Experimental Neurology 238 (2012) 114–121

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Short Communication

Deficits in auditory, cognitive, and motor processing following reversible middle cerebral artery occlusion in mice

Dongnhu T. Truong [a,*], Venugopal R. Venna [b], Louise D. McCullough [b], Roslyn H. Fitch [a]

[a] Department of Psychology, Behavioral Neuroscience Division; University of Connecticut, Storrs, CT 06269-4154, USA
[b] Department of Neuroscience, University of Connecticut Health Center, Farmington, CT 06030, USA

ARTICLE INFO

Article history:
Received 26 June 2012
Accepted 11 August 2012
Available online 19 August 2012

Keywords:
MCAO
Ischemic stroke
Aphasia
Rapid auditory processing
Morris water maze
Rotarod
Rodent model

ABSTRACT

Middle cerebral artery occlusion[1] (MCAO) is a widely used experimental technique in rodents to model both the short-term pathological events and longer term neuroanatomical and functional damage associated with focal ischemia. Various neurobehavioral tasks have been developed to assess the motor and cognitive dysfunctions associated with MCAO in rodents, and these studies have revealed deficits related to long-term sensorimotor function, as well as retention of spatial memory. Assessment of auditory processing in a MCAO model has not been undertaken, despite findings suggesting an auditory processing deficit in humans with stroke induced-aphasia, a common post-stroke deficit. Using a modified pre-pulse inhibition paradigm, and other behavioral tasks thought to tap "language-related processing", adult male C57Bl/6 mice were subjected to 60 minute MCAO or Sham surgery and were behaviorally assessed from P58 to P124 (2 to 65 days post-surgery). Tasks were selected based on evidence that rapid auditory processing[2] (RAP) skills are associated with language processing indices in clinical populations. Cognitive and sensorimotor ability was evaluated using the Morris water maze, non-spatial water maze, and a post-injury rotarod task administered over multiple days (motor learning). Combined behavioral results from post-MCAO mice provide evidence of a RAP deficit as well as deficits in spatial, non-spatial, and motor learning. Overall results support a fuller characterization of behavioral deficits in auditory processing after MCAO.

Introduction

Ischemic stroke is characterized by a loss or alteration of neurological function resulting from 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[3] (MCA)—which supplies blood and oxygen to the temporal, anterolateral frontal, and parietal lobes (Rordorf et al., 1998; Writing Group Members et al., 2008). Stroke-induced deficits may include impairments in cognition (e.g. attention and/or memory) or motor function, but one of the most commonly diagnosed post-stroke disorders is aphasia, with an incidence of 21–38% (Ilvonen et al., 2001; Påhlman et al., 2011; Stephens et al., 2004).

Aphasia is an acquired language disorder involving the disturbance of one or more previously functional modalities of language (Darley, 1982). Clinical research shows that aphasia may be linked to disturbances in auditory processing and phonemic discrimination (Becker and Reinvang, 2007; Hessler et al., 2010; Ilvonen et al., 2001, 2003, 2004; Jauhiainen and Nuutila, 1977; Miceli et al., 1980; Tallal and Newcombe, 1978; Talvitie et al., 2010; Varney, 1984). In particular, patients diagnosed with aphasia showed difficulties in performing tasks associated with auditory temporal processing of verbal and nonverbal stimuli (Hessler et al., 2010; Stefanatos et al., 2007; Tallal and Newcombe, 1978; Talvitie et al., 2010).

Transient occlusion of the MCA in rodents is a widely used experimental technique to model human focal ischemia. Middle cerebral artery occlusion (MCAO) models using rats and mice have revealed consistent evidence of post-injury 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 (Markgraf et al., 1992; Tsai et al., 2011; Wahl et al., 1992; Yamamoto et al., 1988; Yonemori et al., 1996), however, results have been inconsistent (Bouët et al., 2007; DeVries et al., 2001; Gibson and Murphy, 2004; Gibson et al., 2005; Klapdor and Van der Staay, 1998; Li et al., 2004; Van der Staay et al., 1992). These inconsistencies may be secondary to species and strain differences.

* Corresponding author at: University of Connecticut, Department of Psychology, 406 Babbidge Road, Unit 1020, Storrs, CT 06269, USA.
E-mail addresses: dongnhu.truong@uconn.edu (D.T. Truong), venna@uchc.edu (V.R. Venna), lmccullough@uchc.edu (L.D. McCullough), roslyn.h.fitch@uconn.edu (R.H. Fitch).

[1] MCAO—middle cerebral artery occlusion.
[2] RAP—rapid auditory processing.
[3] MCA—middle cerebral artery.

0014-4886/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.expneurol.2012.08.011

Since a small number of published studies have examined the long-term behavioral changes and outcomes in mice with transient MCAO, and none have assessed auditory processing ability to our knowledge, the current study was designed to develop a neurobehavioral profile of experimental focal ischemia in C57Bl/6 male mice focused on assessing tasks thought to tap language-related processing. A modified pre-pulse inhibition paradigm to evaluate rapid auditory processing (RAP) abilities was used to measure behavioral abnormalities that may reflect "aphasia-like" deficits. In addition, cognitive and long-term sensorimotor skills were studied using the Morris water maze, non-spatial water maze, and repeated rotarod assessment without pre-stroke training to evaluate spatial, non-spatial, and motor learning.

Our rationale for choosing an acoustic processing task rests on clinical literature showing that RAP ability is associated with language processing, and that 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 and 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 mice. The Morris water maze is a well-accepted assessment of spatial learning (Hyde et al., 2002; Stoelzel et al., 2002), and we also used a non-spatial version of the maze with internal visual cues to assess non-hippocampal learning (Hyde et al., 2002; Stoelzel et al., 2002). Finally, since a number of studies assessed rotarod ability following pre-training prior to MCAO, we tested subjects repeatedly post-injury without pre-training in order to study post-injury motor learning.

