Age-Dependent Memory Impairments Following Transient Global Ischemia: Relationship to Hippocampal Pathology

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A Thesis

In

The Department

of

Psychology

Presented in Partial fulfillment of the Requirements
for the Degree of Master of Arts (Psychology) at
Concordia University
Montreal, Quebec, Canada

September 2009

Your file Votre référence
ISBN: 978-0-494-63119-5
Our file Notre référence
ISBN: 978-0-494-63119-5

Abstract

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Most human stroke victims are part of an older population while most animal models of ischemia employ young adult rodents. To add generalizability to rodent models of global ischemia, and its associated neuropathology and memory impairments, to the human stroke population, this thesis examines the effect of a 15-minute global ischemia via four vessel occlusion (4VO) in rats that were 8-weeks, 20-weeks, and 50-weeks of age. Rats were tested for object-location memory, using a 24-hour retention delay and object-recognition memory, using 15-minute and 24-hour delays. Rats with sham-ischemia in all age groups showed a preference for the moved object during the novel-object-in-place (NOIP) test of spatial memory, and for the novel object during the 15-minute novel-object-preference (NOP) test and with the exception of the 20-week group, during the 24-hour NOP test. However, rats that received ischemia displayed impairments in all age groups in the NOIP, in the 20-week group during the 15-minute NOP test, and in the 20- and 50-week groups in the 24-hour NOP test of object recognition. Ischemic rats in all age groups had significantly fewer cells in the CA1, CA2, and hilus subfields of the hippocampus, than did sham-ischemia controls. Only the 50-week ischemia group had significant damage in CA3 and the dentate gyrus. Performance in the NOIP test was correlated with cell counts in CA1, CA2, and the dentate gyrus, and with CA1 and CA2 in the 15-minute NOP test. These findings indicate that the severity of memory impairments and neuropathology due to ischemia are influenced by the age of the brain.

Acknowledgements

I would like to acknowledge and extend my heartfelt gratitude to the following persons who have made the completion of this thesis possible. First and foremost I would like to thank my supervisor Dr. Dave G. Mumby who has not only allowed me the opportunity to advance in my career but who has become a trusted friend and colleague. I would like to thank my other committee members Dr. C. Andrew Chapman and Dr. James G. Pfaus for their time and consideration. I would like to extend my appreciation to my fellow lab members Dr. Stephane Gaskin, Pavel Piterkin, Marilyn Tardif and Emily Cole who have all played a strong role in my success. To Dr. David R. Brodbeck I extend my deepest gratitude to for his guidance and for opening the doors to an opportunity of a lifetime. I would also like to thank the other Concordia psychology graduate students, faculty, and secretaries for their generous assistance. Finally, I thank the Bourdage and Tarchuk families to whom, I am eternally thankful for their loving support.

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List of Acronyms

2VO	Two vessel occlusion
4VO	Four vessel occlusion
ANOVA	Analysis of variance
CA	Cornu Ammonis
DMTP	Delayed-matching-to-place
DMTS	Delayed-matching-to-sample
DNMS	Delayed-non-matching-to-sample
GFAP	Gial fibrillary acidic protein
HPC	Hippocampus
IBO	Ibotenate acid
Isc	Ischemia
LTP	Long-term potentiation
MAP	Microtubule associated protein
MCAO	Middle cerebral artery occlusion
mPFC	Medial pre-frontal cortex
NOIP	Novel-object-in-place
NOP	Novel-object-preference
Wk	Week

Age-Dependent Memory Impairments Following Transient Global Ischemia: Relationship to Hippocampal Pathology

Global cerebral ischemia, hereafter referred to simply as ischemia, can cause a variety of memory impairments, and widespread neuropathology. Animal models are an essential tool for studying the relationship between ischemia-induced brain damage and memory impairments. One important difference between animal models of ischemia, and the human condition they are intended to shed light upon, is that most human stroke victims are part of an older population, while most animal models used in ischemia research employ young adult rodents. This difference may result in significant limitations on the ability to generalize from findings in animal studies to humans, given the likelihood that the age of a brain can influence the impact of an ischemic insult.

This thesis examines the effect that the age of the rat at the time of ischemia has on spatial memory and object-recognition memory impairments, as well as the potential relationships between the extent of hippocampal neuropathology and age-related effects of ischemia on these types of memory.

Rats of 8-weeks, 20-weeks, and 50-weeks of age received global ischemia and were tested for spatial memory and for object-recognition memory using novelty-preference tests. In this thesis, a 15-min global ischemia effected performance in object location and object recognition tests differentially between the different age groups. In addition, the age at the time of ischemia altered the neuropathology in some but not all areas of the hippocampus. Correlation analyses revealed significant relationships between ischemia-induced neuropathology of the hippocampus and behaviour both in object-location and object-recognition tests.

Chapter one describes various rodent models of global ischemia that have expanded our knowledge of how the brain controls behaviour, examines typical learning and memory impairments caused by ischemia, inspects the neuropathology associated with ischemia, and the links between memory impairments following global ischemia and the related neuropathology. This chapter ends by highlighting differences in how the brain, of different ages, reacts on a cellular level to ischemia and provides sound reasoning for the present thesis.

Laboratory rat models of global ischemia

The goal of experimental models of ischemia in rats is to gain a better understanding of the effects of ischemia on behaviour and the brain. These models address two broad classes of cerebral ischemia, characterized as focal or global ischemia. Focal ischemia, such as the middle cerebral artery occlusion (MCAO), occurs when blood flow is stopped or significantly reduced to a specific location of the brain. In contrast, global ischemia is caused by cardiac arrest or strangulation and refers to a reduction of blood flow to the entire brain. Laboratory models, such as the two vessel occlusion (2VO) (Smith, Bendek, Dahlgren, Rosen, Wieloch, & Siesjo, 1984) and the four vessel occlusion (4VO) methods, have been designed to study global ischemia.

Pulsinelli and Brierley (1979) developed the 4VO method of transient global cerebral ischemia for rats. Unlike the 2VO, which uses hypotension to reduce collateral blood flow, the 4VO method uses irreversible occlusions of the vertebral arteries. Reversible occlusion of the carotid arteries restricts cerebral blood flow to about 3% causing global ischemia. Individual neuropathological variations in both inter- and intra-

strain are often assumed to be an artefact of differences in collateral circulation (Pulsinelli, & Brierley). This variation in neuropathology is often held responsible for at least part of the variability in the behavioural findings.

Learning and memory impairments following global cerebral ischemia

In rats, studies suggesting that ischemia is responsible for memory impairments, have consistently demonstrated deficits in spatial memory in the T-maze and radial-arm mazes (Volpe, Davis, Towle, & Dunlap, 1992). However, studies showing that ischemia is responsible for performance deficits in spatial memory based on tasks in the water maze are less consistent (Nunn, & Hodges, 1994; Gionet et al., 1991; Jaspers, Block, Heim, & Sontag, 1990). The variability in behaviour may reflect methodological inconsistencies across studies or a variety of different memory impairments, including differences in working and reference memory requirements. Unpublished observations by Piterkin et al. (2009) show that rats, subjected to 15-min global ischemia, subsequently displayed deficits on the delayed-matching-to-place (DMTP) task in a water maze, a test of spatial working-memory, when the retention interval was either 15-seconds or 60-seconds. Ischemic rats were not impaired in the acquisition of the fixed-platform task, a test of spatial reference-memory, although they displayed significant impairments on a probe trial given at the end of training. This suggests that global ischemia impairs spatial working memory and to a lesser extent spatial reference memory.

Evidence suggesting ischemia causes memory impairments also comes from studies that used the delayed-non-matching-to-sample (DNMS) task, which is used to assess object-recognition memory. Using the DNMS task, (Wood, Mumby, Pinel, &

Phillips, 1993) found that rats with a 20-minute global ischemia (2VO) demonstrated significant learning and performance impairments at retention intervals as brief as 15-seconds. These impairments persisted four weeks after surgery and extensive presurgical training did not reduce the impairments. The authors concluded that ischemic damage to the hippocampus results in recognition memory deficits similar to those produced by ischemic damage in humans.

Novel-object-preference paradigms offer greater practicality than the DNMS task and have become a favoured method for assessing object recognition in rats. Not requiring the rats to learn a contingency rule, means the interpretation of the results is not complicated by procedural learning (Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002). In the conventional version of the novel-object-preference (NOP) test, a rat is placed in an open-field arena and allowed to explore two identical sample objects for several minutes. After being removed for a retention delay, the rat is returned to the arena, which now contains two new objects. One object is identical to the sample pair and the other object is novel. Normal rats spend more time exploring the novel object than the familiar one during the first few minutes of the test and through this bias it is inferred that the rat recognizes the sample object (Mumby et al., 2002). Gaskin, Tremblay, & Mumby (2003) showed that rats with hippocampal lesions demonstrate performance impairments in the retrograde, but not the anterograde, direction in the novel-object-preference test. This suggests that extrahippocampal circuitry may be capable of supporting normal object recognition in the absence of the hippocampus. However, if the hippocampus was present during the learning phase, normal performance on novel-object-preference test seems to be dependent on a functional hippocampus in place during testing. Unpublished

observations indicate that rats that received a 15-minute global ischemia (4VO) demonstrated normal performance in the novel-object-preference test of object recognition after a 15-minute delay but were impaired after a 24-hour delay. This suggests that ischemia effects object recognition memory in a delay dependent manner (Piterkin et al. 2009, unpublished).

One advantage of the novel-object-preference paradigm is that it can also be used to assess spatial memory. The novel-object-in-place (NOIP) test of spatial memory requires rats to recognize that a familiar object has changed location. During training, a rat is presented with two identical objects. For the retention test, one of them is moved to a location within the arena that formerly has never housed an object. Normal rats spend more time exploring the object that has changed location than the one that did not move (Mumby et al., 2002). This allows for individual examination of separate, spatial location and object recognition, mnemonic aspects of essentially equivalent familiarization events.

