Memory Impairment Following Transient Global Cerebral Ischemia in Rats: Relationship to Neuropathology in the Hippocampus and Perirhinal Cortex

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ABSTRACT

Memory Impairment Following Transient Global Cerebral Ischemia in Rats: Relationship to Neuropathology in the Hippocampus and Perirhinal Cortex

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Memory impairments following global cerebral ischemia have traditionally been associated with damage to the hippocampus (HPC), but some ensuing impairments, such as object-recognition deficits, suggest the presence of extra-hippocampal neuropathology. In the present study, object-recognition memory, object-discrimination learning, and allocentric-spatial working-memory in rats were assessed following global cerebral ischemia (15 min; 4VO), or sham-ischemia. Ischemic rats were impaired on a delayed-matching-to-place (DMTP) water-maze task, and performed normally on a novel-object-preference (NOP) test of object-recognition memory after a 15-min retention interval, but displayed deficits after a 24-hr interval. Ischemic rats learned object-discrimination problems at normal rates, and displayed normal 24-hr retention of the discriminations, suggesting that their object-memory impairment may have been specific to recognition. Upon completion of behavioral testing, the HPC and perirhinal cortex (PRh) were examined by visual quantification of Nissl-stained neurons, as well as immunohistochemical staining for glial-fibrillary acidic protein (GFAP). Ischemic rats displayed significant cell-loss and increased GFAP expression in both, the HPC and PRh. DMTP deficits were significantly correlated with the degree of cell loss and GFAP expression in the HPC. In addition, the degree of PRh cell loss significantly correlated with the degree of NOP deficits. These findings suggest that PRh damage may make a significant contribution to ischemia-induced impairment of object-recognition memory.

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INTRODUCTION

Global cerebral ischemia is a type of cerebral vascular accident that occurs when blood supply to the brain is interrupted or highly restricted, such as following cardiac arrest or severe hypotension. Many survivors of global ischemia suffer a wide range of long-term complications including motor, behavioral, and cognitive impairments (Grubb, O'Carroll, Cobbe, Sirel, & Fox, 1996; Sauvé, Doolittle, Walker, Paul, & Scheinman, 1996; Wachelder et al., 2009).

Despite its potential for producing widespread cerebral pathology (Grubb et al., 2000; Pulsinelli, Brierley, & Plum, 1982; Smith, Auer, & Siesjö, 1984; Volpe, Pulsinelli, & Davis, 1985), memory impairments induced by global cerebral ischemia have traditionally been attributed to hippocampal (HPC) damage for several reasons. Memory deficits have been reported after global ischemia in rats, humans, and non-human primates with few apparent histological abnormalities outside the HPC (Bachevalier & Mishkin, 1989; Mumby et al., 1996; Rempel-Clower, Zola, Squire, & Amaral, 1996; Volpe, Davis, Towle, & Dunlap, 1992; Wood et al., 1993; Zola-Morgan, Squire, & Amaral, 1986; Zola-Morgan, Squire, Rempel, Clower, & Amaral, 1992). There have not been reports of memory impairment induced by global ischemia without concomitant damage to the HPC. Ischemia in rats impairs performance on spatial-memory tasks, similar to the effects of HPC ablation (Block, 1999; Corbett & Nurse, 1998). The severity of ischemia-induced spatial-memory impairment is sometimes correlated with the severity of HPC cell loss (Block & Schwarz, 1996; Briones & Therrien, 2000; Nelson, Lebessi, Sowinski, & Hodges, 1997; Nelson, Sowinski, & Hodges, 1997; Volpe et al., 1992).

There has been little attention given to the possibility that some aspects of memory impairment following global ischemia result from damage outside the HPC, but some evidence suggests this may be so. Global ischemia impairs rats' performance on tests of object-recognition memory (Gaskin, Amir, & Mumby, 2002; Mumby et al., 1996; Wood et al. 1993), whereas HPC ablation typically does not (Forwood, Winters, & Bussey, 2005; Gaskin et al., 2002; Kesner, Bolland, & Dakis, 1993; Mumby, 2001; Mumby et al. 1996; Rothblat & Kromer, 1991). There is a lack of direct evidence, however, that would implicate any specific extra-hippocampal structure as a locus of the critical ischemia-induced damage.