Material and methods

A total of 22 male C57Bl/6 mice were utilized in this study (Charles River Laboratories, Wilmington, MA) on postnatal day 37 (P37). Only males were used based on prior evidence from rodent models of cortical disruption showing greater behavioral deficits and neural damage in males, coupled with evidence of varying stroke outcomes in females depending on estrous stage (Alkayed et al., 1998; Fitch et al., 1997; Herman et al., 1997; Li et al., 2004, 2011; Peiffer et al., 2002, 2004). Subjects were single housed in standard lab cages (12 h light/dark cycle), with food and water ad lib. On P57–P58, subjects were weighed in preparation for surgery (target weight range 20.3–25.3 g) and were randomly assigned to either a middle cerebral artery occlusion (MCAO), or sham surgery. All procedures were performed blind to surgical condition and conducted in compliance with the National Institutes of Health and approved by the University of Connecticut's Institutional Animal Care and Use Committee (IACUC).

Surgical procedure

Cerebral ischemia was induced via 60 min of reversible MCAO under isoflurane (1%) anesthesia, as described in detail in Li et al. (2004). In brief, a midline ventral neck incision was made, and unilateral right MCAO was performed by advancing a 6.0 silicone-coated nylon monofilament into the internal carotid artery 6 mm from the internal carotid-pterygopalatine artery bifurcation via an external carotid artery stump. Rectal temperatures were carefully monitored and temperature was maintained with heating pad at ~37 °C during surgery and ischemia. Cerebral blood flow measurements by laser Doppler flowmetry (LDF, Moor Instruments Ltd., England) confirmed ischemic occlusion (reduced by 85% of baseline) during MCAO and restoration of blood flow during reperfusion. Mice in the sham condition underwent the same surgical procedure, but the suture was not advanced into the internal carotid artery.

Behavioral testing

All auditory, maze learning, and motor learning behavioral tasks were conducted following the experimental timeline delineated in Fig. 1.

Auditory testing

The startle reduction paradigm (or pre-pulse inhibition) was used to indirectly measure acoustic discrimination via the acoustic startle reflex (ASR)—a large amplitude involuntary response following the presentation of a startle eliciting stimulus (SES) (see Fitch et al., 2008, for details). In brief, when a subject detected a pre-pulse cue prior to an SES, the ASR response should attenuate relative to an uncued SES. Acoustic discrimination was thus assessed by comparing ASRs elicited by cued and uncued SESs (the "attenuation score"). The SES was a 105 dB, 50 ms white noise burst. Subjects were placed on individual load-cell platforms (Med Associates, Georgia, VT), and 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. This system recorded the amplitude of each subject's ASR in mV, and the maximum peak value within the 200 ms signal period following the onset of the SES was extracted and coded for cued and uncued trials. Trials were averaged to provide a mean cued and uncued response amplitude for each trial type. Auditory stimuli were produced using a Dell Pentium IV PC with custom programmed software and a Tucker Davis Technologies real time processor, and sound files were created and played using a custom program (RPvdsEx), and delivered via powered Cambridge Sound Works speakers (~50 cm above each platform).

Normal single tone

The auditory control task included 104 cued/uncued trials presented pseudorandomly through the session, with a variable inter-trial interval (ITI) ranging from 16 to 22 s. Uncued trials consisted of a silent background followed by the SES, and cued trials were defined by the presentation of a 50 ms, 75 dB, 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).

Silent gap

The silent gap task comprised a broadband white noise background, with embedded silent gaps (cues) of variable duration presented 100 ms prior to the SES. The ITI varied from 16 to 24 s throughout the 300 trial sessions. On uncued trials no gaps were used, and for cued trials, two different variations of the task were employed, including a long gap duration and short gap duration task. The long gap task used silent gap cues ranging from 50 to 300 ms in duration, while the short gap task used silent gap cues ranging from 2 to 100 ms. Pre-surgery testing was conducted for long-gap task only (3 days), beginning on P52–54 (3–5 days pre-surgery). Post-surgery testing for long-gap (5 days) and short-gap (8 days) began on P64 and continued through P80 (6–22 days post-surgery).

Maze learning

Visual platform

The visual platform task was administered on P109 (51 days post-MCAO), and provided a control procedure to screen for underlying motor or visual impairments. Subjects were placed in one end of an oval tub (103 cm × 55.5 cm) filled with room temperature water (21 cm), and were required to swim to a visible platform (8.5 cm in diameter) opposite to where they were released.

Morris water maze

The Morris water maze was used to assess subjects' spatial learning and memory ability over a period of 5 testing days. Subjects had to

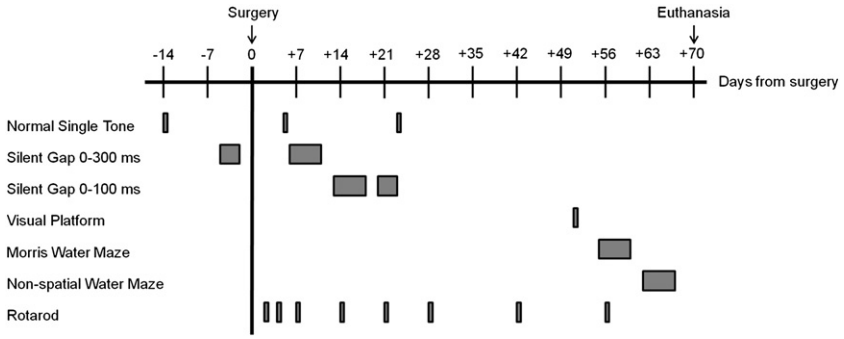

Fig. 1. Outline indicates when each behavioral test was conducted in relation to MCAO or Sham surgery (Day 0). Pre-Surgery testing was performed for Normal Single Tone (day −14) and SG 0–300 ms (days −5 to −3) to provide a baseline for auditory processing prior to surgery. All other behavioral testing occurred after surgery, as outlined.

locate a submerged, invisible, platform (8.5 cm in diameter) located 2 cm below the surface of the water. The escape platform remained in a fixed location within a round black tub (122 cm diameter) surrounded by fixed extra-maze cues (varying shapes painted on testing room wall, location of experimenter, door, etc.). Subjects were given 4 trials a day, with a maximum of 45 s allowed per trial to find the hidden platform (Stoelzel et al., 2002). On each trial, subjects were placed into the tub at a random compass point (north, south, east, west), with each used once per test day. Latency to the platform was measured via stopwatch. Morris water maze testing took place on P113 and continued through P117 (55–59 days post-MCAO).

