-22 days post-MCAO).

2.4 Sensorimotor Assessment

Rotarod Procedure

The rotarod task was used to assess balance and motor coordination of all subjects. Individual mice were placed on a rotating cylindrical drum accelerating from 4 rotations per minute (rpm) to 40 rpm, over a span of 2 minutes. Subjects were given 4 trials on the rotarod per day of testing. The latency for the subject to fall from the rotating drum was recorded on each trial (in seconds), and the average latency was used for further analysis. Pre-surgery testing was not conducted on the rotarod task; however, subjects were assessed for their ability to remain on the rotating drum for at least 20 seconds at the lowest rotational speed (4 rpm) prior to post-surgical testing. All subjects were able to remain on the cylinder under those conditions, and were thus included in further testing. Rotarod testing was performed 2, 4, 7, 14, 21, 28, 42, and 56 days post-surgery.

2.5 Maze Learning

Water Escape (Visual Platform) Procedure

The water escape task occurred on P109 (51 days post-MCAO), and was used as a control procedure to ensure that subjects did not have underlying motor or visual impairments preventing them from effectively performing the subsequent series of water maze tests. Subjects were placed in one end of an oval tub (103 cm x 55.5 cm) filled with room temperature water (21 cm), and were required to swim to a visible platform (8.5 in. cm diameter) at the other end of the tub (i.e., opposite to where they were released). Latency to the visible platform was recorded. Using this task, it was found that one subject had to be dropped from further testing and analyses due to impaired swimming ability. All other subjects effectively performed the task, showing no effect of MCAO as measured by latency to the platform.

Morris Water Maze

Following water escape, the Morris water maze was used to assess subjects' learning and memory ability over a period of 5 testing days. Subjects had to locate a submerged, invisible, platform (8.5 cm in diameter) located 2 cm. below the surface of the water. The submerged platform remained in a fixed location (southeast quadrant) within a round black tub (122 cm diameter). The tub was surrounded by extra maze cues (varying shapes painted on testing room wall, location of experimenter, door, etc.) which remained fixed throughout the 5 testing sessions. Each day, subjects were given 4 trials (separated by approximately 5 minutes) and a maximum of 45 seconds per trial to find the hidden platform. For each trial, subjects were placed into the tub at a random

compass point (north, south, east, west), with each compass point used once per test day. On day 1 of Morris water maze testing (prior to the first trial), subjects were placed on the submerged platform for 10 seconds, removed from the platform, and then placed back into the water at one of the compass locations. Latency to reach the platform on each of the 4 trials was measured using a stopwatch and recorded for all subjects on all days. The total sum of the latencies to reach the target platform during all 4 trials was calculated for each individual subject on each individual testing day, and this measure was used for further statistical analysis. Morris water maze testing took place on P113 and continued through P117 (55-59 days post-MCAO).

Non-spatial Water Maze

The non-spatial water maze is similar goal of finding a submerged, hidden, platform (8.5cm in diameter) within a round tub (122 cm diameter). However, unlike the Morris water maze, the round tub contained a circular, black, rotating insert with 4 painted intramaze cues (i.e., quadrants marked by vertical black and white stripes, horizontal black and white stripes, black polka dots on a white background, and white polka dots on a black background). For this task, the location of the submerged platform was not fixed, but instead was always paired with the vertical black and white striped intramaze cue. Thus, for this task, the subjects could not rely on extra-maze cues to determine the location of the platform, but now had to use local (intra-maze) cues to find the hidden platform. Subjects were given 4 trials and a maximum of 45 seconds per trial to locate the platform during each test day. Subjects were placed into the pool at the same compass location (north). However, after each individual trial, the spatial location of the paired intramaze cue and platform was rotated randomly into one of the four

quadrants of the pool (southwest, southeast, northwest, northeast). On day 1 of non-spatial water maze testing and prior to the first trial, subjects were placed on the submerged platform for 10 seconds, removed from the platform, and then placed back into the water at the north compass point. Latency to reach the platform for each trial (different spatial location of cue/platform pair) was measured using a stopwatch and recorded for all subjects on all days. The total sum of latencies to reach the target platform was calculated for each individual subject on each day of testing, and this score was used for further statistical analysis. Non-spatial water maze testing occurred on P120 and continued through P124 (62-66 days post-MCAO).

2.6 Perfusion and Histology

Following behavioral testing, subjects were weighed, anesthetized with ketamine/xylezine (100mg/kg/15mg/kg), and transcardially perfused with 0.9% saline followed by 10% formalin. Brains were extracted from the skull and post fixed in 10% formalin. For histological preparation, brains were coronally sectioned at 60 µm on a vibratome (Leica VT1000 S). One out of every fourth section was mounted, stained for Nissl using thionine, and then coverslipped for analysis.

Prepared tissue was analyzed using a MicroBright Field (Williston, VT) Stereo Investigator system integrated with a Zeiss Axio Imager A2 microscope with a motorized stage. The Cavalieri's estimator probe, provided by the Stereo Investigator system, was used to assess ventricular and cortical volumes as an index of damage for each individual subject. The right and left hemispheres of each area were estimated separately, with every fourth section sampled for the ventricles and every eighth section sampled for

cortex.

3. Results

3.1 Pre-MCAO Auditory Testing

Silent Gap 0-300 ms

After assignment of each subject to an experimental condition, but before surgery, a 3 (Treatment: Sham, Sham w/cauterized ECA, MCAO) x 3 (Day) x9 (Gap) repeated measures ANOVA was conducted to ensure that all 3 groups performed comparably on the silent gap 0-300 ms task. In fact, all groups showed similar attenuation during cued trials [$F(2,13)=.14$, $p>0.05$]. In addition, a comparison of the mean ASR between the cued and uncued trials using a paired samples t-test showed a significant difference between the raw cued and uncued responses overall ($p<0.05$), indicating effective auditory discrimination of the cue across all gaps for all groups (Figure 1).

3.2 Post-MCAO Auditory Testing

Normal Single Tone

A paired samples t-test was used to compare mean raw cued and uncued ASR responses to ensure detection of the cue for this baseline control task. Results revealed significant detection of the auditory cue on the Normal Single Tone task in all groups, providing evidence for normal hearing and pre-pulse inhibition responses ($p<0.05$). In addition, a one way ANOVA revealed no main effect of Treatment on both days of post-MCAO Normal Single Tone testing [$F(1,14)=0.27$, $p>0.05$] and [$F(1,13)=0.78$, $p>0.05$], with testing on P63 and P81 (5 and 23 post-MCAO, respectively). This result indicated

that both Sham and MCAO mice had comparable hearing and pre-pulse inhibition responses (Figure 2).

Justification for pooling Shams

To ensure that potential behavioral anomalies found in the MCAO induced mice were due to MCAO, and not an artifact of cauterizing the external carotid artery (ECA) during the MCAO procedure, a surgical sham group was used to control for this potential confound. Analyzing the data obtained from the Silent Gap 0-300 ms task with a 2 (Treatment: Sham, Sham ECA) x 5 (Day) x 9 (Gap) repeated measures ANOVA revealed no differences in attenuation score between the surgical shams and surgical ECA shams [$F(1,8)=0$, $p>0.05$]. Similar lack of effects were seen on all behavioral tasks and also for *post mortem* anatomic measures. Thus, the sham groups were pooled for all further analyses. Thus, total n for post-surgery analyses of surgical shams was n=10.

Silent Gap 0-300 ms

A comparison of mean cued and uncued ASR responses for each gap category was conducted using a paired samples t-test, revealing significant detection of the cue at all Gap duration for Sham treated mice ($p<0.05$). For the MCAO treated mice, significant detection of the cue was found at nearly all of the gaps ($p<0.05$) except for the 50 ms gap ($p>0.05$), which was the shortest, and thus most difficult to detect in the given series of trials. Further, a 2 (Treatment: Sham, MCAO) x 5 (Day) x 9 (Gap) repeated measures ANOVA revealed no main effect of Treatment [$F(1,14) = 2.49$, $p>0.05$]. However, there was a significant Treatment x Gap interaction [$F(8,112) = 2.83$, $p<0.01$] and a Treatment x Gap x Day interaction [$F(32,448)=1.53$, $p<0.05$]. The two way

interaction between Treatment x Gap reflects the fact that the Sham group performed significantly better on the longer gaps (175-300 ms) in comparison to the MCAO group (Figure 3), while the shorter gaps (50-150 ms) were too difficult for both the sham and MCAO groups to perform. The three way interaction between Treatment x Gap x Day indicates a greater "learning effect" (i.e., improvement over Days) at the longer gaps for the Sham group (data not shown).