Neuropathology following global cerebral ischemia

Rats and gerbils show similar neuropathology following global ischemia (Kirinao, & Sano, 1984) with CA1, CA2, and hilar neurons of the hippocampus being most vulnerable. Five to 15-minutes after reperfusion a strong cerebral hyperaemia develops followed by a sharp cerebral hyperfusion lasting up to 24-hours (Pulsinelli, & Brierley, 1979). Necrosis of hippocampal neurons occurs three to six hours later, and may continue for more than six weeks after the ischemia (Corbett, & Nurse, 1998). In the rat, although the variability of neuropathology outside the hippocampus is greater, damage is often noted in the striatum and thalamic nuclei among other selectively vulnerable areas

(Kirino, 1982). The duration of ischemia determines the resulting degree of neuropathology (Nunn, LePeillet, Netto, Hodges, Gray, & Meldrum, 1994). A 3-minute occlusion in the gerbil produces a similar degree of neuropathology as does a 12-minute occlusion in the rat (Corbett, & Nurse, 1998). In the rat, even short durations of ischemia of less than 3-minutes can cause measurable damage to selectively vulnerable neurons, with longer durations of 10-20 minutes causing reliable bilateral neuropathology that is essentially, though not exclusively, limited to the to the CA1 region of the hippocampus (Pulsinelli, & Brierley, 1979; Smith et al., 1984). However, effective temperature control is vital to producing consistent pathological effects (Colbourne, & Corbett, 1994).

Because the brain does not store glucose or oxygen, ischemia can lead to irreversible neuropathology of selectively vulnerable neurons, including those of the hippocampus. This may lead to neurological disorders such as memory impairments in as many as 20% of human cardiac arrest survivors (Bedell, Delbanco, Cook, & Epstein, 1983).

Linking neuropathology to learning impairments

The association of permanent memory impairments with selective loss of CA1 neurons in cases of cardiac arrest (Zola-Morgan, Squire, & Amaral, 1986) has been very influential and often misleading. To date there have been no reported global ischemia induced memory impairments in rats that have not suffered damage to the hippocampus. The degree of spatial memory impairments often correlates with CA1 pathology (Auer, Jensen, & Whishaw, 1989; Davis, & Volpe, 1990; Volpe et al., 1992) and treatments that reduce CA1 damage can attenuate spatial-memory impairments (Block, 1999). However,

slight behavioural differences exist between the impact of ischemia and hippocampal lesions on rodent memory tests and may represent major differences in neuropathological states.

Volpe et al., (1992) contrasted rats that had either a 30-minute ischemia (4VO) or low- or high-dose ibotenic acid (IBO) lesions of the dorsal hippocampus. Rats that received ischemia and rats that received high-dose IBO were equally impaired in a spatial task in the T-maze, performance scores correlated with CA1 pathology, and although they shared similar CA1 damage and memory impairments with ischemic rats, those rats with high-dose IBO lesions had the most overall hippocampal damage. In contrast, rats with low-dose IBO lesions performed significantly better than ischemic rats and experienced significantly less CA1 damage, though overall damage to the hippocampus was equivalent to ischemic rats. This may suggest that detectable CA1 damage predicts performance.

A second study (Nunn et al., 1994) assessed spatial memory using water-maze performance in rats that had a 5, 10, 15, or 30 minute ischemia (4VO). CA1 cell loss increased proportionately with the duration of ischemia. However, the correlation between CA1 cell loss and behavioural performance in the water maze was only significant in rats that received 15, or 30-minute occlusions. This reflects that, due to the variable nature of ischemia the strength of the correlation between performance impairments in spatial memory tasks and CA1 damage may only increase to statistically relevant levels after long occlusion times and thus maximal CA1 damage, although extra-CA1 damage may also contribute to spatial memory impairments.

Nunn, Gray, & Hodges (1998) compared the effects of ischemia and ibotenate acid (IBO) CA1 lesions on the DMTP and the fixed platform tasks in the water maze to address the possibility that extra-CA1 neuropathology contributes to ischemia-induced memory impairments in the rat. The ischemic rats were more impaired in the DMTP task, a working spatial memory task and only ischemic rats were impaired during a 1-minute probe trial. On the fixed platform task, a reference-spatial memory task, ischemic rats were less impaired in one trial learning but more impaired after the second trial. Although silver impregnation revealed greater CA1 cell loss in ischemic rats the authors suggest a role for extra-CA1 damage in ischemia induced spatial memory impairments.

Ischemia effects object-recognition as well as spatial memory. Mumby et al. (1996) analysed the effects of ischemia (20-minute, 2VO) and bilateral hippocampal ablation on object recognition (DNMS) in rats. Rats that received ischemia were more impaired than rats that had hippocampal ablations. However, rats that had their hippocampus removed 1-hour post reperfusion were less impaired than those that received ischemia alone. The authors proposed that ischemia manifests its behavioural effects through extra-hippocampal damage, and that this damage is produced by hippocampal-mediated pathogenic processes. This highlights the difficulty in linking ischemic neuropathology and behavioural findings.

Limitations of previous research

The age of the subjects at the time of ischemic attack has long been overlooked in the animal literature although it is often criticised as a limitation of animal models. Ischemia studies often do not indicate the age of rats used, despite the fact that in rats,

age related declines in cerebral blood flow (Berman, Goldman, & Altman, 1988), glucose utilization (Bassant, Jazat-Poindessous, & Lamour, 1994), and plasticity (Lynch, & Baudry, 1982) are all observed. Aging is a major risk factor for stroke. However, how aging effects the response of the brain to ischemia is poorly understood, and the influence of aging on ischemic neuro-recovery is complex.

Badan, Platt, Kessler, & Popa-Wagner, (2003) showed that the time course of cellular processes following focal ischemia is different for 12 week old and 80 week old rats even 28 days after the occlusion. The authors conclude that a temporally anomalous gliotic response to cerebral ischemia, along with late and inadequate up-regulation of neuro-plasticity proteins in addition to a diminished neurogenesis potential, leads to the prevalence of scar tissue that impedes functional recovery from stroke, in aged rats. This study demonstrates differing cellular mechanisms that could influence the degree of memory impairments that are observed after ischemia in young and aged rats, although, cognitive abilities were not studied.

As a result of these findings, the main hypothesis of this thesis is that the age of the rat at the time of ischemia will influence the severity of memory impairments and neuropathology. More specifically, it is expected that as age increases ischemia will have more profound neurological effects and this will cause more severe memory impairments both in spatial and object-recognition tests. This thesis examines the hippocampus for ischemic neuropathology. However, extra-hippocampal or covert damage may contribute to memory impairments. Due to the length of time spent in behavioural testing it is impossible to delineate whether group differences in neuropathology are due to differing

neuropathological impact of ischemia or to the speed and completeness of the recovery of the brain.

Methods

Subjects

Forty three experimentally-naive, male Long-Evans rats (Charles River, St. Constant, QC) were housed individually, with continuous access to water, under a 12-12hr reverse light-dark cycle (20:00-8:00). Feeding was controlled, so that all rats received approximately 20-25 grams of rat chow per day. Rats were randomly assigned to one of three age groups and surgery was performed at 8-weeks (n=11), 20-weeks (n=16), or 50-weeks (n=16) of age. All procedures were conducted in accordance with protocols approved by Concordia University Animal Care, in accordance with guidelines of the Canadian Council on Animal Care.

Global cerebral ischemia

Seven rats from the 8-week group and eight rats from each of the other age groups received transient global cerebral ischemia using a modified version of the 4-vessel occlusion method (4VO) (Pulsinelli, & Brierley, 1979). Each animal was acclimatised to the surgical environment for at least five minutes prior to anesthetisation. Anaesthesia was induced through a mixture of 5% isoflurane and 95% oxygen (Benson Medical Industries, Markham, ON). Incision areas were shaved and cleaned with iodine. Rats were secured into the stereotaxic device and a rostal-caudal incision was made dorsal to

the spinal column on the upper neck to allow access to the C-1 vertebra. The vertebral arteries were permanently occluded by electro-cauterization (Macan, Model MV-8) by way of the alar foramina. The incision was closed with wound clips and treated topically with antibacterial powder (Cicatrin). Rats were removed from the stereotaxic instrument and a second small incision was made on the ventral side of the neck, anterior to the clavicle. The carotid arteries were carefully separated from the vagus nerves, and delimited with dental floss. The incision was closed as before and the animals were returned to their home cage for a 24 hour recovery period.

The next day, animals were put under light isoflurane anesthesia and the ventral incision, made the previous day, was reopened and the carotid arteries were located using the dental floss. The anaesthetic was disconnected and ischemia was induced by placing arterial clamps on the carotid arteries for 15-minutes. Throughout the occlusion, body temperature was controlled at about 36 $^{\circ}$C using a heating pad and measured rectally (Havard Apparatus LTD. Model 50-7053). At the end of the 15-minutes the clamps were removed and the incision was closed and treated as before. Each rat received an injection of *Annafen* (0.5mL/kg) before being returned to its home cage to recover for two weeks.

If an animal regained consciousness during the occlusion, as measured by the self-righting reflex, the carotid arteries were unclamped, anaesthetic was re-administered, the incision was closed and the rats were omitted from the experiment. Success rates of approximately 60%, 80%, and 80% were observed for the 8-week, 20-week, and 50-week groups, respectively. Animals in the sham-ischemia surgery condition group (n=4, n=8, n=8) received all injections, incisions and anaesthetics described above, but, the carotid artery occlusions were omitted. Three rats in the sham-ischemia 8-week group and three

rats in the sham-ischemia 20-week group had all occlusions omitted. All animals were closely monitored after surgery.

Materials

The arena consisted of an empty-white-round arena, (140 cm dia. x 45 cm deep), located in the center of a room with many external cues. The base was covered with a false floor made of corrugated white plastic. Two, imaginary, perpendicular lines divided the arena floor into four equal pie-shaped quadrants, each with one hole about 30 cm from the exterior edge.

The objects used in this experiment were made of glass, plastic, porcelain, or glazed ceramic and varied in height between 10 cm and 20 cm. Three copies of each object were available. Objects were attached with epoxy to identical small glass jars (6 cm tall) that allowed temporary attachment to the floor via jar lids that were screwed on to the plastic floor of the pool no closer than 60 cm apart. This allowed for counterbalancing of object placement to account for any potential spontaneous-preference for a particular quadrant. The holes not in use were covered with a small piece of white tape, which was replaced often to prevent investigation by the rats. The floor and objects were cleaned before and after each session using a 50% ethanol solution.