A major aim of the present study was to determine whether ischemia-induced impairment of object-recognition memory is related to damage in the perirhinal cortex (PRh), a temporal lobe structure that is necessary for object-recognition (Aggleton, Keen, Warburton, & Bussey, 1997; Brown & Aggleton, 2001; Brown & Bashir, 2002; Gaffan & Murray, 1992; Griffiths et al., 2008; Massey et al., 2008; Meunier, Bachevalier, Mishkin, & Murray, 1993; Mumby & Pinel, 1994). In previous studies that reported object-recognition deficits following global ischemia in rats, no significant damage was observed in the PRh (Mumby et al. 1996; Wood et al., 1993). The histological methods used in those studies, however, relied exclusively on estimation of neuronal-density from Nissl-stained tissue. It is reasonable to suspect that ischemia may cause damage that is sufficient to impair brain function and behavioral performance yet insufficient to induce cell death and thus be detected by conventional histological methods (Bachevalier & Meunier, 1996; Corbett & Nurse, 1998; Squire & Zola, 1996).

In the present study, PRh and HPC integrity was examined using conventional neuronal-density measures and immunohistochemical staining for an astrocyte marker, glial-fibrillary acidic protein (GFAP). GFAP is found in microfilaments of glial astrocytes. When damage occurs in the adult brain, astrocytes become reactive, proliferating near the site of damage, thus upregulating the expression of GFAP (Norenberg, 1994; Panickar & Norenberg, 2005).

Rats' object-recognition memory was assessed following transient global cerebral ischemia or sham-ischemia. The rats were also trained on object-discrimination tasks in order to determine whether ischemia produced a general impairment in the ability to learn about objects. Additionally, rats' allocentric-spatial memory was assessed in order to confirm the functional effectiveness of the ischemia in the event that no impairments were observed on the object-recognition or object-discrimination tasks.

The main behavioral findings were that ischemic rats were impaired on object-recognition and allocentric-spatial memory tasks. Histological examination revealed significant cell loss and elevated GFAP expression in the HPC and PRh of ischemic rats. Furthermore, the severity of object-recognition deficits correlated with the severity of PRh cell loss, and the degree of allocentric-spatial memory impairments correlated with histopathology in both the HPC and PRh.

The remainder of this introduction is structured as follows: Section 1.2 presents an overview of stroke and global ischemia. Section 1.3 describes animal models of global ischemia. Section 1.4 discusses mechanisms of ischemic neuronal damage. Section 1.5 describes memory impairment following global cerebral ischemia. Section 1.5 presents

the rationale for the present study, and section 1.6 outlines the experimental design of this thesis.

1.2 Stroke and Global Cerebral Ischemia

Stroke, or cerebral vascular accident, is the third leading cause of death and the primary cause of permanent disability in Canada and the United States (American Stroke Association, 2009; Canadian Stroke Network, 2007). It is estimated that in 2009 the United States alone will pay $68.9 billion for stroke-related medical and disability costs (American Stroke Association, 2009). Given that one's risk of suffering stroke significantly increases with age, and we are an increasingly aging population, this economic burden is expected to escalate considerably in the near future (American Stroke Association, 2009; Canadian Stroke Network, 2007; Flynn, MacWalter, & Doney, 2008).

There are two types of stroke: hemorrhagic and ischemic. Hemorrhagic stroke occurs when a blood vessel ruptures inside the brain (intracerebral hemorrhage), or on its surface (subarachnoid hemorrhage). Bleeding can disrupt normal brain function via compression of the surrounding tissue caused by the expanding hematoma, direct toxic effects due to disruption of the blood-brain barrier, and development of edema and inflammation. Hemorrhagic strokes account for 10 - 20% of all strokes (Fingas, Penner, Silasi, & Colbourne, 2009; Thrift, Donnan, & McNeil, 1995; Xi, Keep, & Hoff, 2006). The other 80 - 90% of all strokes are ischemic, which occur when blood flow to the brain is interrupted or severely restricted. The brain is extremely vulnerable to fluctuations in blood flow as it depends on aerobic metabolism for production of energy and relies on approximately 25% of cardiac output in order to sustain its metabolic requirements. Thus, even a relatively brief disruption in cerebral circulation can lead to rapid depletion of

glucose and oxygen levels and consequently, to brain damage (Briones & Therrien, 2000; Flynn, MacWalter, & Doney, 2008). Cerebral ischemia can be focal or global. Focal ischemia occurs when an artery is occluded by a thrombus or embolus, thereby preventing perfusion of brain tissue downstream of the obstruction. Global ischemia occurs when blood flow to the entire brain is severely compromised, such as during cardiac arrest.

Approximately 50% of individuals that undergo successful cardiopulmonary resuscitation never regain consciousness. About 30% become severely disabled, and are unable to stand, sit, walk, or perform basic personal necessities. Additionally, these patients experience devastating mental deterioration, losing basic understanding of time, place, and the roles of other individuals, as well marked impairment in the ability to retain and recall experiences (Jørgensen & Holm, 1998).