Silent Gap 0-100 ms

Using a paired samples t-test to assess mean raw cued and uncued ASR responses, sham mice were found to show significant detection of the auditory cue at most of the silent gaps ($p<0.05$), excepting the shorter (more difficult) gaps of 2, 5, and 20 ms duration ($p>0.05$). MCAO treated mice did not show significant detection *at any* of the silent gaps, except for the longer (and thus easier to detect) gaps of 75 and 100 ms ($p<0.05$). A marginal, but not significant, main effect of Treatment was found between the Sham and MCAO groups using a 2 (Treatment: Sham, MCAO) x 8 (Day) x 9 (Gap) repeated measures ANOVA [$F(1,13) = 3.60$, $p<0.1$], suggesting that Shams were performing better overall on the task. In addition, there was a significant Treatment x Gap interaction [$F(8,104) = 3.21$, $p<0.01$] (Figure 3), as well as a significant Treatment x Gap x Day interaction [$F(56,728)=1.57$, $p<0.001$]. As in the Silent Gap 0-300 ms task, the two way interaction between Treatment x Gap indicates the Sham group more effectively performed the task at the longer gaps (40-100 ms) in comparison to the MCAO group, while the shorter gaps (2-30 ms) were more difficult for both groups. The three way interaction between Treatment x Gap x Day, again indicates a stronger learning (Day) effect at the longer gaps for the Sham group (data not shown).

3.3 Sensorimotor Task: Rotarod

A 2 (Treatment: Sham, MCAO) x 8 (Day) repeated measures ANOVA revealed a main effect of Treatment [$F(1,13)=21.23$, $p<.001$], indicating that Shams performed better overall on the task. A one-way ANOVA was used to further analyze potential group differences for each individual day of rotarod testing. While no significant difference in performance was found on the first day of rotarod testing (2 days post-surgery) [$F(1,14)=2.41$, $p>0.05$], subsequent days of testing showed statistically better performance of the Sham treated mice in comparison to MCAO treated mice (Figure 5).

3.4 Maze Tasks

Morris Water Maze

Analysis using a 2 (Treatment: Sham, MCAO) x 5 (Day) repeated measures ANOVA revealed a main effect of Treatment [$F(1,12)=8.06$, $p<.05$] on the Morris water maze. This main effect suggests that Shams overall performed significantly better than the MCAO mice, with a shorter mean latency to find the target platform. Simple effects also showed a significant effect of Day [$F(4,48)=9.41$, $p<.001$], indicating that as the days of testing progressed, both the Sham and MCAO treated groups improved in performance. Further analysis using a one-way ANOVA found significant Treatment differences in performance on days 3 and 5 of Morris water maze testing, with Shams completing the task faster than MCAO mice.

Non-Spatial Water Maze

A significant main effect of Treatment was found between the Sham and MCAO groups when using a 2 (Treatment: Sham, MCAO) x 5 (Day) repeated measures ANOVA [$F(1,12)=7.13$, $p<0.05$], showing that Sham treated mice performed significantly better in comparison to MCAO treated mice. As in the Morris water maze task there was a significant effect of Day [$F(4,48)=2.75$, $p<0.05$], indicating that as the days of testing progressed on the non-spatial water maze both the Sham and MCAO treated mice improved significantly on the maze. A one-way ANOVA analyzing group differences within each individual day found a significant difference in performance on the fourth day of non-spatial water maze testing, with the Shams performing significantly better than the MCAO group (Figure 7).

3.5 Histology

Cortical Volume

A 2 (Treatment: Sham, MCAO) x 2 (Side: Right, Left) repeated measures ANOVA found a marginal main effect of Treatment on the volume of the cortex [$F(1,13) = 3.78$, $p<0.1$], with larger volumes in Shams. A significant effect of Side [$F(1,13) = 9.41$, $p<0.01$] and a Treatment x Side interaction [$F(1,13) = 9.14$, $p<0.01$] was also found. The effect of Side may indicate that in general, the right cortex was smaller than the left cortex overall. However, the significant two way interaction reveals that the differential relationship between the cortical volumes of the right and left sides are likely "pulled" by asymmetry resulting from unilateral injury in MCAO. However, additional investigation using a paired samples t-test to compare the volume of the right and left

cortex in MCAO treated mice found no significant difference in size between the two hemispheres ($p>0.05$), although this may be attributable to the small n (Figure 8).

Ventricles

A main effect of Treatment was found using a 2 (Treatment: Sham, MCAO) x 2 (Side: Right, Left) repeated measures ANOVA [$F(1,13) = 10.67$, $p<0.01$]. This effect showed a significant overall difference in ventricular size between Sham and MCAO groups. In addition to the main Treatment effect, a significant effect of Side [$F(1,13) = 22.96$, $p<0.001$], as well as a significant Treatment x Side interaction [$F(1,13) = 18.82$, $p<0.001$], were also seen. The effect of Side suggests that the right ventricle overall was larger than the left ventricle, but differences in size between the right and left ventricles in the MCAO treatment mice may have specifically "pulled" this effect. Further analysis using a paired samples t-test comparing right and left ventricular size in the MCAO treated mice in fact revealed a significant difference in ventricular size ($p<0.05$), with larger values on the right side (Figure 9).

Correlation between index of damage and behavior

An index of damage as illustrated by ventricular volume and cortical volume was obtained for each subject by determining the ratio between right hemisphere volume versus left hemisphere volume for the given area. This index was then used in a bivariate linear regression analysis to determine the relationship between the index of damage of the ventricles and cortex separately (x), versus behavioral indices as measured by: the mean of longer gaps (40-100 ms) in the Silent Gap 0-100 ms task; the rotarod task; the Morris water maze; and non-spatial water maze (y). Results revealed no significant

linear relationships between any indices of damage to any behavioral task, likely due to the very small n.

4. Discussion

4.1 Auditory Processing

Prior to any surgical manipulation on the subjects, a pre-MCAO auditory screening using the Silent Gap 0-300 ms task was conducted on all mice to determine baseline rapid auditory processing and pre-pulse inhibition ability. It was found during pre-MCAO testing that both Treatment groups were not only able to significantly detect the rapid auditory cues for each silent gap condition, but that both groups performed the task comparably (with no significant differences in auditory discrimination and pre-pulse inhibition ability). After surgery, MCAO treated mice showed a significant deficit in rapid auditory processing ability as compared to Sham treated mice on the Silent Gap 0-300 ms task, with Sham treated mice still able to effectively discriminate the silent gap at all levels of the 0-300 ms paradigm, but MCAO treated mice unable to discriminate the 50 ms tone (and consequently the most difficult condition within that series). Such a finding alone comparing the pre and post test on the 0-300 ms task provides strong evidence that MCAO may alter subsequent rapid auditory processing ability.

Results from the Silent Gap 0-100 ms task also support a rapid auditory processing deficit associated with MCAO in mice. In the task, not only were MCAO treated mice not performing the task by discriminating the shorter gaps (i.e., they showed significant discrimination only at the 75 and 100 ms gaps), but they were performing

significantly worse than the Sham treated mice on the conditions they were able to effectively discriminate.

The rapid auditory processing results presented here appear to compliment the work of Stefanatos *et al.* (2007), who assessed the auditory temporal processing of aphasic patients using similar auditory stimuli. In that study, it was found that aphasics presented with difficulties identifying silent gaps within white noise when the breaks in acoustic stimuli were short in duration as compared to long, and aphasics performed worse in the discrimination task compared to healthy controls. However, Stefanatos *et al.* (2007) also raised concerns in terms of parsing out any rapid auditory processing deficits from potential confounding attention deficits. That is, significant processing deficits could reflect subjects' inability to attend to the task. To help address this issue in the current paradigm, our subjects were given an auditory control task (Normal single tone). Results of this task showed that all subjects could discriminate a pre-pulse cue and could effectively attenuate their startle response, arguing against attention deficits. However, to provide a stronger argument for a rapid auditory processing deficit, additional means to assess preattentive auditory discrimination in mice would be useful. Within the human literature, the use of ERPs and MMN paradigms have given added means to parse out the aspects of rapid auditory processing and attention at a neural level. For example, Ilvonen *et al.* (2001) and (2003) used MMN to show impairment in the discrimination of temporally dependent auditory information at the preattentive level within the auditory cortex of aphasics. Since these and related studies in humans have shown impairments in rapid auditory processing at both the preattentive (MMN) and active discrimination (respond upon conscious discrimination) level in aphasics (thus eliminating attention

deficits), it would be useful for future studies to likewise assess preattentive rapid auditory processing in the MCAO mouse using an ERP signal analogous to the MMN component in humans (Amann *et al.*, 2010; Umbricht *et al.*, 2005).