A video camera positioned over the arena digitally recorded the entire arena during the sample and test phases. For these experiments, animals were observed in a separate room via a monitor connected to the over-head camera. The light level in the testing room was dimmed and held constant throughout the experiment.

Behavioural testing

Novel object-in-place test of spatial memory (NOIP). The novel-object-in-place test of spatial memory (NOIP) is a non-rewarded test of spatial memory, which exploits rats' natural propensity to investigate novelty. Although the object itself is already familiar, normal rats identify that the spatial location of the object has changed and investigate the object in the new location more than the object that remained stationary (Mumby et al., 2002).

The day after the two-week recovery period, all animals were allowed a 7-minute habituation session in the empty arena. Twenty-four hours after habituation, each rat was placed in the arena with two identical objects in adjacent quadrants for their first 5-minute training session. The rats each received three identical training sessions, each separated by 24-hours. One day after the last training session, the rats were tested.

During the delay between the last training session and the test session, the location of one object was moved to the quadrant opposite to where the object was located during training. As a result, during the test session the animals were exposed to the same two identical objects for 5-minutes, one in the same location as during the training sessions, and one in a new location.

Quadrants were named, and will be referred to hereafter, according to the position of the objects during the test; one quadrant contained an object in the same location as during training (Same), one quadrant contained an object during training but was empty during testing (Former), one quadrant was empty during training, but contained an object during testing (New), and one quadrant was empty during both

training and testing (Never). The position of the objects was designed to counterbalance any spontaneous preference for a specific quadrant.

Novel-object-preference (NOP). Novel-object-preference test of object recognition is a test that assesses object recognition through rats' natural tendency to explore novel objects more than familiar ones. If an animal displays this bias behaviour it is inferred that they remember the familiar object. This object-recognition task was initiated 21-days post surgery. Animals were not given a habituation period, as they were already accustomed to the arena and testing room from the NOIP test. Each rat was exposed to two identical objects and allowed to explore for five minutes. They were then returned to their home cage for either a 15-minute or 24-hour retention delay. During the delay the experimenter replaced one object with an identical copy, and the other with a novel object. After the delay the rats were returned to the arena and allowed to explore for a 5-minute test session.

Six test sessions, three at each delay, were administered, alternating between the 15-minute and 24-hour delays. Each test sessions was separated by 24-hours, and each test session used new objects. Approximately half the rats in each group received object-pair A as a sample, while the other half of the animals received object-pair B as a sample. This was reversed during the first test session, such that a copy of object A was used as the novel object for those that received object B in the sample session, and *vice versa.* This counterbalancing of objects was repeated with new objects, for all test sessions. In a prior experiment with different rats, object-pairs A and B were assessed to ensure that the baseline level of investigation of objects A and B were relatively similar. Placement of

the objects was designed to counterbalance any spontaneous preference for a certain area of the arena.

Measurements and statistical analysis. Spontaneous activity was video recorded and object-exploratory behaviour was later manually coded. A rat was considered to be exploring an object when its head was oriented within 45 degrees, and within 4 cm, of the object. Rearing on, climbing over or sitting on the objects was excluded unless it fulfilled the prior requirement. The main dependent measures were the exploration ratios of each test: the proportion of total object exploration during the test phase that was spent exploring the novel-object ($t_{Novel}/t_{Novel}+t_{Sample}$) or object in the new-location ($t_{New\ location}/t_{New\ location}+t_{Same\ location}$). To determine if rats demonstrated a preference, ratios were compared to the level expected by chance (.5) using one-sample *t*-tests. Previous findings suggest that novelty preference may only be robust during the first few minutes and decays over the length of the test, presumably because both objects become equally familiar (Mumby et al., 2002). For this reason, object investigation ratios were calculated for the first two minutes, the last two minutes and all five minutes of the tests.

For the novel-object-in-place test of spatial memory, time spent in each of the four quadrants, was also assessed using ratios: time spent in the quadrant containing the object in the new location/ time spent in quadrants containing either object ($t_{New}/t_{New}+t_{Same}$), or time spent in the quadrant that formerly held the object that moved/ time spent in quadrants not containing objects ($t_{Former}/t_{Former}+t_{Never}$). One-sample *t*-tests were used to assess whether rats in each group showed a preference during the first two minutes and all five minutes combined.

Separate analyses of variance (ANOVA) were conducted on the exploration times of the final familiarization and test phases. The between-subject independent variables were the surgery (Ischemic vs. Sham-ischemia) and age (8-, 20-, or 50-weeks).

Histology

Immediately following the behaviour testing, five weeks post reperfusion, animals from the 8-week and 50-week groups were perfused transcardially with 150 mL of 0.9% saline followed by 150 mL of 10% paraformaldehyde in 0.1M phosphate buffer (PB). The 20-week group was perfused during the sixth week following ischemia. Brains were carefully removed and post-fixed in 4% paraformaldehyde in 0.1M PB containing 30% sucrose for 48 hours then stored at -80℃.

Brains were serially-sectioned at 20μm using a cryostat. Slices were mounted on slides and stained with cresyl violet. Light microscopy was used to identify and count stained neurons. Cell counts were averaged across left and right hemispheres. Cell counts of the hippocampus were correlated with performance in the memory tests. An analysis of variance (ANOVA) was conducted on cell counts of the CA1, CA2, CA3, hilus, and dentate gyrus subfields of the hippocampus.

Results

Behavioural results

Novel-object-in-place test of spatial memory. Figure 1 shows the mean object investigation ratios of the novel-object-in-place test of spatial memory. Data are shown

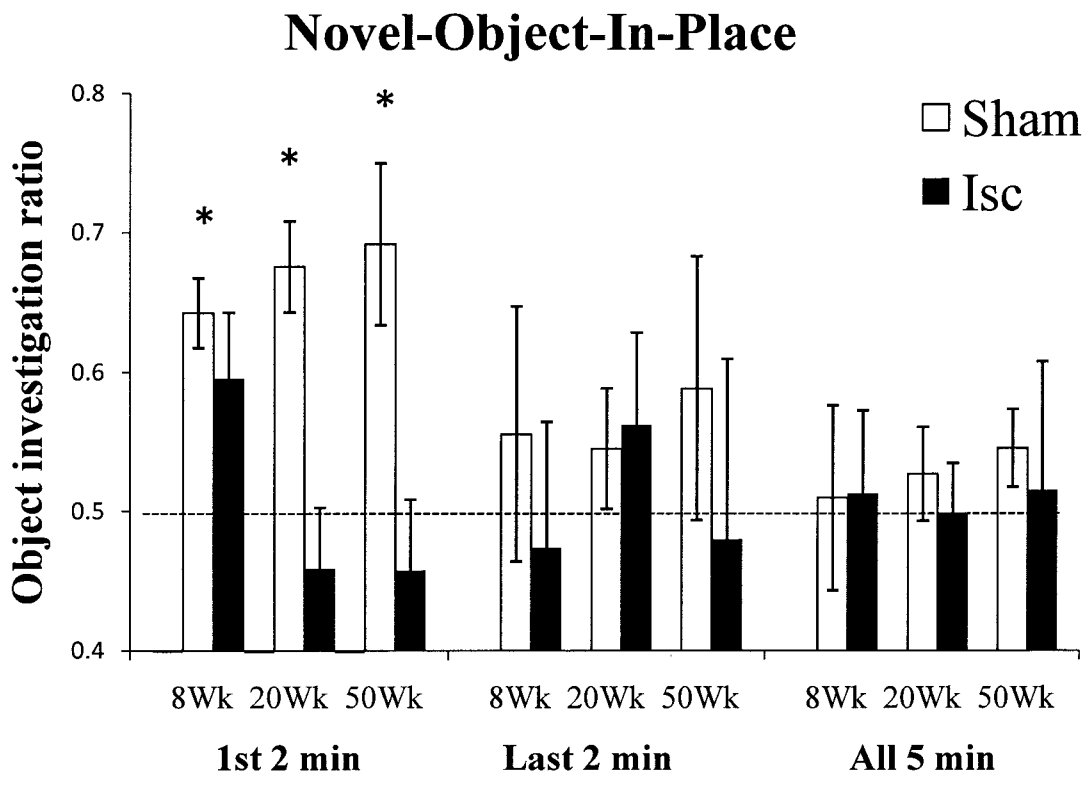


Figure 1 Mean exploration ratios during the test phase on the novel-object-in-place test of spatial memory. The ratio represents the proportion of object-exploration time that was spent exploring the object in a new location; $t_{New\ location}/ (t_{New\ location} + t_{Same\ location})$. Dashed line represents no discrimination between objects. Data are shown separately, by age group, for the first two minutes, last two minutes, and all five minutes of the test. Asterisks denote mean ratios that are significantly above .5 (one-sample *t*-test, $p < .05$). Error bars represent SEM.