Follow-up studies have shown that the patients who show considerable recovery may nevertheless experience a variety of complications months or even years after cardiopulmonary resuscitation. Many report severe fatigue, feelings of anxiety and depression, and an overall reduction in quality of life compared to the general population (Jørgensen & Holm, 1998; O'Reilly, Grubb, O'Carroll, 2003; Wachelder et al., 2009). Furthermore, clinically significant (i.e. moderate to severe) and persistent cognitive impairments have been observed in 20-50% of cardiac arrest survivors. These include impairments in orientation, attention, as well as verbal, spatial, recall, and recognition memory (Drysdale, Grubb, Fox, & O'Carroll, 2000; Grubb et al., 2000; Grubb et al., 1996; O'Reilly et al., 2003; Pußwald, Fertl, Faltl, & Auff, 2000; Roine, Kajaste, & Kaste, 1993; Sauvé et al., 1996; Sunnerhagen, Johansson, Herlitz, & Grimby, 1996).

1.3 Animal Models of Global Cerebral Ischemia

Research on global ischemia is conducted for two general purposes: to understand the pathophysiology and mechanisms of brain damage, and to promote the development and evaluation of treatments and potential neuroprotective agents. Research with animal models plays an indispensable role in these endeavors since invasive procedures and highly controlled experimental measures are not possible with human participants. Furthermore, given the complexity of the brain's response to injury, and given that the evaluation of any treatment will ultimately rest on assessment of functional outcome, use of in-vitro techniques alone cannot sufficiently address these goals.

The majority of research on global ischemia with animal models has been conducted on rodents, which offer a number of benefits over the use of larger species such as dogs, pigs, and non-human primates. Rodents are relatively easy to purchase and maintain, and are less costly than the larger species. Rodents' smaller brain-size allows for a more complete, yet less financially burdensome and time consuming histological examination of the entire brain. Researchers interested in assessment of functional outcome can make use of a large variety of behavioral and sensorimotor tests that have been developed for use with rodents, especially rats. Finally, from an ethical perspective, there is more public acceptance of experimentation on rodents compared to the larger species (Graham, McCullough, & Murphy, 2004).

Surgical procedures used to induce global cerebral ischemia fall into two general categories: those that produce complete global ischemia, and those that produce incomplete global ischemia. During complete global ischemia, cerebral blood flow is entirely interrupted for a desired period of time, which can be achieved by decapitation

(Lowry, Passonneau, Hasselberger, & Schulz, 1964), aortic and inferior vena caval occlusion (Jackson & Dole, 1979), neck tourniquet (Kofke et al., 1979), neck-cuff inflation (Grenell, 1946), or ventricular fibrillation (Safar, Stezoski, & Nemoto, 1976). During incomplete global ischemia, cerebral blood flow is sufficiently reduced so that the brain can no longer sustain its metabolic requirements. The most widely used procedures that induce incomplete global cerebral ischemia are the rat model four-vessel occlusion (4VO), and the two-vessel occlusion (2VO) procedure supplemented with hypotension (Graham et al., 2004; Traystman, 2003).

The 4VO procedure was introduced by Pulsinelli and Brierley (1979), and involves two stages. On the first day, the vertebral arteries are permanently occluded using electrocoagulation via the alar foramen of the first cervical vertebra, and atraumatic clasps are loosely placed around both common carotid arteries by way of a neck incision. On the second day, the neck incision is reopened while the animal is awake, and the carotid arteries are clamped for a given period of time to induce global ischemia of a desired duration. Onset of unconsciousness and the loss of the righting reflex upon clamping are taken as indices that the procedure is successful. The 2VO procedure is performed under a general anesthetic and also involves bilateral occlusion of the common carotid arteries. At the same time, the animal is subjected to systemic hypotension in order to reduce the cerebral blood flow below the ischemic threshold (Eklof & Siesjo, 1972; Smith et al., 1984). Systemic hypotension is commonly achieved by controlled exsanguination (followed by reinfusion), which is sometimes supplemented by administration of vasodilators (McBean & Kelly, 1998). The 4VO and 2VO+hypotension

procedures in the rat model induce cerebral pathology of comparable size and location (McBean & Kelly, 1998), and both have been widely used and validated.

The vessel-occlusion procedures have also been used with other rodent species. The gerbils' lack of posterior communicating artery (i.e. incomplete Circle of Willis) is a unique vascular feature witch permits induction of complete global ischemia by way of bilateral common carotid artery occlusion alone (Levine & Sohn, 1969). Unfortunately, this anatomical feature is found in only 50% of gerbils (Harukuni & Bhardwaj, 2006), which inevitably inflates the 2VO procedure's failure rate in this species. Attempts to use vessel-occlusion procedures in mice have had limited success, particularly due to high mortality rates (Traystman, 2003). Furthermore, the gerbils' and mice' smaller size makes the surgical procedures and physiological monitoring (i.e. blood pressure, electroencephalogram (EEG) activity, or core body and brain temperature) more difficult (McBean & Kelly, 1998).