4.2 Sensorimotor Assessment: Rotarod

The current results showing a sensorimotor deficit in MCAO treated mice on the rotarod task are consistent with those found in previous studies. However, no study using mice has reported long term sensorimotor deficits (greater than 7 days post-MCAO) using the rotarod task (Bouët *et al.*, 2007; Ferrara *et al.*, 2009; Freret *et al.*, 2009; Hunter *et al.*, 2000). Previous studies typically found impaired performance within the first couple of days post MCAO surgery, with MCAO mice showing full recovery of function and comparable performance to shams by approximately 7 days after surgery (Bouët *et al.*, 2007; Ferrara *et al.*, 2009; Freret *et al.*, 2009; Hunter *et al.*, 2000). Our results, conversely, showed continued sensorimotor deficits in the MCAO treated mice on the rotarod 56 days post surgery, with no sign of improvement on the task.

Such findings do bring up the issue that impaired performance could have been attributed to poor initial coordination, regardless of surgery condition, thus explaining the lack of improvement on MCAO mice rotarod ability. In the present study, subjects were not pre-tested on the rotarod prior to any surgical manipulation. Because of this, the argument could be made attributing the poor performance post-MCAO to poor coordination prior to MCAO treatment. Although this could contribute to the overall results found during the 8 test sessions ranging from 2-56 days post-MCAO, it should be noted that the first day of testing (2 days post-surgery) revealed comparable performance

on the rotarod task across both groups. This indicates that regardless of MCAO treatment, the sham operated mice showed similar levels of balance and coordination on the first exposure to the task. If there was unintentional grouping of all the "uncoordinated" mice into the MCAO treated group, and all the "well coordinated" mice into the sham treated group, then even on the first day exposure to the task, a significant difference in ability to remain on the rod should have been found. Interestingly enough, as the days of testing continued, sham treated mice progressively improved on the task until their abilities showed a "ceiling" 7 days post-surgery. Conversely, the MCAO treated mice never improved on the task. These results support a long term sensorimotor deficit in MCAO treated mice.

4.3 Water Maze Assessment

The Morris water maze is a widely used cognitive assessment of spatial learning and memory. Moreover, Morris water maze performance has been well studied in the *rat* model of MCAO, with results indicating increased latencies to reach the hidden platform in comparison to sham operated controls (Markgraf *et al.*, 1992; Modo *et al.*, 2000; Sadamoto *et al.*, 1998; Yamamoto *et al.*, 1988; Yonemori *et al.*, 1996). In contrast, within the limited body of research assessing Morris water maze ability in MCAO operated mice, results have been variable, thus making a general consensus on performance related to MCAO in mice inconclusive (Bouët *et al.*, 2007; Gibson & Murphy, 2004; Gibson *et al.*, 2005; K. Klapdor & van der Staay, 1998; Van der Staay *et al.*, 1992). The current study using the Morris water maze found a significant overall impairment in the MCAO mice. Specifically, in Morris water maze, treated mice took a longer time to locate the submerged platform in comparison to sham controls, indicating

difficulty in using and remembering spatial cues to obtain the relative location of the platform. Although impaired swimming speed secondary to MCAO could have proven to be a potential cofound to the significant results found in the current study, the use of the water escape task (visual platform) prior to water maze testing eliminated underlying deficits in swimming speed as well as visual acuity in MCAO subjects as causal factors for subsequent group differences.

Morris water maze data from the present study concurs with results obtained from Gibson *et al.* (2004) and (2005), but conflicts with studies conducted by Bouët *et al.* (2007), Klapdor *et al.* (1998), and Van de Staay *et al.* (1992) which all found no differences on Morris water maze performance for MCAO treated mice. Though surgical methods to induce MCAO in mice differed slightly from study to study, including differences in hemisphere receiving focal ischemia, the end result still led to effective occlusion of the middle cerebral artery with measurable infarction at the respective side of occlusion. Interestingly, when comparing methods between studies that did versus did not find an effect of MCAO performance, variations in mouse strains used could account in part for inconsistencies. Specifically, in the studies that found differential performance in the MCAO group (Gibson & Murphy, 2004; Gibson *et al.*, 2005), C57Bl/6 mice were used for testing (including the present study), while the other studies used either CFW1 or Swiss mice[1] (Bouët *et al.*, 2007; Van der Staay *et al.*, 1992).

[1] The study conducted by Klapdor *et al.* (1998) used C57Bl/6 as well, yet found no differences in post-MCAO performance. Unfortunately, this study differed from others since subjects received extensive training on a modified working memory Morris water maze task prior to surgery, adding potential factors that could confound interpretations. Therefore this study will not be addressed.

Regardless of MCAO treatment, a strain difference in Morris water maze performance is present (Klapdor & Van Der Staay, 1997). It had been found that C57Bl/6 mice in general were proficient on the Morris water maze task while the other strains assessed (CFW1, BALB, and NMRI), while competent on the task, were not as efficient as C57Bl/6. Since strain differences in performance were present in the Morris water maze prior to any manipulation, the rationale that the Morris water maze may not be sensitive enough to assess learning and memory in the MCAO model in certain strains is possible. If one strain was inherently not good on the task, a focal manipulation may not elicit an impairment in performance (floor effect). Thus a potential explanation for the inconsistencies using a mouse model of MCAO in Morris water maze could be that strains used were inherently capable of doing the task, but were not proficient enough to allow differences in Morris water maze performance to emerge in MCAO mice.

4.3.2 Non-Spatial Water Maze

The Non-spatial water maze used here was developed as a variation of the Morris water maze, but with the difference of a rotating insert containing salient painted pictures to act as a local cue for the location of the platform (Hyde *et al.*, 2002; Stoelzel *et al.*, 2002). By requiring the use of local cues to find the platform, the non-spatial water maze this provides a hippocampally independent task as compared to the hippocampally dependent Morris water maze (Hyde *et al.*, 2002; Stoelzel *et al.*, 2002). The present study found both Morris water maze and non-spatial water maze deficits in MCAO treated mice, thus suggesting that the deficits found in both tasks were associated with a global injury beyond focal infarct of the hippocampus secondary to MCAO.

4.4 Histology: Correlation between index of damage to behavior

A correlation analysis is useful in better understanding how neuroanatomical damage due to MCAO may relate to behavioral outcome, and studies have illustrated such a relationship between degree of damage and performance on a behavioral task (Bouët *et al.*, 2007; Grabowski *et al.*, 1991; Hudzik *et al.*, 2000). However, in the present study, no correlation was found between damage index and behavior despite significant behavioral findings. One likely explanation for the lack of correlation between significant behavioral results and index of damage could be attributed to the small n. For post-MCAO assessment, only 6 mice (out of the 12 original MCAO treated mice) survived to initial testing. Moreover, by the end of behavioral testing, only 5 remained for analysis. Because of this, there may have not been enough subjects to effectively predict behavior from damage index using either cortical or ventricular volumes.

5. Conclusions

In summary, a mouse model of ischemic stroke using C567Bl/6 mice reveals not only rapid auditory processing deficits similar to those seen in aphasic patients, but also long term sensorimotor and cognitive deficits. With the growing popularity and convenient use of transgenic and knockout mice to understand molecular mechanisms of stroke, the need to better understand the MCAO mouse model and its validity to effectively study clinical ischemic stroke is crucial. The results obtained from the current study are a step toward realizing the potential of this mouse model by effectively translating the auditory processing deficits found in human aphasics to MCAO mice. To better validate MCAO in mice as a model of rapid auditory processing deficits in aphasia, additional studies should assess potential contributions of MCAO impact on information

processing *per se* versus effects on auditory attention, for example by using ERP paradigms. When modeling ischemic stroke in MCAO from a cognitive and sensorimotor perspective, additional studies also need to be performed in order to examine potential strain differences. In addition, MCAO models utilizing both males and females need to be studied and compared, since previous investigations in clinical populations provide evidence of sexual dimorphism in not only behaviors and stroke severity/outcome, but rodent models of ischemic stroke have also found gender differences in the molecular mechanisms associated with neural cell death (Li et al. 2004; McCullough *et al.*, 2005; Niewada *et al.*, 2005; Siegel *et al.*, 2010).

In conclusion, the behavioral data obtained from the series of auditory processing, sensorimotor, and learning and memory experiments provides strong evidence supporting the ongoing use of mice as a model for functional impairments in clinical ischemic stroke.

References

Alkayed, N. J., Harukuni, I., Kimes, A. S., London, E. D., Traystman, R. J., Hurn, P. D., & Grady, P. A. (1998). Gender-linked brain injury in experimental stroke • editorial comment. *Stroke, 29*(1), 159-166.