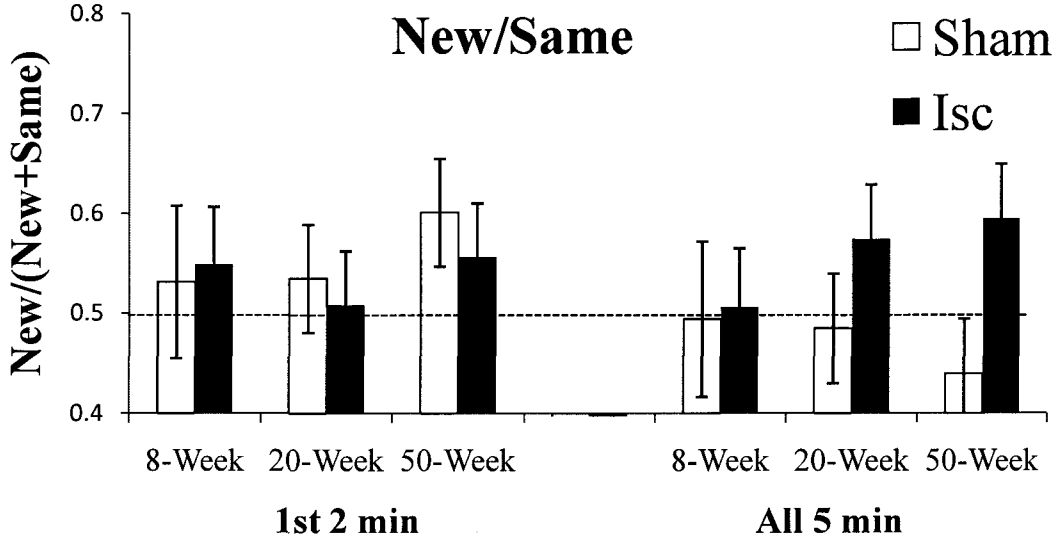
separately, by age group (8-week, 20-week, 50-week), for the first 2-minutes, the last 2-minutes and all 5-minutes of the test. Exploration ratios indicate the degree of preferential exploration for either object while accounting for variations in time spent investigating the objects across rats and trials. Asterisks denote the explorations ratios that are significantly different from 0.5 or chance. Rats that received sham-ischemia had investigation ratios that were significantly higher than .5, indicating a preference for the object in a new location in all three age groups (8-week, $t(3) = 5.67, p < .05$, 20-week, $t(7) = 5.42, p < .05$, 50-week, $t(7) = 3.31, p < .05$) during the first 2-minutes of the test. None of the ischemic rats had ratios that were different than .5 (8-week, $t(6) = 2.01, p > .05$, 20-week, $t(7) = -0.94, p > .05$, 50-week, $t(7) = -0.83, p > .05$). This suggests that ischemia impaired object-recognition memory at all ages.

An analysis of variance (ANOVA) was conducted to evaluate differences in exploration ratios between ischemic and sham-ischemia rats in the 8-week, 20-week and 50-week age groups for the first 2-minutes of the novel-object-in-place test of spatial memory. The result yielded a significant main effect of ischemia, $F(1,37) = 17.07, p < .05$) and a nonsignificant effect of age ($F(2, 37) = .56, p > .05$) and a nonsignificant interaction ($F(2, 37) = 1.91, p > .05$). Bonferroni corrected planned pairwise comparisons reveal a significant impairment of the ischemic rats in the 20-week ($p < .05$) and 50-week ($p < .05$) groups relative to the age-matched sham-ischemia rats. This highlights the reason for the choice of main dependent measure. Although all rats that received ischemia had object investigation ratios that were not different than 0.5 in the novel-object-in-place test of spatial memory, the 8-week ischemic rats do not have ratios significantly different than their age-matched sham-ischemia counterparts ($p > .05$).

Figure 2 shows the mean quadrant preference ratios, for the first 2-minutes and all 5-minutes of the test, plotted separately by age group and condition, for rats in the novel-object-in-place test of spatial memory. Ratios indicate A: (New/Same) time spent during the test phase in the quadrant containing the object in the new location/ time spent in quadrants containing either object ($t_{New}/(t_{New}+t_{Same})$), B:(Former/Never) time spent in the quadrant that formerly held the object that moved/ time spent in quadrants not containing objects ($t_{Former}/(t_{Former}+t_{Never})$). For the New/Same ratio considering all 5-minutes of the test one-sample-*t*-tests for rats that received sham-ischemia reveal no significant differences from chance levels (8-week, $t(3) = -0.19, p > .05$, 20-week, $t(7) = -0.45, p > .05$, 50-week, $t(7) = -0.64, p > .05$) nor for the ischemic rats (8-week, $t(6) = 0.13, p > .05$, 20-week, $t(7) = 2.18, p > .05$, 50-week, $t(7) = 1.88, p > .05$) indicating that neither sham-ischemia nor ischemic rats showed preferences for the global region of the moved object. For the Former/Never ratio, one-sample-*t*-tests for the sham-ischemia rats reveal no significant differences from chance levels (8-week, $t(3) = -0.41, p > .05$, 20-week, $t(7) = 0.52, p > .05$, 50-week, $t(7) = 1.22, p > .05$) nor for the ischemic rats (8-week, $t(6) = -0.52, p > .05$, 20-week, $t(7) = -0.17, p > .05$, 50-week, $t(7) = -1.19, p > .05$) indicating that neither sham-ischemia nor ischemic rats showed preferences for the global region of the arena that do not contain an object.

Separate analyses of variance (ANOVA) were conducted on each of the quadrant ratios to evaluate differences in the degree of preferential place preference around the objects during all 5-minutes of the test. For the New/Same ratio, this yielded a

A



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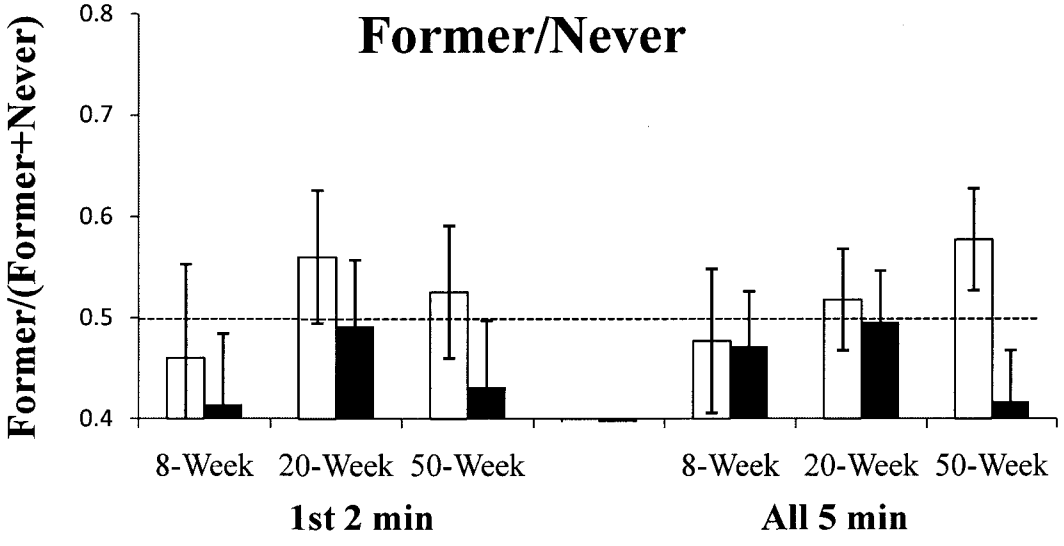

Figure 2 A: (New/Same) Time spent in the quadrant containing the object in the new location/ time spent in quadrants containing either object (t_{New} / (t_{New}+t_{Same})). B:(Former/Never) Time spent in the quadrant that formerly held the object that moved/ time spent in quadrants not containing objects (t_{Former} / (t_{Former}+t_{Never})). Data shown separately, by age group, for the first two minutes and all five minutes combined. Error bars represent the SEM.

nonsignificant main effects of surgery $F(1,37) = 3.06, p > .05$) and age $F(2,37) = 0.11, p > .05$), and a nonsignificant interaction $F(2,37) = 0.67, p > .05$). For the Former/Never ratio, this yielded nonsignificant main effects of surgery $F(1,37) = 1.92, p > .05$) and age $F(2,37) = 0.16, p > .05$), and a nonsignificant interaction $F(2,37) = 1.29, p > .05$).

Figure 3 shows the time spent exploring objects during the final familiarization and test phases for the novel-object-in-place test of spatial memory. Results from a separate ANOVA conducted on the overall time spent investigating the objects during the familiarization and test phases of the novel-object-in-place test of spatial memory indicate for the familiarization phase, a significant main effect of age ($F(2, 37) = 3.37, p < .05$) and a nonsignificant main effect of ischemia ($F(1,37) = 0.03, p > .05$), and a nonsignificant interaction ($F(2, 37) = 1.21, p > .05$). The results for the test phase were a nonsignificant main effect of age ($F(2, 37) = 2.46, p > .05$), a nonsignificant main effect of ischemia ($F(1,37) = .75, p > .05$), and a nonsignificant interaction ($F(2, 37) = .67, p > .05$). The 8-week group spent more time with the objects during the familiarization than the 50-week group.

Novel-object-preference test of object recognition memory. Figure 4 shows the object investigation ratios of the 24-hour novel-object-preference test of object recognition memory. Sham-ischemia rats in 8-week ($t(3) = 4.30, p < .05$) and 50-week,($t(7) = 2.87, p < .05$) age groups had ratios that were significantly above .5, indicating a preference for the novel object during the first 2-minutes. Rats in the 20-week group did not show a preference, ($t(7) = 0.40, p > .05$). Ischemic rats in the 8-week, ($t(6) = 4.77, p < .05$) group had investigation ratios significantly above .5 thus,

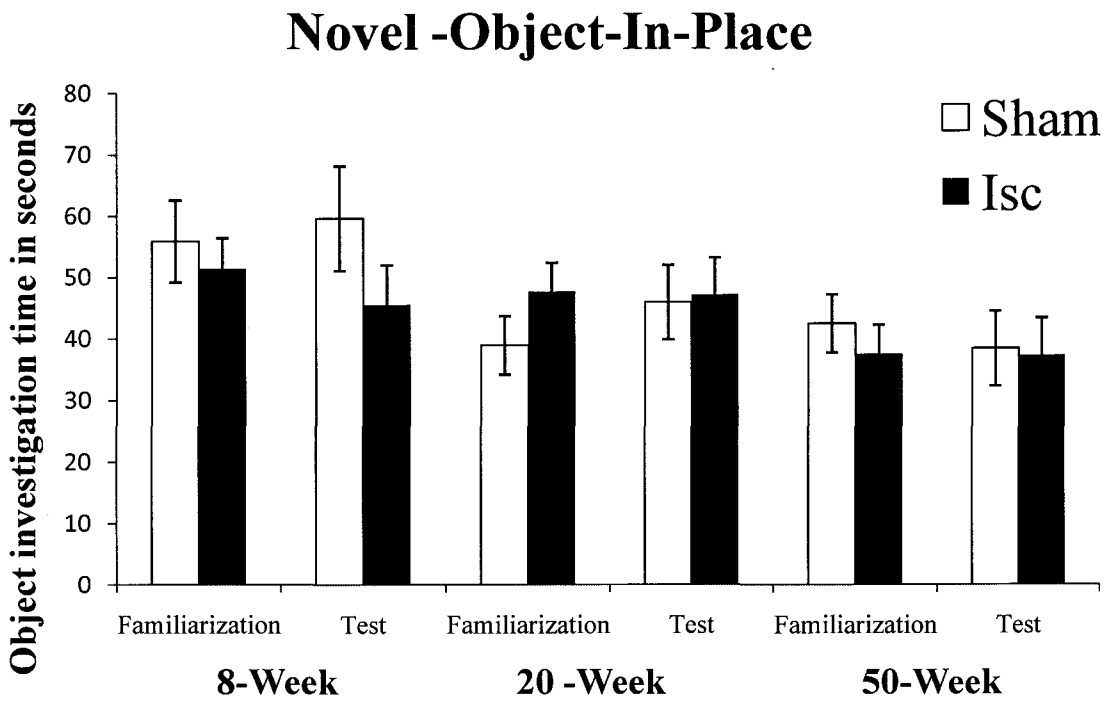

Figure 3 Average time spent engaged in object exploration during the familiarization and test phases of the novel-object-in-place test of spatial memory. Error bars represent the SEM.