Non-spatial water maze

In contrast to the Morris water maze, the non-spatial water maze provides an assessment of non-spatial (associative) learning and memory. Here, the location of the submerged platform was paired with a salient intramaze cue painted within a rotating insert containing 3 additional intramaze cues (i.e., quadrants marked by vertical black and white stripes, horizontal black and white stripes, black dots on a white background, and white dots on a black background). Subjects were given 4 trials and a maximum of 45 s per trial to locate the platform during each test day (Stoelzel et al., 2002). Subjects were placed into the pool at a fixed compass location (north). However, for each trial, the spatial location of the paired intramaze cue and platform was rotated randomly into one of the four quadrants of the pool, thus dissociating the goal from spatial (room) cues. Latency to reach the platform for each trial was measured via stopwatch. Non-spatial water maze testing occurred on P120 and continued through P124 (62–66 days post-MCAO).

Motor learning

Rotarod

Mice were placed on a rotating cylindrical drum accelerating from 4 rotations per minute (rpm) to 40 rpm, over a span of 2 min (Bouët et al., 2007). Subjects were given 4 trials on the rotarod per day of testing. The latency of the subject to fall from the rotating drum was recorded for each trial (in seconds), and the average latency was used for further analysis. Rotarod testing was performed 2, 4, 7, 14, 21, 28, 42, and 56 days post-surgery.

Perfusion and histology

Following behavioral testing, subjects were weighed, anesthetized with ketamine/xylezine (100 mg/kg/15 mg/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). Sections were mounted, stained for Nissl using thionine, and coverslipped for analysis.

Due to the chronic nature of these experiments, tissue atrophy was used as an indirect measure of prior injury, reflecting the fact that individual structures are not easily delineated in a chronic MCAO model due to cystic atrophy (Bland et al., 2000). Prepared tissue was analyzed using a MicroBright Field (Williston, VT) Stereo Investigator system integrated with a Zeiss Axio Imager A2 microscope. To assess tissue atrophy, Cavalieri's estimator was used to measure both hemispheres and lateral ventricles. Total tissue atrophy (mm^3) was calculated by subtracting the total volume of the contralateral (unaffected) hemisphere (contraleral hemisphere volume–contralateral lateral ventricle volume) by the total volume of the ipsilateral (infarcted) hemisphere (ipsilateral hemisphere volume–ipsilateral lateral ventricle volume). Percent atrophy was calculated by dividing total tissue atrophy from the total volume of the contralateral (unaffected) hemisphere and multiplied by 100.

Statistical analysis

Following behavioral testing and histological analysis, individual statistical analyses were performed across all behavioral assays and histology. For auditory processing tasks, subjects were assessed for cue discrimination using a paired samples *t*-test comparing mean cued and uncued raw startle reflex response. Group differences for NST were assessed using a univariate ANOVA, while the silent gap tasks each utilized a repeated measures ANOVA with the following variables: Surgery (2 levels: Sham, MCAO); Day (5 levels: 5 days silent gap 0–300 ms; 8 levels: 8 days silent gap 0–100 ms); and Gap (9 levels: gap durations). Rotarod, Morris water maze, and non-spatial water maze data was analyzed using a repeated measures ANOVA with the following variables: Surgery (2 levels: Sham, MCAO); and Day (8 levels: 8 session rotarod; 5 levels: 5 days Morris water maze; 5 levels: 5 days non-spatial water maze). Following surgery, the total n was as follows: n = 10 Sham; and n = 5 MCAO.

All statistical analyses were conducted using SPSS 19 with an alpha criterion of 0.05.

Results

Pre-surgery auditory testing

Silent gap 0–300 ms

After subject assignment to experimental conditions (but before surgery), behavioral results revealed similar attenuation during cued trials [$F(1,13) = .01$, $p > 0.05$] for MCAO and shams, and comparison of mean ASR between cued and uncued trials showed a comparable and significant difference between raw cued and uncued responses overall for both groups, indicating discrimination ($p < 0.05$) (Fig. 2.A).