Amann, L. C., Gandal, M. J., Halene, T. B., Ehrlichman, R. S., White, S. L., McCarren, H. S., & Siegel, S. J. (2010). Mouse behavioral endophenotypes for schizophrenia. *Brain Research Bulletin, 83*(3-4), 147-161.

Becker, F., & Reinvang, I. (2007a). Mismatch negativity elicited by tones and speech sounds: Changed topographical distribution in aphasia. *Brain and Language, 100*(1), 69-78.

Becker, F., & Reinvang, I. (2007b). Successful syllable detection in aphasia despite processing impairments as revealed by event-related potentials. *Behavioral and Brain Functions, 3*(1), 6.

Benasich, A. A., & Tallal, P. (2002). Infant discrimination of rapid auditory cues predicts later language impairment. *Behavioural Brain Research, 136*(1), 31-49.

Bouët, V., Freret, T., Toutain, J., Divoux, D., Boulouard, M., & Schumann-Bard, P. (2007). Sensorimotor and cognitive deficits after transient middle cerebral artery occlusion in the mouse. *Experimental Neurology, 203*(2), 555-567.

Choudhury, N., Leppanen, P. H. T., Leevers, H. J., & Benasich, A. A. (2007). Infant information processing and family history of specific language impairment: Converging evidence for RAP deficits from two paradigms. *Developmental Science, 10*(2), 213-236.

Darley, F. L. (1982). *Aphasia*. Philadelphia: W.B. Saunders Company.

DeVries, A. C., Nelson, R. J., Traystman, R. J., & Hurn, P. D. (2001). Cognitive and behavioral assessment in experimental stroke research: Will it prove useful? *Neuroscience & Biobehavioral Reviews, 25*(4), 325-342.

Ferrara, A., El Bejaoui, S., Seyen, S., Tirelli, E., & Plumier, J. (2009). The usefulness of operant conditioning procedures to assess long-lasting deficits following transient focal ischemia in mice. *Behavioural Brain Research, 205*(2), 525-534.

Fitch, R., Brown, C. P., Tallal, P., & Rosen, G. D. (1997). Effects of sex and MK-801 on auditory-processing deficits associated with developmental microgyric lesions in rats. *Behavioral Neuroscience, 111*(2), 404-412.

Freret, T., Bouet, V., Leconte, C., Roussel, S., Chazalviel, L., Divoux, D., Schumann-Bard, P., Boulouard, M. (2009). Behavioral deficits after distal focal cerebral

ischemia in mice: Usefulness of adhesive removal test. *Behavioral Neuroscience, 123*(1), 224-230.

Gibson, C. L., Bath, P. M. W., & Murphy, S. P. (2005). G-CSF reduces infarct volume and improves functional outcome after transient focal cerebral ischemia in mice. *Journal of Cerebral Blood Flow & Metabolism, 25*(4), 439.

Gibson, C. L., & Murphy, S. P. (2004). Progesterone enhances functional recovery after middle cerebral artery occlusion in male mice. *Journal of Cerebral Blood Flow & Metabolism, 24*(7), 813.

Grabowski, M., Nordborg, C., & Johansson, B. B. (1991). Sensorimotor performance and rotation correlate to lesion size in right but not left hemisphere brain infarcts in the spontaneously hypertensive rat. *Brain Research, 547*(2), 249-257.

Herman, A. E., Galaburda, A. M., Fitch, R. H., Carter, A. R., & Rosen, G. D. (1997). Cerebral microgyria, thalamic cell size and auditory temporal processing in male and female rats. *Cerebral Cortex, 7*(5), 453-464.

Hessler, D., Jonkers, R., & Bastiaanse, R. (2010). The influence of phonetic dimensions on aphasic speech preception. *Clinical Linguistics & Phonetics, 24*(12), 996.

Hudzik, T. J., Borrelli, A., Bialobok, P., Widzowski, D., Sydserff, S., Howell, A., Gendron, P., Corbett, D., Miller, J., Palmer, G. C. (2000). Long-term functional end points following middle cerebral artery occlusion in the rat. *Pharmacology Biochemistry and Behavior, 65*(3), 553-562.

Hunter, A. J., Hatcher, J., Virley, D., Nelson, P., Irving, E., Hadingham, S. J., & Parsons, A. A. (2000). Functional assessments in mice and rats after focal stroke. *Neuropharmacology, 39*(5), 806-816.

Hyde, L. A., Stavnezer, A. J., Bimonte, H. A., Sherman, G. F., & Denenberg, V. H. (2002). Spatial and nonspatial morris maze learning: Impaired behavioral flexibility in mice with ectopias located in the prefrontal cortex. *Behavioural Brain Research, 133*(2), 247-259.

Ilvonen, T., Kujala, T., Tervaniemi, M., Salonen, O., Näätänen, R., & Pekkonen, E. (2001). The processing of sound duration after left hemisphere stroke: Event-related potential and behavioral evidence. *Psychophysiology, 38*(4), 622-628.

Ilvonen, T., Kujala, T., Kozou, H., Kiesiläinen, A., Salonen, O., Alku, P., & Näätänen, R. (2004). The processing of speech and non-speech sounds in aphasic patients as reflected by the mismatch negativity (MMN). *Neuroscience Letters, 366*(3), 235-240.

Ilvonen, T., Kujala, T., Kiesilainen, A., Salonen, O., Kozou, H., Pekkonen, E., Roine, R.; Kaste, M., Naatanen, R. (2003). Auditory discrimination after left-hemisphere stroke: A mismatch negativity follow-up study. *Stroke, 34*(7), 1746-1751.

Jauhiainen, T., & Nuutila, A. (1977). Auditory perception of speech and speech sounds in recent and recovered cases of aphasia. *Brain and Language, 4*(4), 572-579.

Klapdor, K., & Van Der Staay, F. J. (1997). *Physiology & Behavior, 60*(5), 1247-1254.

Klapdor, K., & van der Staay, F. J. (1998). Repeated acquisition of a spatial navigation task in mice: Effects of spacing of trials and of unilateral middle cerebral artery occlusion. *Physiology & Behavior, 63*(5), 903-909.

Li, J., Siegel, M., Yuan, M., Zeng, Z., Finnucan, L., Persky, R., Hurn, P.D., McCullough, L. D. (2011). Estrogen enhances neurogenesis and behavioral recovery after stroke. *Journal of Cerebral Blood Flow & Metabolism, 31*(2), 425.

Li, X., Blizzard, K. K., Zeng, Z., DeVries, A. C., Hurn, P. D., & McCullough, L. D. (2004). Chronic behavioral testing after focal ischemia in the mouse: Functional recovery and the effects of gender. *Experimental Neurology, 187*(1), 94-104.

Markgraf, C. G., Green, E. J., Hurwitz, B. E., Morikawa, E., Dalton Dietrich, W., McCabe, P. M., Ginsberg,M.D., Schneiderman, N. (1992). Sensorimotor and cognitive consequences of middle cerebral artery occlusion in rats. *Brain Research, 575*(2), 238-246.

McCullough, L., Wu, L., Haughey, N., Liang, X., Hand, T., Wang, Q., Breyer, R., Andreasson, K. (2004). Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. *The Journal of Neuroscience, 24*(1), 257-268.

McCullough, L. D., Zeng, Z., Blizzard, K. K., Debchoudhury, I., & Hurn, P. D. (2005). Ischemic nitric oxide and poly (ADP-ribose) polymerase-1 in cerebral ischemia: Male toxicity, female protection. *Journal of Cerebral Blood Flow & Metabolism, 25*(4), 512.

Miceli, G., Gainotti, G., Caltagirone, C., & Masullo, C. (1980). Some aspects of phonological impairment in aphasia. *Brain and Language, 11*(1), 159-169.

Modo, M., Stroemer, R. P., Tang, E., Veizovic, T., Sowniski, P., & Hodges, H. (2000). Neurological sequelae and long-term behavioural assessment of rats with transient middle cerebral artery occlusion. *Journal of Neuroscience Methods, 104*(1), 99-109.

Niewada, M., Kobayashi, A., Sandercock, P. A. G., Kamiński, B., & Członkowska, A. (2005). Influence of gender on baseline features and clinical outcomes among 17,370 patients with confirmed ischaemic stroke in the international stroke trial. *Neuroepidemiology, 24*(3), 128.

Påhlman, U., Gutiérrez-pérez, C., Sävborg, M., Knopp, E., & Tarkowski, E. (2011). Cognitive function and improvement of balance after stroke in elderly people: The gothenburg cognitive stroke study in the elderly. *Disabil Rehabil,* , 1-11.