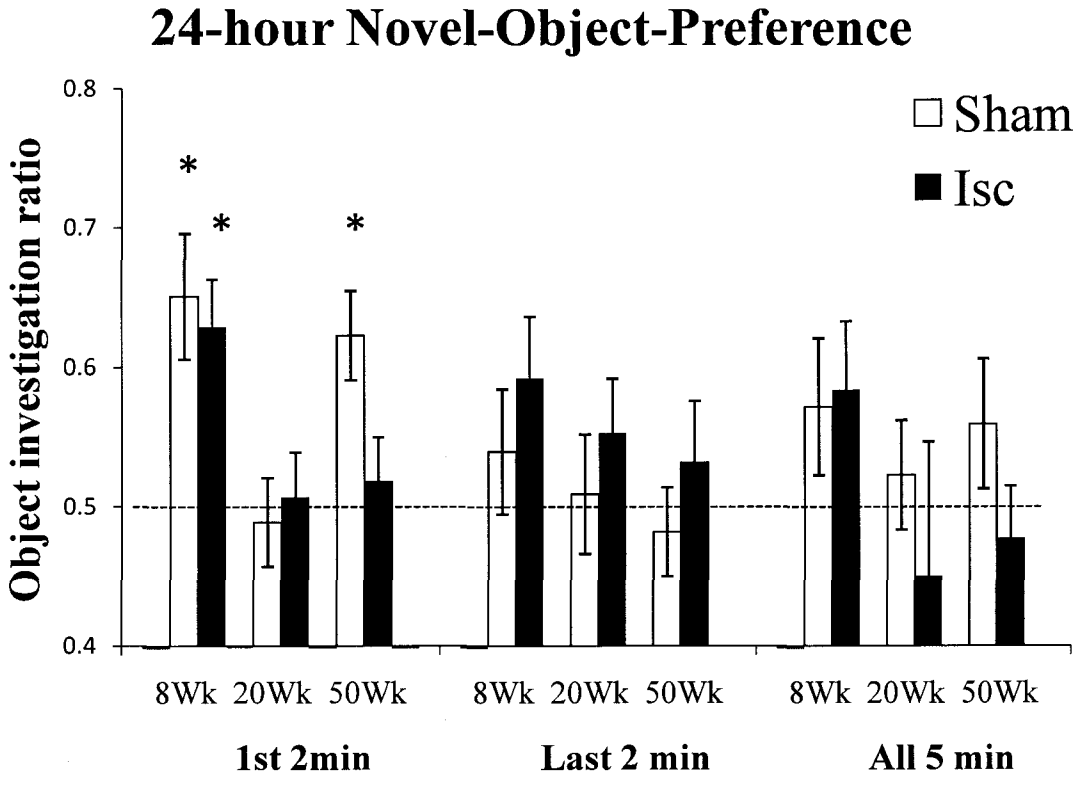

Figure 4 Mean exploration ratios during the retention test of the 24-hour novel-object-preference test for each age group. The ratio represents the proportion of object-exploration time that was spent exploring the novel object; $t_{Novel}/ (t_{Novel}+t_{Sample})$. Dashed line represents no discrimination between objects. Data are shown separately for the first two minutes, last two minutes, and all five minutes of the test. Asterisks denote mean ratios that are significantly above .5 (one-sample *t*-test, $p < .05$). Error bars represent SEM.

demonstrating a significant preference for the novel object during the first 2-minutes. Rats in the 20-week, ($t(7) = 0.27, p > .05$), and 50-week, ($t(7) = 1.21, p > .05$) ischemic groups did not have ratios significantly different than chance.

Figure 5 shows the object investigation ratios of the 15-minute novel-object-preference test of object-recognition memory. Rats that received sham-ischemia in all age groups had ratios above chance (.5) during the first 2-minutes (8-week, $t(3) = 11.505, p < .05$, 20-week, $t(7) = 15.99, p < .05$, 50-week, $t(7) = 4.67, p < .05$). Ischemic rats in both the 8-week ($t(6) = 7.75, p < .05$) and 50-week groups $t(7) = 2.99, p < .05$) had object investigation ratios above .5 during the first 2-minutes of the test while ischemic rats in the 20-week condition did not show a preference ($t(7) = 0.27, p > .05$).

An ANOVA was conducted on the investigation ratios during first 2-minutes of the 24-hour and 15-minute novel-object-preference test, to evaluate whether there was an interaction between the factors of age and delay on ischemic memory impairments for object recognition. This revealed a significant main effect of delay ($F(1, 37) = 20.22, p < .05$), surgery ($F(1, 37) = 11.82, p < .05$), age ($F(2, 35) = 10.14, p < .05$), and delay x surgery x age interaction ($F(2, 37) = 5.96, p < .05$). Bonferroni corrected pairwise comparisons, revealed an ischemic impairment in the 50-week group ($p < .05$) for the 24 hour delay and an ischemic impairment in the 20-week group in the 15-minute delay ($p < .05$). This suggests that as the age of the rat at the time of ischemia increases, object recognition memory impairments are more severe. This also suggests that the 8-week group of ischemic rats demonstrate normal object recognition memory for up to 24-hours.

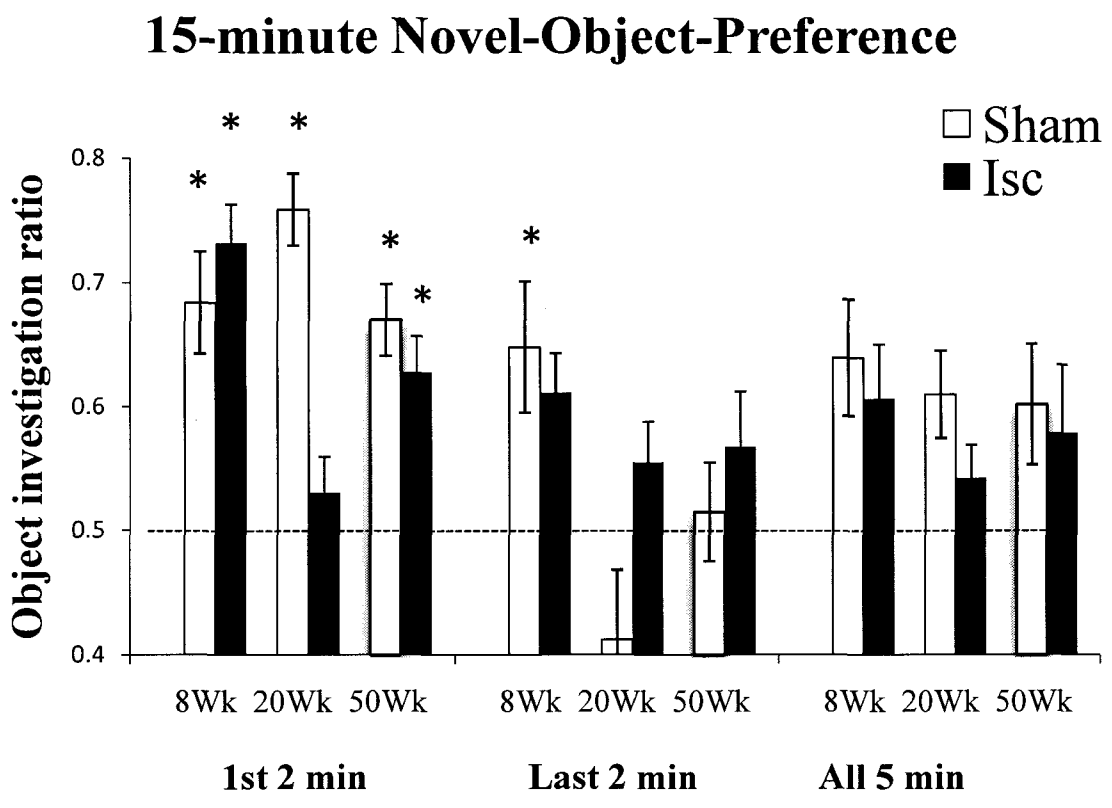

Figure 5 Mean exploration ratios during the 15-minute delay of the novel-object-preference test for each age group. The ratio represents the proportion of object-exploration time that was spent exploring the novel object; $t_{Novel}/(t_{Novel}+t_{Sample})$. Dashed line represents no discrimination between objects. Data are shown separately for the first two minutes, last two minutes, and all five minutes of the test. Asterisks denote mean ratios that are significantly above .5 (one-sample *t*-test, $p < .05$). Error bars represent SEM.