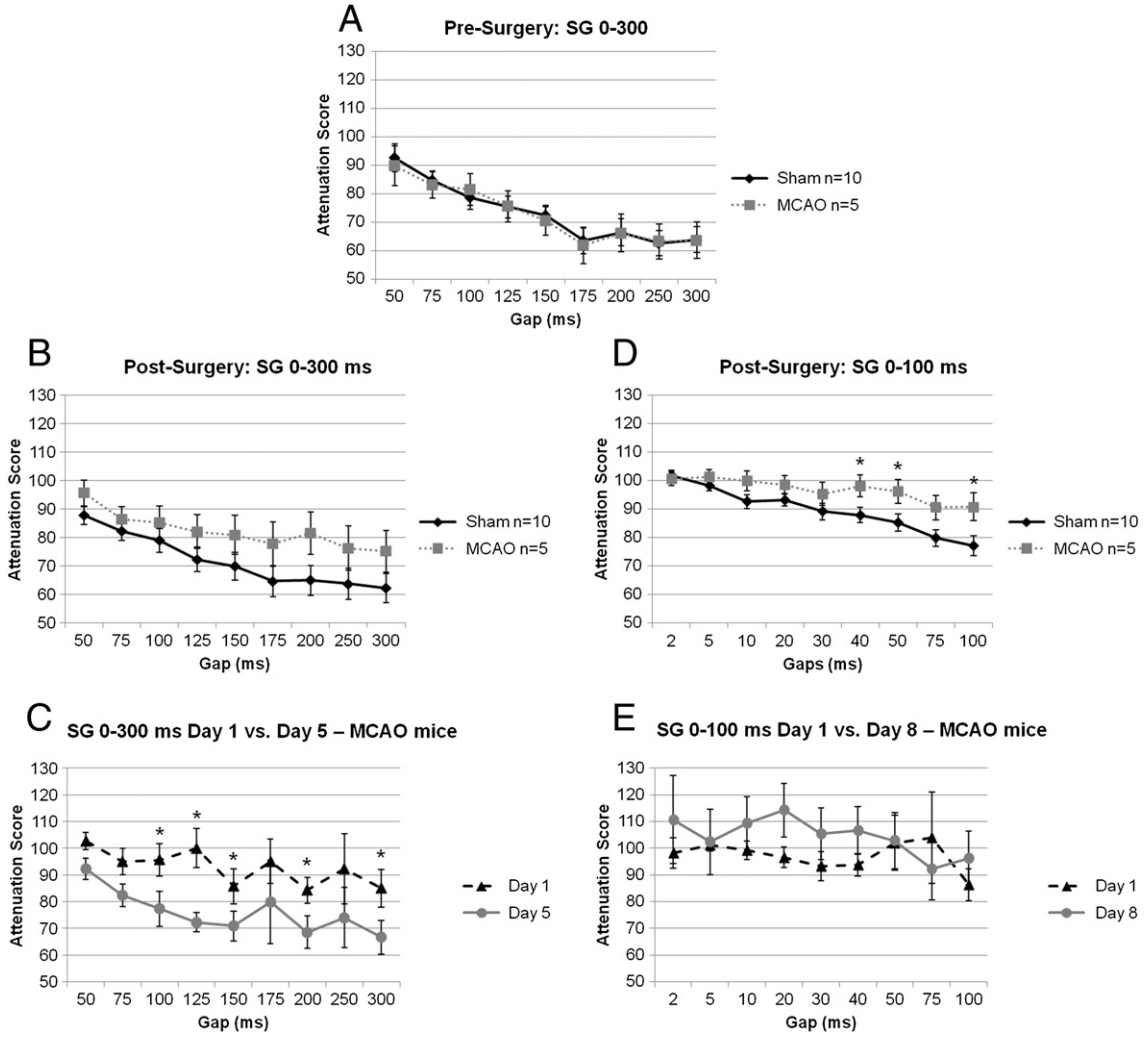

Fig. 2. Silent Gap data are presented as mean attenuation score (attenuation score = [cued trial/uncued trial] * 100), with a score of 100 indicating chance response, while a score below 100 indicates a reduction of the startle response during a cued trial. A) Pre-surgery Silent Gap 0–300 ms averaged across 3 days revealed no significant differences in RAP ability between MCAO and Sham mice. B) Post-surgery Silent Gap 0–300 ms did not show overall significance averaged across 5 days of testing, however a significant Surgery × Gap × Day interaction revealed that C) MCAO mice significantly improved RAP performance on the last day of post-surgery SG 0–300 ms testing in comparison to the first day of post-surgery MCAO testing, while sham performance remained static across test days (data not shown). D) Post-surgery Silent Gap 0–100 ms revealed a marginally significant effect of Surgery when performance was averaged across 8 days of testing, however a significant Surgery × Gap interaction was found, suggesting that MCAO mice exhibited poorer RAP ability in comparison to Shams at the longer, easier to detect, silent gap durations (75–100 ms). E) Although a significant 3-way interaction between Surgery × Gap × Day was found, further analysis did not suggest that MCAO mice improved SG 0–100 ms performance across days of testing. *$p<0.05$.

Post-surgery auditory testing

Normal single tone

Results revealed significant detection of the single tone cue for both groups, providing evidence for normal hearing and pre-pulse inhibition responses ($p<0.05$). No effect of Surgery condition was found on either day of post-surgery normal single tone testing ([$F(1,13)=0.73$, $p>0.05$] and [$F(1,13)=0.78$, $p>0.05$], 5 and 23 days post-MCAO, respectively).

Silent gap 0–300 ms

No overall main effect of Surgery condition was found [$F(1,13)=1.90$, $p>0.05$] when SG 0–300 ms performance was examined across five days of testing (Fig. 2.B). However, a significant Surgery × Gap × Day interaction [$F(32,448)=1.53$, $p<0.05$] was revealed, reflecting that across Days of SG 0–300 ms testing, MCAO performance improved at the longer (i.e., easier) Gap durations. Conversely, sham performance was initially very good at all Gap levels and therefore did not show marked improvement between Days 1 and 5 of SG 0–300 ms testing. Moreover, further analysis of MCAO scores on Day 1 versus Day 5 (repeated measures ANOVA) revealed a significant difference in MCAO mice performance [$F(1,4)=14.50$, $p<0.05$], with overall improved RAP ability from Days 1 to 5 (Fig. 2.C). A paired samples *t*-test comparing cue and uncued startle responses at each Gap duration (to determine threshold detection on Day 1 and Day 5 in MCAO mice) also revealed a shift in threshold for auditory discrimination, from 200 ms on Day 1 to 125 ms on Day 5. This shift in gap detection threshold suggests that MCAO subjects recovered some rapid auditory processing ability, although not to the level of shams who showed a threshold of auditory discrimination at 50 ms (shortest gap duration) on Days 1–5 of testing (sham data not shown).