Peiffer, A. M., Rosen, G. D., & Fitch, R. H. (2002). Sex differences in rapid auditory processing deficits in ectopic BXSB/MpJ mice .*13*(17), 2277-2280.

Peiffer, A. M., Rosen, G. D., & Fitch, R. H. (2004). Sex differences in rapid auditory processing deficits in microgyric rats. *Developmental Brain Research, 148*(1), 53-57.

Pettigrew, C., Murdoch, B., Kei, J., Ponton, C., Alku, P., & Chenery, H. (2005). The mismatch negativity (MMN) response to complex tones and spoken words in individuals with aphasia. *Aphasiology, 19*(2), 131-163.

Rordorf, G., Koroshetz, W. J., Copen, W. A., Cramer, S. C., Schaefer, P. W., Budzik, R. F., Schwamm, L.H.; Buonanno, F., Sorensen, A.G., Gonzalez, G. (1998). Regional ischemia and ischemic injury in patients with acute middle cerebral artery stroke as defined by early diffusion-weighted and perfusion-weighted MRI. *Stroke, 29*(5), 939-943.

Sadamoto, Y., Igase, K., Sakanaka, M., Sato, K., Otsuka, H., Sakaki, S., Masuda, S., Sasaki, R. (1998). Erythropoietin prevents place navigation disability and cortical infarction in rats with permanent occlusion of the middle cerebral artery. *Biochemical and Biophysical Research Communications, 253*(1), 26-32.

Siegel, C., Turtzo, C., & McCullough, L. D. (2010). Sex differences in cerebral ischemia: Possible molecular mechanisms. *Journal of Neuroscience Research, 88*(13), 2765-2774.

Stefanatos, G. A., Braitman, L. E., & Madigan, S. (2007). Fine grain temporal analysis in aphasia: Evidence from auditory gap detection. *Neuropsychologia, 45*(5), 1127-1133.

Stephens, S., Kenny, R. A., Rowan, E., Allan, L., Kalaria, R. N., Bradbury, M., & Ballard, C. G. (2004). Neuropsychological characteristics of mild vascular cognitive impairment and dementia after stroke. *International Journal of Geriatric Psychiatry, 19*(11), 1053-1057.

Stoelzel, C. R., Stavnezer, A. J., Denenberg, V. H., Ward, M., & Markus, E. J. (2002). The effects of aging and dorsal hippocampal lesions: Performance on spatial and nonspatial comparable versions of the water maze. *Neurobiology of Learning and Memory, 78*(2), 217-233.

Tallal, P., & Newcombe, F. (1978). Impairment of auditory perception and language comprehension in dysphasia. *Brain and Language, 5*(1), 13-24.

Talvitie, S. S., Matilainen, L. E., Pekkonen, E., Alku, P., May, P. J. C., & Tiitinen, H. (2010). The effects of cortical ischemic stroke on auditory processing in humans as indexed by transient brain responses. *Clinical Neurophysiology, 121*(6), 912-920. doi:DOI: 10.1016/j.clinph.2010.03.003

Umbricht, D., Vyssotki, D., Latanov, A., Nitsch, R., & Lipp, H. (2005). Deviance-related electrophysiological activity in mice: Is there mismatch negativity in mice? *Clinical Neurophysiology, 116*(2), 353-363.

Van der Staay, F. J., Stollenwerk, A., Horvath, E., & Schuurman, T. (1992). Unilateral middle cerebral artery occlusion does not affect water-escape behavior of CFW1 mice. *Neuroscience Research Communications, 11*(1), 11-18.

Varney, N. R. (1984). Phonemic imperception in aphasia. *Brain Lang, 21*, 85-94.

Walton, J. P. (2010). Timing is everything: Temporal processing deficits in the aged auditory brainstem. *Hearing Research, 264*(1-2), 63-69.

Writing Group Members, Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N.; Hailpern, S., Ho, M., Howard, V., Kissela, B., Kittner, S., Lloyd-Jones, D., McDermott, M., Meigs, J., Moy, C., Nichol, G., O'Donnell, C., Roger, V., Sorlie, P., Steinberger, J., Thom, T., Wilson, M., Hong, Y., and for the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. (2008). Heart disease and stroke statistics--2008 update: A report from the american heart association statistics committee and stroke statistics subcommittee. *Circulation, 117*(4), e25-146.

Yamamoto, M., Tamura, A., Kirino, T., Shimizu, M., & Sano, K. (1988). Behavioral changes after focal cerebral ischemia by left middle cerebral artery occlusion in rats. *Brain Research, 452*(1-2), 323-328.

Yonemori, F., Yamada, H., Yamaguchi, T., Uemura, A., & Tamura, A. (1996). Spatial memory disturbance after focal cerebral ischemia in rats. *Journal of Cerebral Blood Flow & Metabolism, 16*(5), 980.

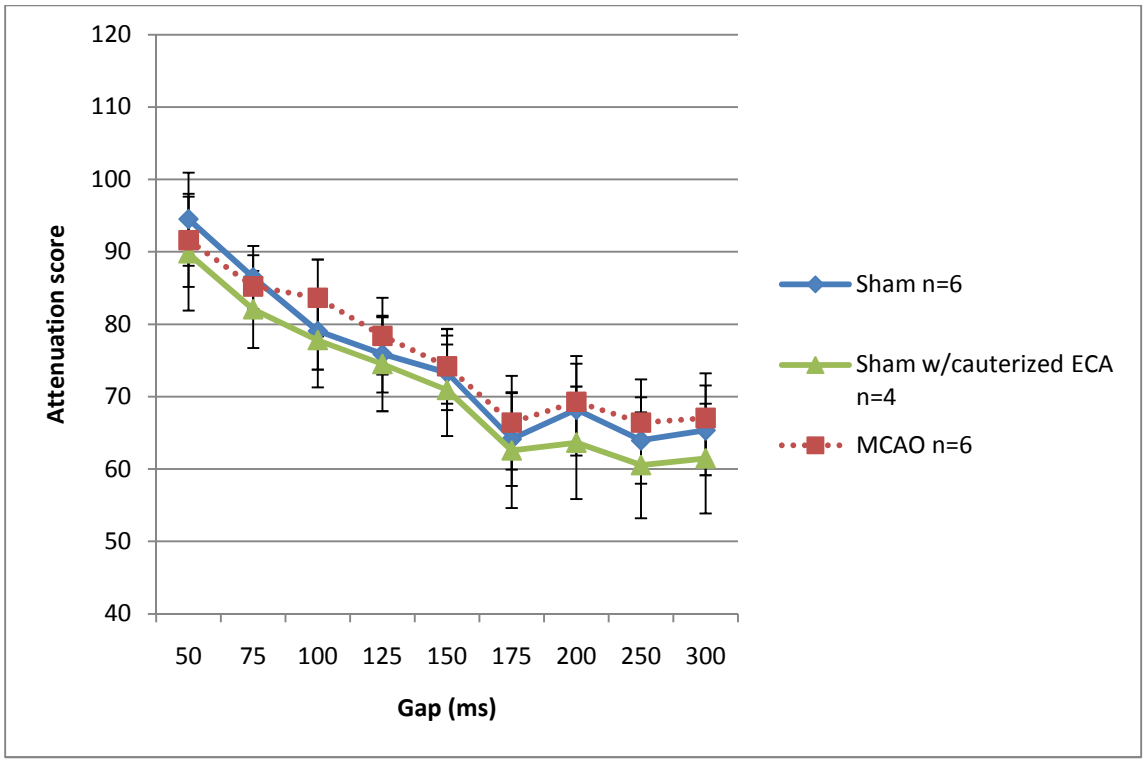

Figure 1: Pre-MCAO surgery - Silent Gap 0-300 ms attenuation scores averaged over 3 days. There were no significant differences in baseline rapid auditory processing ability prior to surgery on the Silent Gap 0-300 ms task (p>0.05).