Figure 6 and 7 show the time spent exploring objects during the familiarization and test phases for the 24-hour and 15-minute novel-object-preference test of object recognition memory. To exclude the possibility that group differences in object-investigation ratios during the test phases were due to differences in time spent investigating the objects during the familiarization phases, separate analyses of variance (ANOVA) were conducted on the overall time spent investigating either object during the familiarization phases of the 24-hour and 15-minute novel-object-preference tests. Results for 24-hour delay indicate a significant main effect of age ($F(2, 37) = 9.53, p < .05$) and nonsignificant main effect of surgery ($F(1,37) = .40, p > .05$) and a nonsignificant interaction ($F(2,37) = .21, p > .05$). The 50-week group spent significantly less time with the objects than did either the 8-week or 20-week groups. Results for the 15-minute delay indicate a significant main effect of age ($F(2, 37) = 3.99, p < .05$) and nonsignificant main effect of surgery ($F(1, 37) = 3.91, p > .05$) and a nonsignificant interaction ($F(2,37) = .014, p > .05$). The 8-week group spent significantly more time with the objects than the 50-week group.

To exclude the possibility that differences in investigation ratios during the test phases were related to of differences in time spent with the objects during the test session separate ANOVA were conducted on the times spent with the objects in the test phases of the 24-hour and 15-minute novel-object-preference tests. Results for the 24-hour delay indicate a significant main effect of age ($F(2, 37) = 15.80, p < .05$) and nonsignificant main effect of surgery ($F(1,37) = 2.12, p > .05$) and a nonsignificant interaction ($F(2,37) = .55, p > .05$).The 8-week group spent the most time with objects. Results for the 15-minute delay indicate a significant main effect of age

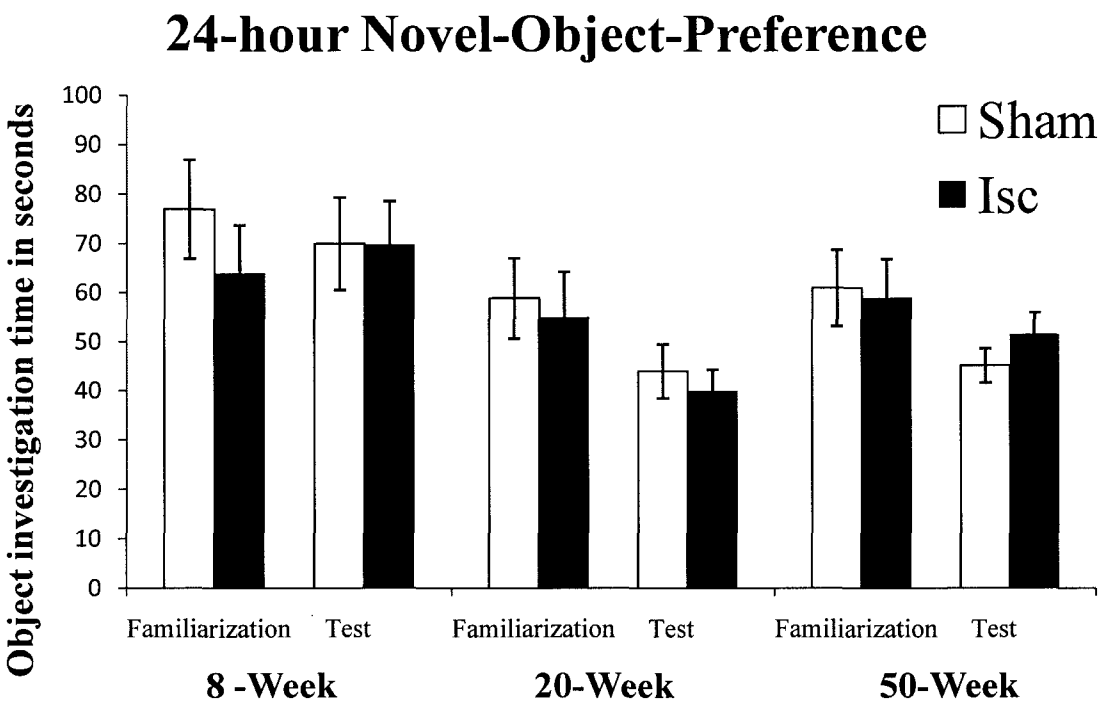

Figure 6 Average time spent engaged in object exploration during the familiarization and test phases of the 24-hour novel-object-preference test of object recognition memory. Error bars represent the SEM.

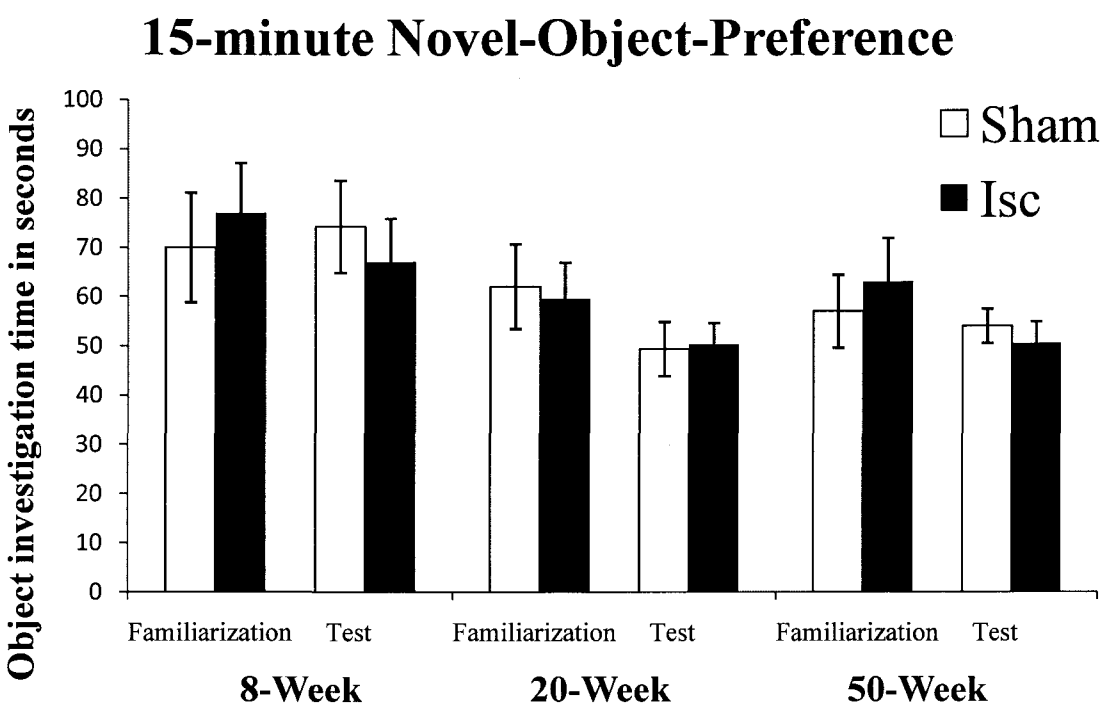

Figure 7 Average time spent engaged in object exploration during the familiarization and test phases of the 15-minute novel-object-preference test of object recognition memory. Error bars represent the SEM.

($F(2, 37) = 6.06, p < .05$) and nonsignificant main effect of surgery ($F(1, 37) = .68, p > .05$) and nonsignificant interaction ($F(2,37) = .096, p > .05$). The 8-week group spent significantly more time with the objects than the 20-week or 50-week group. Although age effects the amount of time the rats spent with the objects, the lack of group differences in surgery groups times indicates that rats that received sham-ischemia and those that received ischemia spent equal and comparable time investigating the objects during the test and familiarization phases.

Histological results

Figure 8 shows examples of typical coronal hippocampal sections of sham-ischemia and ischemic rats from all age groups, taken at approximately -3.00 mm from bregma. Tissue was stained with cresyl violet and digital pictures were acquired through digital imaging software (Scion Image).

Figure 9 shows the mean cell counts of precise pixelated subsection of hippocampal sections, averaged across left and right hemispheres. A Wilks' Lambda 2x3x5 repeated measures ANOVA run on the cell counts of the hippocampus using group (ischemia vs. sham-ischemia), age (8-week, 20-week, and 50-week) and subfield (CA1, CA2, CA3, hilus, and dentate gyrus) as independent variables revealed significant main effects of subfield ($F(4,34) = 2.43, p < .05$), surgery ($F(1,37) = 139.39, p < .05$), and a significant subfield x age x surgery interaction ($F(8, 70) = 3.17, p < .05$) as well as a nonsignificant main effect of age ($F(2,37) = 1.20, p > .05$). Bonferroni corrected analysis revealed that rats in all age groups had significant ischemic differences in the CA1 ($p < .01$), CA2 ($p < .05$), and the dentate gyrus ($p < .05$). Rats in the 8 week and 20-week