Silent gap 0–100 ms

Averaged across eight days of testing, sham mice displayed 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 ($p>0.05$). Overall, MCAO 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 Surgery was found between the sham and MCAO groups [$F(1,13)=3.60$, $p<0.1$]. In addition, there was a significant Surgery × Gap interaction [$F(8,104)=3.21$, $p<0.01$], suggesting that shams

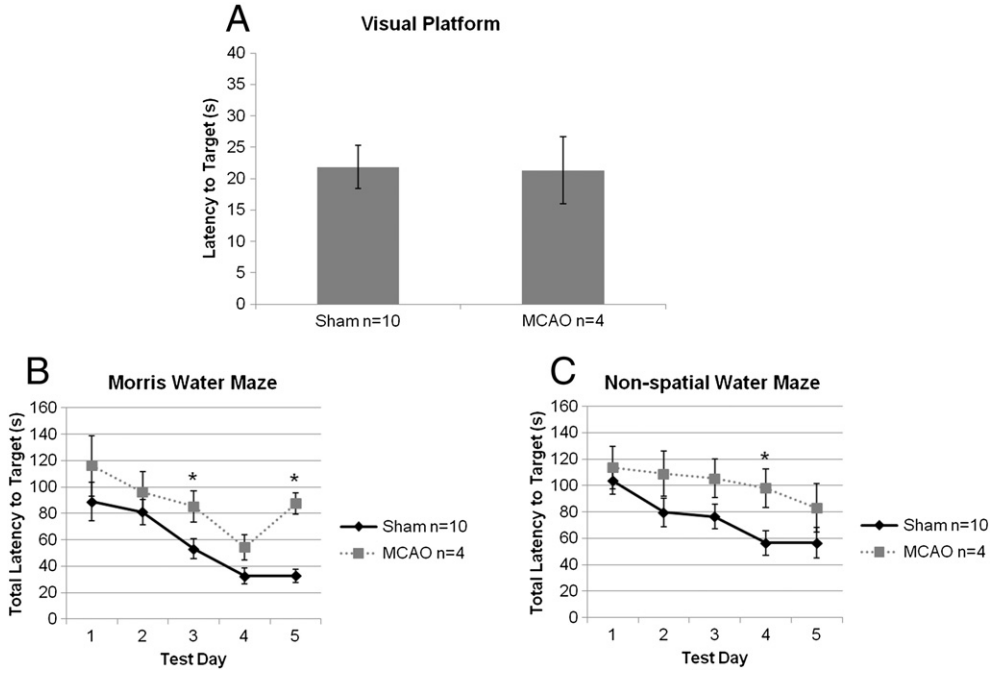

Fig. 3. Water maze data are presented as total latency to escape platform. A) Visual platform (water maze control task) indicated comparable ability to visually detect and swim to the exposed escape platform. MCAO mice overall exhibited significantly longer total latencies to locate the hidden escape platform over 5 consecutive days of testing in both the B) Morris and C) non-spatial water mazes, revealing impaired spatial and associative learning and memory performance in comparison to shams for the Morris and non-spatial water maze tasks, respectively. However, despite differences in latency, both groups showed significant improvement of performance with experience as revealed by the test Day effect in both water mazes. *$p<0.05$.

more effectively performed the task at the longer Gap durations (30–100 ms) in comparison to MCAO mice, while the shorter Gap durations (2–20 ms) were more difficult for both groups of subjects to detect and therefore did not differentiate groups (Fig. 2.D). A significant Surgery × Gap × Day interaction [$F(56,728)=1.57$, $p<0.05$] was also found. However, unlike the 3-way interaction revealed in SG 0–300 ms testing, further examination did *not* reveal any recovery of RAP ability in MCAO mice between Days 1 and 8 of SG 0–100 ms testing (Fig. 2.E). This absence of RAP recovery in the SG 0–100 gap detection task suggests that the Gap durations were too short and difficult for the MCAO subjects to detect despite 8 days of experience on the task.

Maze tasks

Visible platform

One subject had to be dropped from further water maze testing and analyses due to impaired swimming ability. All other subjects performed the task. Univariate ANOVA revealed no effect of Surgery on latency to the exposed platform in this task [$F(1,12)=0.01$, $p>0.05$], indicating comparable swimming ability and visual acuity to locate the platform (Fig. 3.A).

Morris water maze

Across five days of Morris water maze testing, a main effect of Surgery was found [$F(1,12)=8.06$, $p<.05$] in addition to a significant effect of Day [$F(4,48)=9.41$, $p<.001$] (Fig. 3.B). The Day effect suggests that subjects (regardless of surgery) improved on the task across Days of testing. However, despite improvement on the Morris water maze, the MCAO group continued to perform consistently worse on the task in comparison to shams. Further analysis using a univariate ANOVA found significant group differences in performance on Days 3 and 5 of testing, with shams locating the platform faster than MCAO mice.

Non-spatial water maze

Across five days of non-spatial water maze testing, a main effect of Surgery was found between sham and MCAO groups [$F(1,12)=7.13$, $p<0.05$], as well as a significant effect of Day [$F(4,48)=2.75$, $p<0.05$] (Fig. 3.C). As in the Morris water maze, the effect of Day suggests that subjects overall were taking less time to locate the hidden escape platform as testing progressed. However, despite improved performance over days, the MCAO group did not reach the performance level of shams. Finally, a univariate ANOVA analyzing group differences within each Day found a significant difference in performance on Day 4 of testing, with shams performing better than MCAO mice.

Rotarod

Analysis of latencies across the eight testing sessions revealed a main effect of Surgery [$F(1,13)=21.23$, $p<.001$] (Fig. 4), and a

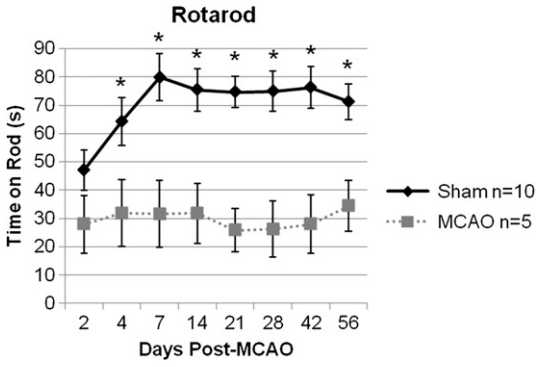

Fig. 4. Rotarod data are presented as mean latency on rod. Rotarod assessment showed impaired long term sensorimotor function in MCAO treated mice, with shorter latencies on the rod in comparison to Shams across all days of testing, excepting the first day (2 days post-surgery). *$p<0.05$.

univariate ANOVA was used to further analyze potential group differences for each Day (session). While no significant difference in performance was found on Day 1 of testing (2 days post-surgery) [$F(1,13)=2.41$, $p>0.05$], subsequent days of testing (4–56 days post-MCAO) revealed statistically better sham performance in comparison to MCAO mice (Fig. 4).