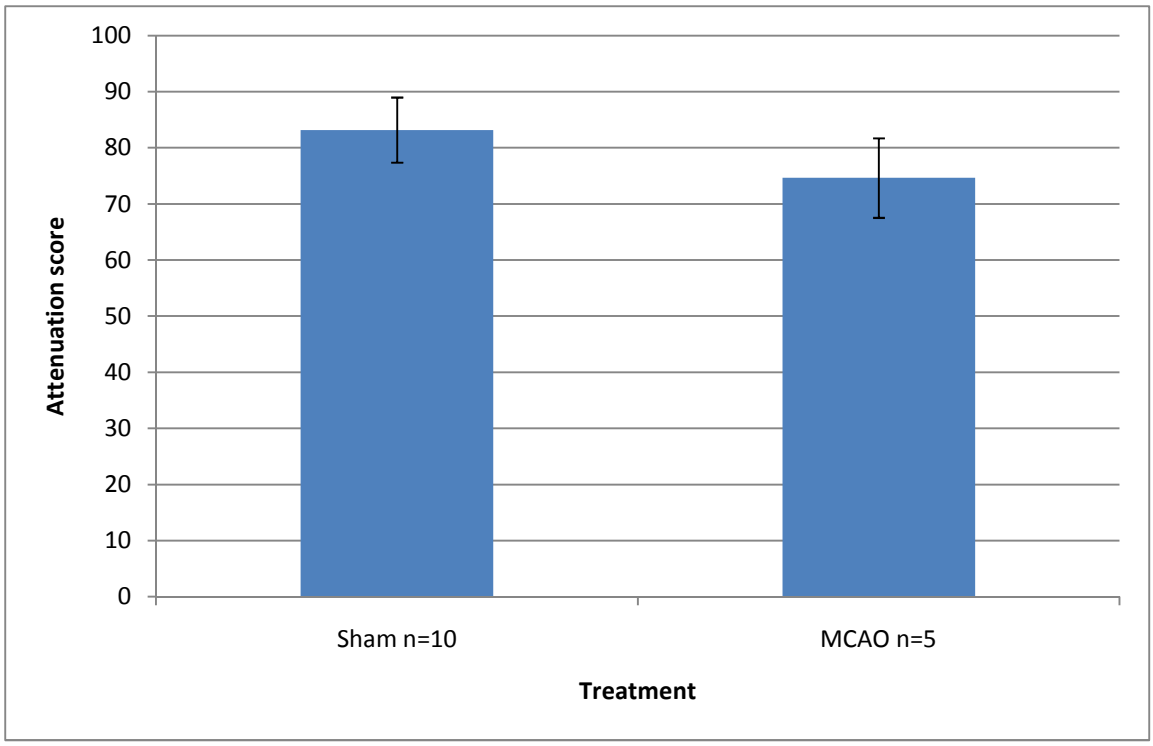

Figure 2: Post-MCAO Surgery – Normal Single Tone attenuation scores on P81 (23 days post MCAO). Both groups showed significant detection of the auditory cue ($p<0.05$). One way ANOVA results revealed no main effect of Treatment [$F(1,13)=0.78$, $p>0.05$], indicating that both groups had comparable hearing and pre-pulse inhibition responses.

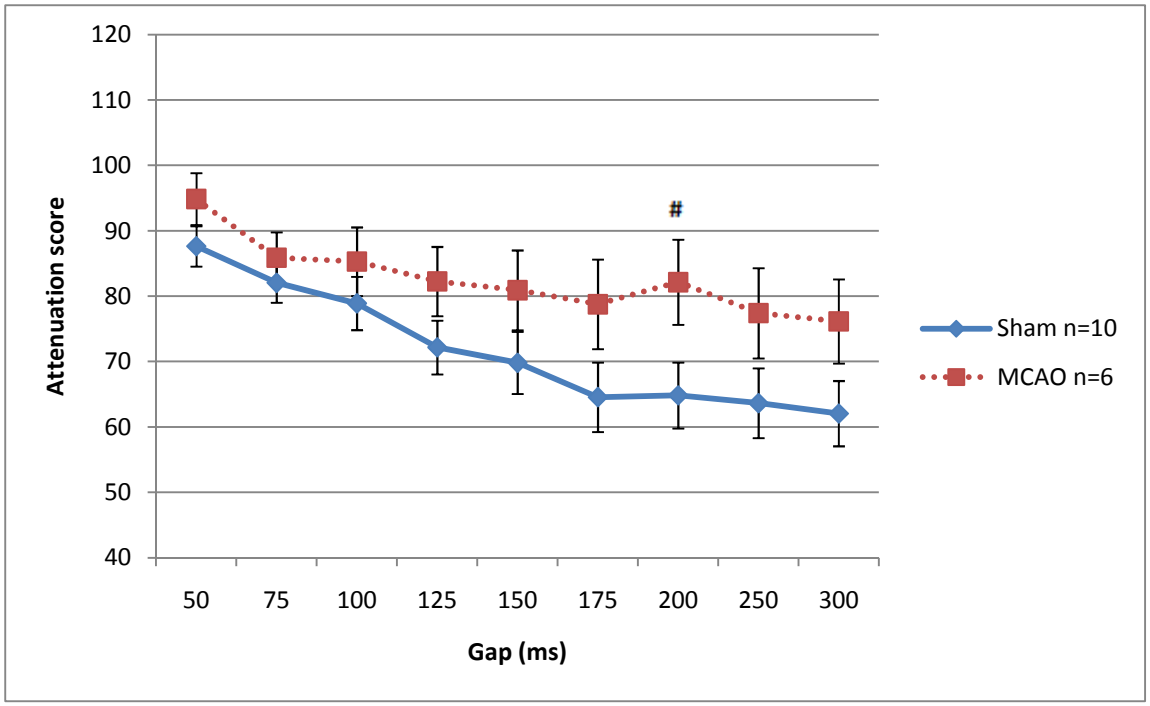

Figure 3: Post-MCAO surgery – Silent Gap 0-300 ms attenuation score averaged over 5 days (6-10 days post-MCAO). Results show no main effect of Treatment [$F(1,14) = 2.49$, $p>0.05$]. A Treatment x Gap interaction was found [$F(8,112) = 2.83$, $p<0.01$], reflecting that the Sham group performed significantly better on the longer gaps (175-300 ms) in comparison to the MCAO group. *Note: an attenuation score of 100 indicates chance performance.*

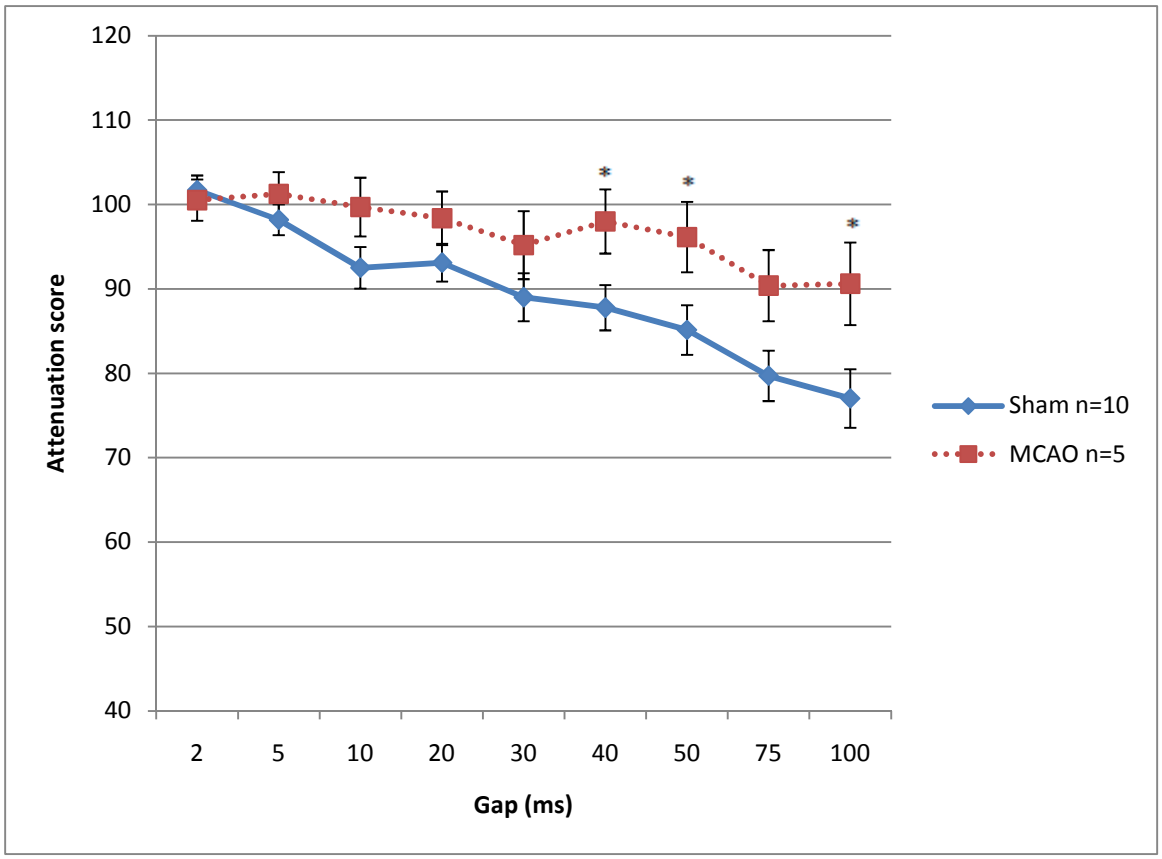

Figure 4: Post-MCAO – Silent Gap 0-100 ms attenuation score averaged over 8 days (13-22 days post-MCAO). A marginal main effect of Treatment [$F(1,13) = 3.60$, $p<0.1$] was found, suggesting that Shams were performing better on the task. In addition, a Treatment x Gap interaction [$F(8,104) = 3.21$, $p<0.01$], suggests that the Sham group performed significantly better on the longer gaps (40-100 ms) in comparison to the MCAO group, possibly indicating that the shorter gaps (2-30 ms) were more difficult for both groups. *Note: an attenuation score of 100 indicates chance performance.*