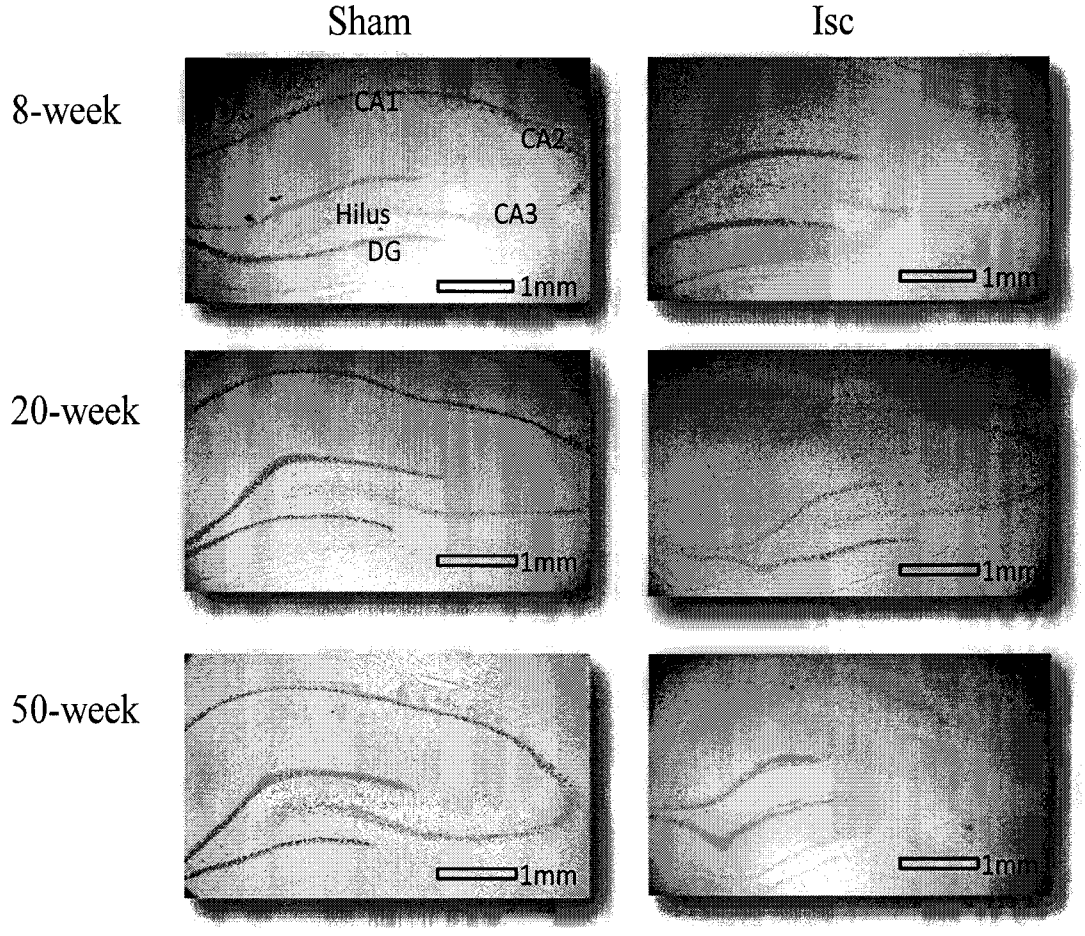

Figure 8 Examples of representative coronal hippocampal sections taken at approximately -3.00 mm bregma. Subfields of interest are indicated on the upper left photo and include CA1, CA2, CA3, dentate gyrus (DG), and hilus.

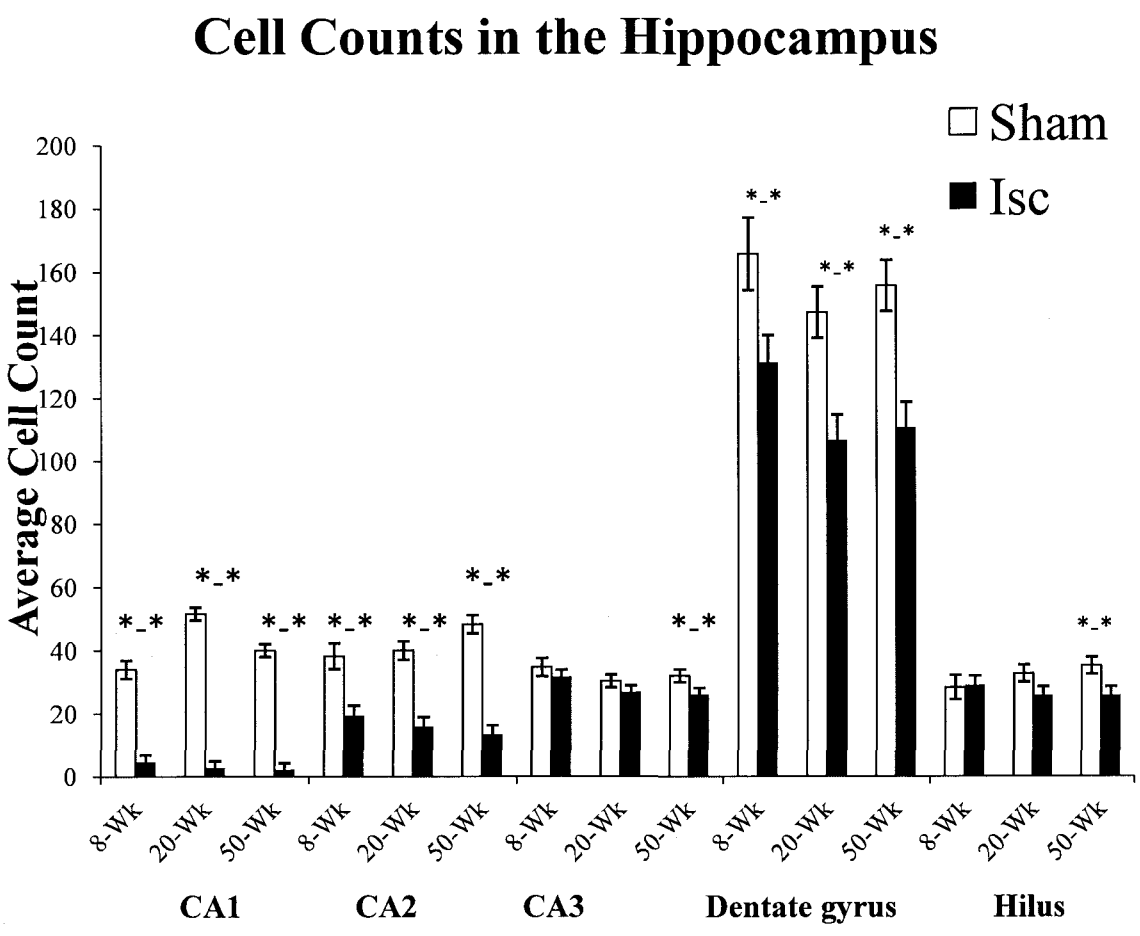

Figure 9 Average cell count of coronal hippocampal sections taken at approximately -3.00 mm bregma, averaged across hemispheres. Data plotted separately for CA1, CA2, CA3, dentate gyrus, and the hilus. Asterisks denote significant differences between sham-ischemia and ischemic rats at $p < .05$

ischemic groups did not have significantly different cell counts from age-matched sham-ischemia rats in the CA3 ($p > .05$) and hilus subfields ($p > .05$). This suggests that ischemia has more severe neuropathological impact on older rats.

Table 1 shows the Pearson correlation values per individual subfield of the hippocampus and the object investigation ratios for first two minutes of the tests. Performance in the novel-object-in-place test of spatial memory was significantly correlated with cell counts of the CA1, CA2, and dentate gyrus. Performance in the15-minute delay of the novel-object-preference test correlated significantly with cell counts made in CA1 and CA2. However, performance in the 24-hour novel-object-preference test did not correlate significantly with any measured subfield.

Discussion

Behavioural findings

Novel-object-in-place test of spatial memory. The main findings of the novel-object-in-place test of spatial memory were that rats that received sham-ischemia in all age groups spent more time during the first 2-minutes of the test exploring the object that had moved than exploring the stationary object, whereas rats that had received ischemia did not. Although rats in the 8-week ischemic group did not show a significant preference for the moved object during the first 2-minutes, their preference ratios were not significantly lower than those of age-matched sham-ischemia control rats. These findings

Table 1.

Pearson correlation values for individual subfields and performance on memory tests.

		CA1	CA2	CA3	DG	Hilus
Novel-object-in-place test	Pearson Correlation	0.57	0.53	0.20	0.62	0.28
	Sig. (2-tailed)	<.05	<.05	0.20	<.05	0.06
Novel-object-preference test 24-hour	Pearson Correlation	0.06	0.17	0.05	0.25	0.13
	Sig. (2-tailed)	0.70	0.27	0.75	0.10	0.41
Novel-object-preference test 15-min	Pearson Correlation	0.39	0.30	0.27	0.24	0.27
	Sig. (2-tailed)	<.05	<.05	0.08	0.13	0.07

are consistent with previous data indicating that ischemia impairs spatial memory as measured by a task in a water maze (Nunn et al., 1998).

Neither sham-ischemia nor ischemic rats showed a preference for the quadrant that contained the moved object over the quadrant that contained the object that was not moved, or for the quadrant that formerly held an object over the quadrant that never did. This pattern of behaviour differs from rats that have had neurotoxic lesions of the ventral hippocampus, who do demonstrate these preferences (Gaskin, Gamliel, Tardif, Cole, & Mumby, 2009). There was, however, a trend towards significance in older (20- and 50-week) ischemic rats for the quadrant that contained the moved object over the quadrant that housed the stationary object when considering all five test minutes. This may represent a shift of the behavioural expression of the memory and not a total memory impairment. It should be noted that rearing may also represent a valid measure of memory in the novel-object-in-place test and future work on changes in rearing may provide important information on how the brain reacts to ischemia at different ages, and how memory for an objects location is expressed.

Novel-object-preference test of object recognition memory. Ischemia produced delay- and age-dependent impairments in the novel-object-preference test. Rats that received sham-ischemia in all age groups as well as ischemic rats in both the 8- and 50-week groups demonstrated a preference for the novel object in the 15-minute delay condition. With the exception of the 20-week group, all rats that received sham-ischemia had significant preference ratios, whereas only ischemic rats in the 8-week group showed a preference for the novel object, at the 24-hour delay. Although, the 8-week ischemia

group preformed as well as age matched sham-ischemia rats, the 24-hour delay may not have been long enough to view impairments and only the 20-week ischemic rats failed to perform normally at the 15-minute delay suggesting that age influences memory impairments in object recognition following ischemia in a delay dependent manner. These results support previous findings of delay-dependent performance impairments on the DNMS task and novel-object-preference test following ischemia (Mumby et al., 1996; Wood & Phillips, 1991; Wood et al., 1993; Piterkin et al., 2009, unpublished). However, some statistical results may have been compromised due to the sham-ischemia rats in the 20-week group failing to show a preference in the 24-hour novel-object-preference test.

Sham-ischemia rats in the 20-week group did not show a preference during the 24-hour delay of the novel-object-preference test. One explanation is that the two minute time span was too long and that rats in the sham-ischemia group became familiar quickly with the novel object, leading to equal exploration times by the end of the first 2-minutes of testing. This seems unlikely, since these same rats demonstrated a preference during this time of the test during the 15-minute delay condition. A different explanation is that the 24-hour delay was too challenging, and that the rats demonstrated equal investigation of both objects since neither was remembered.