Histology

An assessment of percent tissue atrophy revealed a main effect of Surgery [$F(1,13)=17.926$, $p<0.001$], indicating that MCAO mice presented with significant tissue atrophy while shams, as expected, did not have tissue loss (Fig. 5). This suggests that ischemic damage due to MCAO at earlier ages results in tissue loss in necrotic regions when chronic end points are used.

Discussion

Auditory processing

During pre-MCAO testing both sham and MCAO subjects were able to detect rapid auditory cues for each gap condition, and both groups performed the task comparably (no significant differences in auditory discrimination and pre-pulse inhibition ability were seen). After surgery, although MCAO mice did not show an overall significant deficit in RAP ability as compared to sham mice on the Silent Gap 0–300 ms task over 5 days of testing, the lack of overall effect could have been pulled by the recovery of RAP performance in MCAO mice between Day 1 of post-surgery SG 0–300 ms testing (6 days post-surgery) and Day 5 of post-surgery SG 0–300 ms testing (10 days post-surgery). Results from the Silent Gap 0–100 ms task do support a RAP deficit associated with MCAO in mice, with MCAO mice failing to discriminate the shorter gaps (i.e., significant discrimination only at the 75 and 100 ms gaps), and performing significantly worse than sham mice on conditions they *were* able to discriminate. Further, MCAO mice did not show a recovery of RAP ability on the more difficult SG 0–100 ms task, which was most likely due to the difficulty of the task (shorter, harder to detect gap durations) in comparison to the SG 0–300 ms task (where recovery was observed). These collective findings provide strong evidence that MCAO alters subsequent RAP ability.

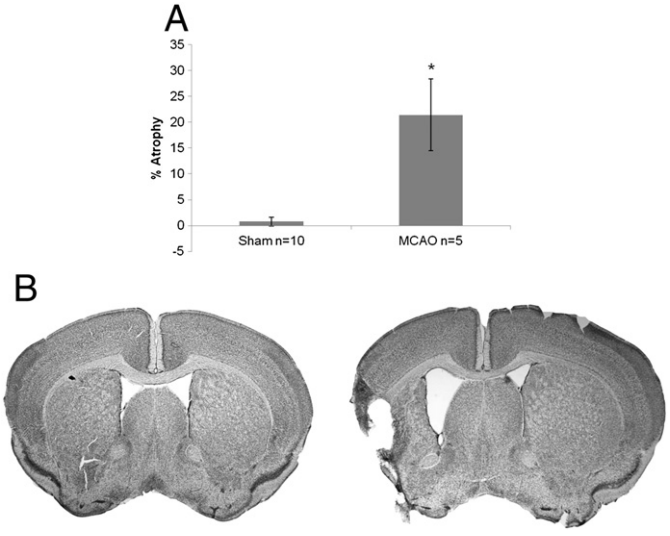

Fig. 5. A) Tissue loss due to cerebral ischemia following surgery is expressed as percent atrophy by comparing the total tissue volume of the ipsilateral (infarcted) hemisphere to the total tissue volume of the contralateral (unaffected) hemisphere. B) Representative Nissl stained coronal sections of mice subjected to sham surgery (left) and MCAO surgery (right).

With regard to neural substrates that may underlie these RAP deficits, the central auditory pathway is conserved neuroanatomically among mammalian species (Webster, 1992) and involves an auditory processing stream that ascends from the cochlea through various brain stem nuclei. The central auditory pathway begins in the cochlear nuclear complex, ascending to the superior olivary complex, and then the lateral lemniscal nuclei (Webster, 1992). The ascending processing stream continues on to the inferior colliculus and then medial geniculate nucleus (MGN) of the thalamus, which relays information to primary auditory cortex, among other targets (Webster, 1992). Studies of bilateral auditory cortical lesions, as well as functional deactivation, have suggested that auditory cortex contributes in rodents to temporal processing and acuity of gap detection tasks similar to those used in the current study (Bowen et al., 2003; Threlkeld et al., 2008). These studies showed that loss of cortical function within the region of auditory cortex increased the threshold for gap detection, indicating that subjects were less sensitive to detecting more rapid auditory information (Bowen et al., 2003; Threlkeld et al., 2008).

MCAO primarily produces infarcts in the frontal, parietal, and temporal cortex (which contains primary auditory cortex and associated cortices) as well as areas of the striatum (see Carmichael, 2005, for review). In the current study, gross histological analysis revealed tissue atrophy largely in the temporoparietal cortex with some frontocortical and striatal atrophy (concurrent with previous findings, see Carmichael, 2005, for review). This suggests that the auditory cortex of MCAO mice was certainly damaged (if not entirely eliminated) by tissue damage following MCAO surgery in the ipsilateral (damaged) hemisphere. Conversely, the contralateral hemisphere (unaffected by MCAO) showed intact A1, a composite injury profile that could be comparable to unilateral auditory cortex lesions. The unilateral loss of auditory cortex could partially explain the impaired performance of MCAO mice in gap detection of more rapid cues (<50 ms) in comparison to intact shams, as well as recovery on the easier tasks (given that contralateral A1 was spared).