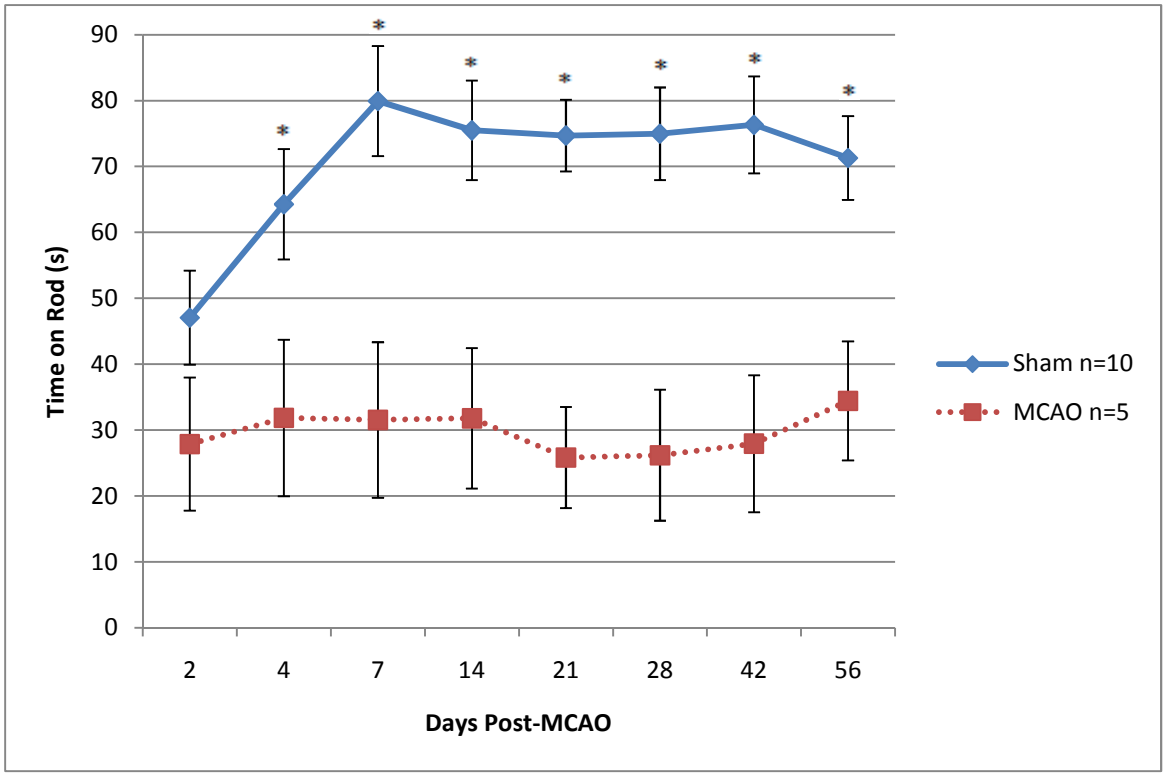

Figure 5: Post-MCAO – Mean time on rotarod. A main effect of Treatment [$F(1,13) = 20.66$, $p<0.001$] was found, reflecting that Shams performed better on the task in comparison to MCAO treated mice.

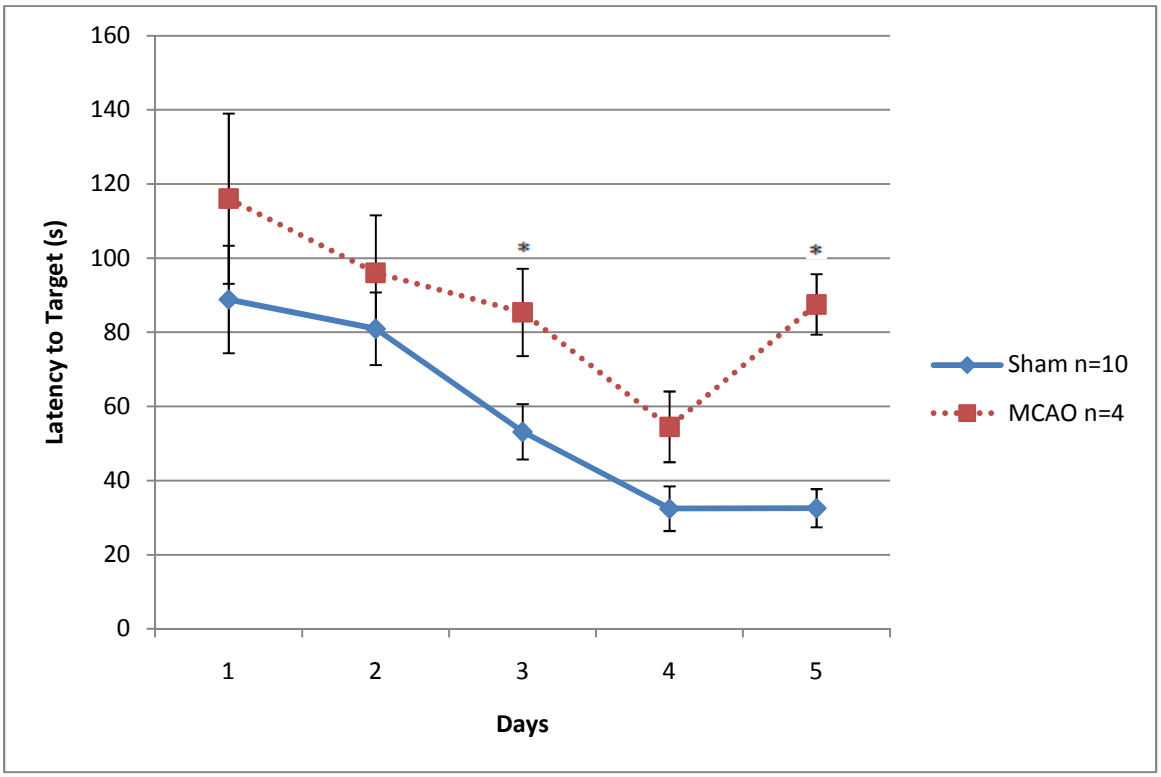

Figure 6: Post-MCAO – Morris water maze mean latency to target over 5 days (55 59 days post-MCAO). A main effect of Treatment [$F(1,12)=8.06$, $p<0.05$] was found, indicating that MCAO treated mice performed worse on the Morris water maze in comparison to Sham operated mice.