These findings also suggest that memory impairments may be attenuated in younger rats. The 8-week ischemic rats demonstrated a significant preference for novel object at both the 15-minute and 24-hour delays conditions. Although this suggests normal object recognition memory in the 8-week ischemic rats it is highly probable that the 24-hour delay was not long enough for the rats to demonstrate memory impairments.

It is impossible to resolve whether the underlying neuropathological process responsible for these age differences in memory test performance is as a result of reduced ischemic pathology or as a result of age differences in the recovery process. Differences in cell counts between groups can only suggest that ischemia resulted in neuropathology in the hippocampus in all ages in comparable ways.

Neuropathology

Ischemia caused major damage to CA1, CA2, and dentate gyrus in all age groups. Ischemic rats in the 8-week and 20-week groups did not have significantly fewer neurons in CA3 and hilus subfields than their age-matched sham-ischemia counterparts, where as ischemic rats the 50-week group had a significant reduction in cell counts in these areas. This suggests that the 8-week and the 20-week groups had less severe ischemic damage within the hippocampus than the older 50-week rats. Only a single anterior-coronal section was examined and it is possible that the degree of neuropathology was different in other sections. However, an interesting possibility for the differences in cell counts is that ischemia, induced increased rates adult neurogenesis in the 8-week and 20-week ischemic rats and that these rats did not solely sustain less ischemic damage.

Inhibition of adult neurogenesis within the hippocampus can cause performance impairments in the novel-object-preference test in rats (Jessberger et al., 2009). Others have shown that hippocampal neurogenesis is enhanced as a consequence of ischemia (Nakatomi et al., 2002). However, the adult neurogenesis process slows over the rats first year (Kuhn, Dickison-Anson, & Gage, 1996). It is possible that the 8-week and 20-week ischemic rats did not solely incur less ischemic damage but had more capability to

replace damaged neurons due to increased rates of neurogenesis than did the older 50-week group.

Adult neurogenesis may also explain observed differences in cell counts in the CA1 region of the hippocampus between rats that received sham-ischemia. Ischemic rats in all ages had comparable cell counts in the CA1 subfield of the hippocampus. Sham-ischemia rats in the 20-week group appeared to have significantly more CA1 neurons than the other age groups. This also could have resulted from the adult neurogenesis process, which presumably would continue adding cells to the CA1 subfield of the hippocampus throughout early adulthood, making cell counts in 20-week sham-ischemia rats appear larger than those in the 8-week group. The gradual decline of neurogenesis over the first year of age (Bizon, Lee & Gallagher, 2004) in addition to gradual cell death due to aging (Landfield, 1999; Bizon & Gallagher, 2005) would explain the difference between the 20-week and 50-week groups. However, again it remains unclear whether these potential processes had any influence on the behaviours measured in this thesis.

Linking neuropathology to behavioural findings

Ischemia caused damage to hippocampal subfields including CA1, CA2, and the dentate gyrus. It is possible that other anatomically distinct areas, such as the thalamus, may have also suffered damaged because of ischemia. In the present study, only the hippocampus was examined, making it impossible to discern whether impairments on the novel-object-in-place or novel-object-preference tests reflect the same fundamental functional impairment or distinct impairments that resulted from unique aspects of the brain damage. New experimental designs are needed to firmly establish causal links

between ischemia induced neuropathology and memory impairments. However, correlations between test performance and cell counts in the hippocampus, and comparisons of these results with those of studies involving surgical lesions, and synaptic protein quantification will yield valuable information about the effect that age at the time of ischemia has on neuropathology after ischemia.

A significant correlation was observed between cell counts of CA1, CA2, and the dentate gyrus and object investigation ratios of the first 2 minutes of the novel-object-in-place test of spatial memory. This result is congruent with previous spatial memory findings linking CA1 damage with memory impairments in the novel-object-in-place test and other spatial memory tasks (Gaskin et al., 2003). Although there have been highly neuroprotective treatments that have failed to prevent spatial memory impairments, as well as highly effective treatments of spatial memory impairments that have little or no neuroprotective effect in the hippocampus (Benetoli et al., 2007; Roberge, Bernard, Messier, & Plamondon, 2008), suggesting that extrahippocampal damage can contribute to ischemia-induced spatial-memory deficits.

Significant relationships between the object-investigation ratios of the first 2-minutes of the 15-minute novel-object-preference test and cell counts in CA1 and CA2, suggest that the hippocampus may also be involved in object recognition memory. This is in accordance with studies showing significant correlations between impaired object recognition using the DNMS task and hippocampal pathology in rats that received a 20-minute global ischemia (2VO) (Wood et al., 1993). As previously mentioned, however, the evidence from studies involving surgical ablation of the hippocampus suggests otherwise, as DNMS and NOP performance are typically unaffected by hippocampal

ablation or neurotoxic lesion (Mumby et al., 1996; Mumby et al., 2002). Performance on the 24-hour novel-object-preference test did not correlate significantly with any neuronal region measured nor was it significantly correlated with any other test. These findings are consistent with reports that object recognition memory impairments are due to covert or extrahippocampal ischemic damage (Mumby et al., 1996). These possibilities were not quantified at this time thus, it is impossible to delineate these differences. Further histological examination of the tissue may add significant value in interpreting the behavioural findings.

Dendritic damage caused by ischemia may interfere with the neuron's synaptic qualities and cause functional abnormalities while still allowing it to stain and appear normal. Several studies have reported evidence of impaired neuronal functioning in the gerbil brain following global ischemia, even when there was no evidence of significant cell loss (Corbett, & Nurse, 1992). Previous findings using immunocytochemistry show specific cellular synaptic proteins, such as microtubule associated proteins (MAP) and activated astrocytic markers such gial fibrillary acid protein (GFAP), are upregulated differently in the hippocampus after ischemia in young and aged rats (Kharlamov, Kharlamov & Armstrong, 2000). This suggests that the brain damage responsible for memory impairments following ischemia may be undetectable by the means used in the present thesis.

Rats with hippocampal lesions demonstrate normal performance on the novel-object-preference test of object recognition memory in a variety of conditions (Gaskin et al., 2003; Mumby et al., 2002). Other brain areas such as the thalamus may be damaged by ischemia and result in impairments in successful object recognition in the novel-

object-preference test. Extra-hippocampal structures may represent excellent candidates for further examination using advanced electrophysiological and imunocytochemical staining techniques.

It is difficult to suggest that age-dependent ischemic-differences in behavioural performance in memory tests are a result solely of differences in damage within the hippocampus. Even if damage within hippocampus was solely responsible for all of the observed memory impairments, the staining methods used were not sensitive to functionally impaired or covertly damaged neurons. However, it does appear that damage to the hippocampus is correlated with performance in the novel-object-in-place test of spatial memory and to a lesser degree the 15-minute novel-object-preference test of objection recognition memory. Adult neurogenesis may explain differences in cell counts and some differences in performance in object recognition tests, though it should be noted that covert damage or damage to extra hippocampal areas may have contributed to memory impairments in rats of this thesis.

Limitations

One limitation of the present thesis is that each age group (8-week, 20-week, and 50-week) was tested sequentially and individually, such that the 8-week group was tested first and the 50-week group was tested last. This methodology was adopted due to time constraints and limits the generalizability of the findings across age groups. Here it can be noted that all rats in all age groups had the same general housing, were handled and tested by the same experimenter and all testing and surgical procedures were identical, though the precision of the surgeon improved as the experiment continued.

Summary of Findings and General Conclusions

Ischemic rats in all age groups demonstrated performance impairments in the novel-object-in-place (NOIP) test of spatial memory, although, the 8-week group did not differ significantly from age-matched sham-ischemia rats. The cell counts of CA1, CA2, and the dentate gyrus were all significantly correlated with object investigation ratios in this test. This suggests that a 15-minute global ischemia (4VO) produces significant spatial memory impairments in all ages and these impairments may be related to hippocampal damage.

Rats that received ischemia in the 20-week group, failed to demonstrate a preference for the novel object during the 15-minute novel-object-preference test of object recognition where as only ischemic rats in the 8-week group demonstrated a preference for the novel object during the 24-hour delay condition. Although, sham-ischemia rats in the 20-week hinder clear interpretation, this seems to suggest that the age of the rat influences delay-dependent object-recognition memory impairments following global ischemia. Performance in the 15-minute delay condition of the novel-object-preference test correlated with CA1 and CA2 cell counts, suggesting that the hippocampus may play a role in object recognition at short delays. However, the object investigation ratios of the 24-hour delay condition were not correlated with any hippocampal subsection measured suggesting that extra-hippocampal or covert damage may be responsible for object recognition memory impairments at long delays.

Rats in the 50-week ischemic group had significantly lower cell counts from aged-matched sham-ischemia control rats in all measured hippocampal subfields,

whereas rats in the 8-week and 20-week groups did not have significantly different cell counts in CA3 and the hilus subfields. This suggests that the age of a brain influences the resulting neuropathology or ability to repair damaged areas following global ischemia.

This thesis presents data that suggests that the age of the rat influences the resulting effects of ischemia on hippocampal cell counts more severely in the old than the young. However, it remains unclear if these resulting differences are responsible for any differences in behaviour. More studies are needed examining age-dependent differences in behaviour following global ischemia to clarify the influence that age of the brain has on the neuropathology and memory impairments following global ischemia.

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Appendix

Tests of Between-Subjects Effects

Dependent Variable: Object investigation ratios of the first two minutes of the novel-object-in-place test of spatial memory.

Source	F	df	Sig.
Isc	17.07	1	$p < .05$
Age	.56	2	$p > .05$
Isc * Age	1.91	2	$p > .05$

Tests of Between-Subjects Effects

Dependent Variable: New/Same quadrant preference ratios of the first two minutes of the novel-object-in-place test of spatial memory.

Source	F	df	Sig.
Isc	3.06	1	$p > .05$
Age	0.11	2	$p > .05$
Isc * Age	0.66	2	$p > .05$

Tests of Between-Subjects Effects

Dependent Variable: Former/Never quadrant preference ratios of the first two minutes of the novel-object-in-place test of spatial memory.

Source	F	df	Sig.
Isc	1.92	1	$p > .05$
Age	0.16	2	$p > .05$
Isc * Age	1.29	2	$p > .05$