Disruption of temporal auditory processing could also be secondary to retrograde degeneration of the MGN in the ipsilateral hemisphere. Previous research has shown that chronic MCAO in rodents results in significant shrinkage of the thalamus beginning as early as two weeks post-MCAO, potentially a result of the interruption of neuronal connections to and from the cortex and thalamus by cerebral necrosis (Fujie et al., 1990). Under this hypothesis, we could infer that necrosis of the auditory cortex would lead to shrinkage of the auditory specific thalamic nucleus (MGN; Games and Winer, 1988). To examine this hypothesis, further histological analysis would be necessary to determine whether morphological changes did occur in the MGN following chronic MCAO injury, a possible venue for future study.

At a clinical level, findings from the current study compliment the clinical work of Stefanatos et al. (2007), who assessed the auditory temporal processing of aphasic patients using a gap detection task. These authors found that aphasics had difficulties in identifying silent gaps within white noise when the breaks in acoustic stimuli were short in duration as compared to long, and aphasics performed worse on the task compared to healthy controls. However, Stefanatos et al. (2007) also raised concerns about parsing out RAP deficits from potential confounding attention deficits. That is, significant processing deficits could reflect subjects' inability to attend to the task. To address this issue in the current paradigm, our subjects were given an auditory control task (normal single tone), and results of this task showed that all subjects could discriminate a pre-pulse cue and effectively attenuate their startle response—thus arguing against attention deficits as the cause of difficulties in RAP.

Water maze

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, data assessing Morris water maze ability in MCAO operated mice have been variable, thus making a general consensus for this model inconclusive (Bouët et al., 2007; Gibson and Murphy, 2004; Gibson et al., 2005; Klapdor and van der Staay, 1998; Van der Staay et al., 1992). The current study using the Morris water maze found a significant overall impairment in MCAO mice. Specifically, MCAO mice took a longer time to locate the submerged platform in comparison to sham controls, indicating difficulty in using and remembering spatial cues to assess the location of the platform.

The non-spatial water maze was developed as a variation of the Morris water maze, with the difference of local cues providing information to the platform location (Hyde et al., 2002; Stoelzel et al., 2002). By requiring a reliance on local cues to find the platform, the non-spatial water maze 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 mice, thus suggesting that the deficits found were associated with a global injury beyond focal infarct of the hippocampus secondary to MCAO.

Although impaired swimming speed secondary to MCAO could be a potential cofound to the significant water maze results found in the current study, the use of the visual platform task prior to water maze testing eliminated underlying deficits in short-term swimming speed as well as visual acuity in MCAO subjects as causal factors for subsequent group differences. However, we must acknowledge that the visible platform task is much shorter in duration than both the Morris spatial and non-spatial water mazes and that the effects of fatigue on swim speed could potentially play a role in the significant water maze results presented. Although swim speed during the water maze tasks was not directly examined in the current study, previous research has shown that MCAO mice have comparable swim speed to sham mice in the Morris water maze despite presenting significant impairments in Morris water maze performance (longer latency to hidden platform; Gibson et al., 2005).

Motor learning: rotarod

Although a sensorimotor deficit in MCAO mice is consistent with data from previous studies, no study using mice has reported long-term motor learning 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 found impaired performance within the first few days post-MCAO, but a 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 MCAO mice on the rotarod 56 days post-surgery, with no sign of improvement on the task. Importantly, in the present study, subjects were not pre-tested on the rotarod prior to surgical manipulation. Although an argument could be made that the MCAO group had worse coordination prior to MCAO surgery, scores from the first day of testing (2 days post-surgery) revealed comparable performance on the rotarod across both groups, and animals were randomly assigned to surgical condition. This indicates that regardless of surgical condition, mice actually showed similar levels of balance and coordination on the first exposure to the task. Interestingly, however, as the days of testing continued, sham mice progressively improved on the task to an asymptote 7 days post-surgery. Conversely, the MCAO mice never improved on the task, with surgery condition effects getting larger over time. These results indicate a long term motor learning deficit in MCAO mice.

Conclusion

In summary, a mouse model of ischemic stroke using C567Bl/6 mice reveals the presence of RAP deficits similar to those described in aphasic patients, in addition to long-term motor and cognitive learning deficits. As our population ages and the incidence of stroke continuing to rise (leaving more stroke survivors in communities), the need to better understand the MCAO rodent model and its validity to study ischemic stroke and therapeutic options is crucial. In order to provide therapeutically informative data, animal models of stroke must be able to capitalize on the most common and deleterious behavioral symptoms associated with stroke, and demonstrate effective outcomes specifically for these measures. A mouse model of "aphasia-like" features—i.e. one that captures measures associated with core underlying components of human language ability—represents an advancement that may shift rodent MCAO research away from motor performance assessments (where few deficits are seen, likely due to the paucity of white matter in rodents as compared to humans; Bailey et al., 2009) and toward clinically informative tasks such as RAP assessments that relate to language-based symptomatology (i.e., aphasias) as seen in clinical practice. The results obtained from the current study are preliminary but represent a step toward realizing the potential of this mouse model by translating auditory processing deficits found in human aphasics to MCAO mice.

Although right MCAO was studied, the presence of auditory processing deficits may suggest that temporal processing is not as lateralized to the left hemisphere as in humans (Bryden, 1982), and deficits found may highlight a general temporal processing deficit. Future studies will need to assess left versus right MCAO deficits in mice to better understand the relationship between hemispheric location of MCAO and auditory processing outcome.

In conclusion, the behavioral data obtained provides strong evidence supporting the use of mice as a model for functional impairments in clinical ischemic stroke and its potential to further assess therapeutic options.

Acknowledgments

This work was supported by funding from the University of Connecticut Research Advisory Council, UCHC/Storrs, and Regional Campus Incentive Award to RHF and LM and an American Heart Association Fellowship to VRV.