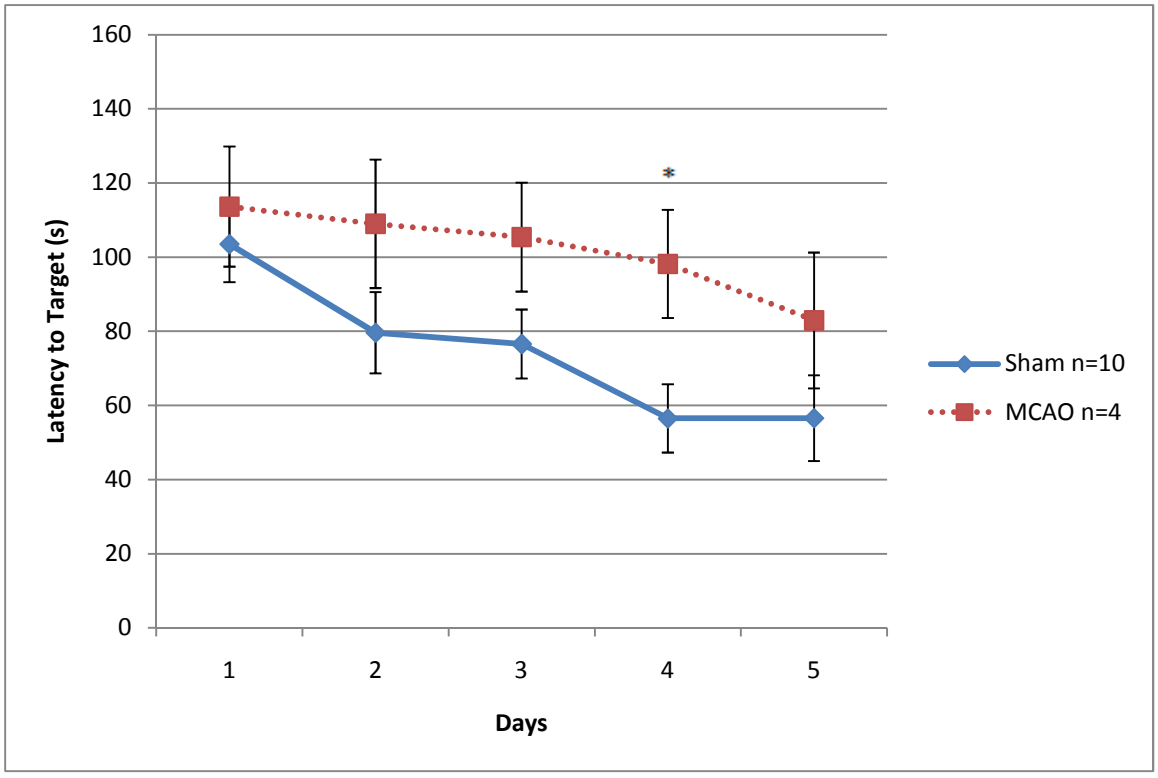

Figure 7: Post-MCAO – Non-spatial water maze mean latency to target over 5 days (62-66 days post-MCAO). A significant main effect of Treatment was found [$F(1,12)=7.13$, $p<0.05$], revealing that MCAO treated mice performed worse on the task in comparison to Sham operated controls.

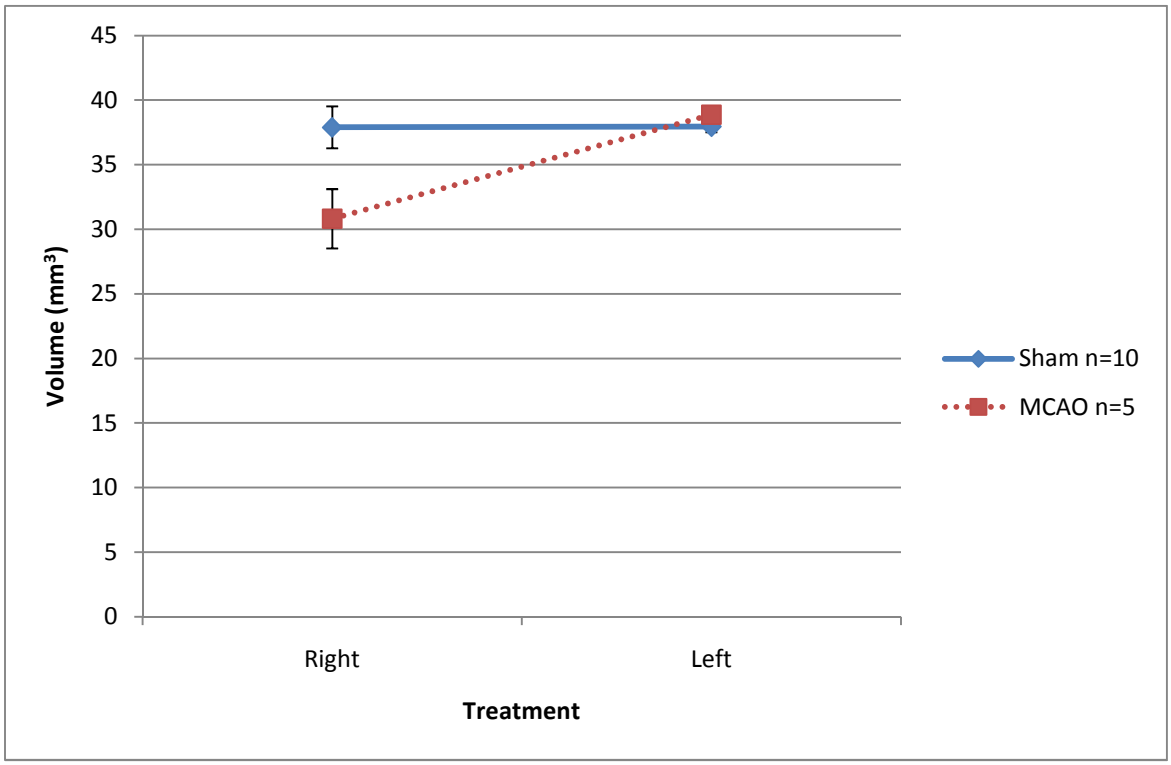

Figure 8: Histology – Volumetric analysis of the Cortex (mm^3) comparing right and left hemispheres. A marginal main effect of Treatment was found [$F(1,13) = 3.78$, $p<0.1$], however there was a significant effect of Side [$F(1,13) = 9.41$, $p<0.01$] and a Treatment x Side interaction [$F(1,13) = 9.14$, $p<0.01$] indicating a difference in the relationship between the right and left hemisphere volumes in the MCAO treated mice. A paired samples t-test comparing the mean volume of Right and Left cortex in MCAO mice revealed no significant differences between Right and Left cortical volumes [$t(4)=-2.11$, $p>0.05$].

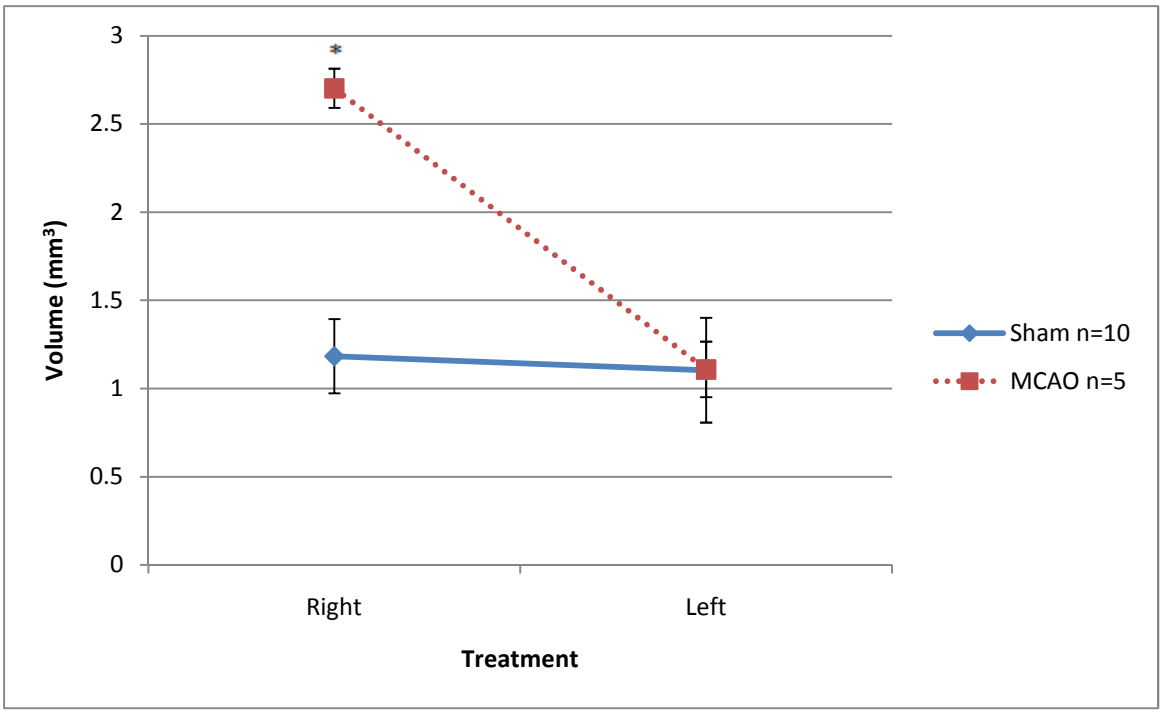

Figure 9: Histology – Volumetric analysis of the Ventricle (mm^3) comparing right and left hemispheres. A significant main effect of Treatment was found [$F(1,13) = 10.67$, $p<0.01$] in addition to a significant simple effect of Side [$F(1,13) = 22.96$, $p<0.001$] and a Treatment x Side interaction [$F(1,13) = 18.82$, $p<0.001$]. Results indicate an overall difference in ventricular volume between the MCAO and Sham treated mice with the indication of a difference in relationship between left and right ventricular volumes in MCAO induced subjects. A paired samples t-test comparing the mean Right and Left ventricular volumes in MCAO mice revealed a significant difference between the Right and Left ventricle, revealing that the Right ventricle was significantly larger than the Left ventricle [$t(4)=3.24$, $p<0.05$].