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. Stroke 29, 159–166.
Bailey, E.L., McCulloch, J., Sudlow, C., Wardlaw, J.M., 2009. Potential animal models of lacunar stroke. Stroke 40, e451–e458.
Becker, F., Reinvang, I., 2007. Successful syllable detection in aphasia despite processing impairments as revealed by event-related potentials. Behav. Brain Funct. 3, 6.
Benasich, A.A., Tallal, P., 2002. Infant discrimination of rapid auditory cues predicts later language impairment. Behav. Brain Res. 136, 31–49.
Bland, S.T., Schallert, T., Strong, R., Aronowski, J., Grotta, J.C., Feeney, D.M., 2000. Early exclusive use of the affected forelimb after moderate transient focal ischemia in rats: functional and anatomic outcome. Stroke 31, 1144–1152.
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. Exp. Neurol. 203, 555–567.
Bowen, G.P., Lin, D., Taylor, M.K., Ison, J.R., 2003. Auditory cortex lesions in the rat impair both temporal acuity and noise increment thresholds, revealing a common neural substrate. Cereb. Cortex 13, 815–822.
Bryden, M.P., 1982. Laterality: Functional Asymmetry in the Intact Brain, first ed. Academic Press, New York.
Carmichael, S.T., 2005. Rodent models of focal stroke: size, mechanism, and purpose. NeuroRx 2, 396–409.
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. Dev. Sci. 10, 213–236.
Darley, F.L., 1982. Aphasia. W.B. Saunders Company, Philadelphia.
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? Neurosci. Biobehav. Rev. 25, 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. Behav. Brain Res. 205, 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. Behav. Neurosci. 111, 404–412.
Fitch, R.H., Threlkeld, S.W., McClure, M.M., Peiffer, A.M., 2008. Use of a modified prepulse inhibition paradigm to assess complex auditory discrimination in rodents. Brain Res. Bull. 76, 1–7.
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. Behav. Neurosci. 123, 224–230.
Fujie, W., Kirino, T., Tomukai, N., Iwasawa, T., Tamura, A., 1990. Progressive shrinkage of the thalamus following middle cerebral artery occlusion in rats. Stroke 21, 1485–1488.
Games, K.D., Winer, J.A., 1988. Layer V in rat auditory cortex: projections to the inferior colliculus and contralateral cortex. Hear. Res. 34, 1–25.
Gibson, C.L., Murphy, S.P., 2004. Progesterone enhances functional recovery after middle cerebral artery occlusion in male mice. J. Cereb. Blood Flow Metab. 24, 813.
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. J. Cereb. Blood Flow Metab. 25, 439.
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. Cereb. Cortex 7, 453–464.
Hessler, D., Jonkers, R., Bastiaanse, R., 2010. The Influence of phonetic dimensions on aphasic speech preception. Clin. Linguist. Phon. 24, 996.
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, 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. Behav. Brain Res. 133, 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, 622–628.
Ilvonen, T., Kujala, T., Kiesilainen, A., Salonen, O., Kozou, H., Pekkonen, E., Roine, R.O., Kaste, M., Naatanen, R., 2003. Auditory discrimination after left-hemisphere stroke: a mismatch negativity follow-up study. Stroke 34, 1746–1751.
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). Neurosci. Lett. 366, 235–240.
Jauhiainen, T., Nuutila, A., 1977. Auditory perception of speech and speech sounds in recent and recovered cases of aphasia. Brain Lang. 4, 572–579.
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. Physiol. Behav. 63, 903–909.
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. Exp. Neurol. 187, 94–104.
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. J. Cereb. Blood Flow Metab. 31, 425.
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 Res. 575, 238–246.
Miceli, G., Gainotti, G., Caltagirone, C., Masullo, C., 1980. Some aspects of phonological impairment in aphasia. Brain Lang. 11, 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. J. Neurosci. Methods 104, 99–109.
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. Neuroreport 13, 2277–2280.
Peiffer, A.M., Rosen, G.D., Fitch, R.H., 2004. Sex differences in rapid auditory processing deficits in microgyric rats. Dev. Brain Res. 148, 53–57.
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, 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. Biochem. Biophys. Res. Commun. 253, 26–32.
Stefanatos, G.A., Braitman, L.E., Madigan, S., 2007. Fine grain temporal analysis in aphasia: evidence from auditory gap detection. Neuropsychologia 45, 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. Int. J. Geriatr. Psychiatry 19, 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. Neurobiol. Learn. Mem. 78, 217–233.
Tallal, P., Newcombe, F., 1978. Impairment of auditory perception and language comprehension in dysphasia. Brain Lang. 5, 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. Clin. Neurophysiol. 121, 912–920.
Threlkeld, S.W., Penley, S.C., Rosen, G.D., Fitch, R.H., 2008. Detection of silent gaps in white noise following cortical deactivation in rats. Neuroreport 19, 893–898.
Tsai, Y.W., Yang, Y.R., Wang, P.S., Wang, R.Y., 2011. Intermittent hypoxia after transient focal ischemia induces hipppocampal heurogenesis and c-Fos expression and reverses spatial memory deficits in rats. PLoS One 6 (8), e24001.
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. Neurosci. Res. Commun. 11, 11–18.
Varney, N.R., 1984. Phonemic imperception in aphasia. Brain Lang. 21, 85–94.
Wahl, F., Allix, M., Plotkine, M., Boulu, R., 1992. Neurological and behavioral outcomes of focal cerebral ischemia in rats. Stroke 23, 267–272.
Walton, J.P., 2010. Timing is everything: temporal processing deficits in the aged auditory brainstem. Hear. Res. 264, 63–69.
Webster, D.B., 1992. The Mammalian Auditory Pathway: Neuroanatomy (Springer Handbook of Auditory Research). Springer, New York.
Writing Group Members, Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N., Hailpern, S.M., 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., 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, e25–e146.
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 Res. 452, 323–328.
Yonemori, F., Yamada, H., Yamaguchi, T., Uemura, A., Tamura, A., 1996. Spatial memory disturbance after focal cerebral ischemia in rats. J. Cereb. Blood Flow Metab. 16, 980.