1.4 Mechanisms of Neuronal Damage

Global cerebral ischemia produces selective neuronal damage in a variety of neuronal populations. The HPC is a structure that is most vulnerable to damage following global ischemia, especially through loss of neurons in the CA1 subfield and hilus of the dentate gyrus (Kirino & Sano, 1984; Smith, et al., 1984). If duration of ischemia is sufficiently brief (3 minutes and 10 minutes in the gerbil and rat models, respectively), detectable histologic injury appears exclusively in the HPC (Pulsinelli et al., 1982; Kirino, 1982). Longer durations of ischemia produce cell loss in the striatum, thalamus, and cortex (Chesselet et al., 1990; Grisham & Granger, 1989; Pulsinelli et al., 1982).

Normal cerebral blood flow is approximately 50 to 75 ml/100g of tissue per minute. When it is reduced to 18 mL/100g of tissue per minute, ischemic depolarization begins to occur (Harukuni & Bhardwaj, 2006). Lack of fresh glucose and oxygen supply induces anaerobic glycolysis and mitochondrial inhibition of adenosine triphosphate (ATP) synthesis. Energy stores are rapidly depleted and ATP-reliant ion transport systems such as the Na^+/K^+ATPase and $Ca2^+$ATPase ion pumps fail. Neuronal plasma membrane becomes depolarized; K^+ is released into extracellular space and intracellular Na^+ and $Ca2^+$ concentrations rise. (Doyle, Simon, & Stenzel-Poore, 2008). Depolarization and an increase in intracellular $Ca2^+$ trigger the release of glutamate, which is the most abundant excitatory neurotransmitter in the brain. Due to its release and the concomitant impairment of its ATP-dependent reuptake mechanisms, synaptic glutamate concentration is elevated causing activation of *N*-methyl-*D*-aspartate (NMDA), kainate, and α-amino- 3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which produces further membrane depolarization and a further increase of intracellular $Ca2^+$ levels. Extended calcium overload causes production of harmful elements such as free radicals, reactive oxygen species, and $Ca2^+$-dependent catabolic enzymes, which cumulatively contribute to excitotoxic cell injury (Bhardwaj, Alkayed, Kirsch, & Hurn, 2003; Doyle, et al., 2008).

It was traditionally accepted that excitotoxic injury results in necrotic cell death, which is characterized by severe mitochondrial injury, production of toxins, cell swelling, membrane rupture, and tissue inflammation due to spilling of cell contents into the surrounding environment. Necrosis normally occurs as a result of acute cellular stress, and is not considered a regulated or programmed event (Gorman, 2008). Apoptosis is a

process of programmed cell death, or "cell suicide", characterized by preservation of ATP and mitochondrial function, and initiation of biochemical pathways that cause cell shrinkage, genomic DNA fragmentation, and chromatin condensation, without membrane lysis and inflammation of the surrounding tissue (Ankarcrona et al., 1996; Harukuni & Bhardwaj, 2006). It has been shown that neurons that survive acute excitotoxic injury and experience restoration of energy and partial recovery of mitochondrial function may nevertheless undergo delayed cell death by apoptosis (Ankarcrona et al., 1996). Additionally, factors such as cell type, age, and condition at the time of the ischemic incident are believed to play a role in determining which mode of cell death is undertaken (Lipton, 1999).

Despite reoxygenation of tissue and normalization of blood flow soon after the cessation of the ischemic period, cerebral reperfusion can contribute to brain injury via oxidative and nitrative stress. Increased production of free radicals such as superoxide, nitric oxide, and peroxynitrate leads to degradation of the basal lamina, compromising structural integrity of the vascular wall (Doyle, et al., 2008). Additionally, ischemic cells release inflammatory cytokines (interleukin [IL-1], tumor necrosis factor α [TNF-α]) triggering production of cell adhesion molecules, which can further damage the blood-brain barrier and increase likelihood of intracerebral hemorrhage and vasogenic brain edema (Crack & Taylor, 2005; Harukuni & Bhardwaj, 2006).

1.5 Memory Impairment following Global Cerebral Ischemia

Numerous studies have shown that rats subjected to global cerebral ischemia display deficits on tests of spatial reference and working memory. On any given test of spatial learning and memory, reference memory is memory for location or position in the

environment that remains constant over trials; spatial working memory is memory for trial-specific information. The radial arm maze (Olton & Samuelson, 1976) and the Morris water maze (Morris, Garrud, Rawlins, & O'Keefe, 1982) have been designed to asses these memory abilities in rats, and both continue to be widely used for this purpose.

The 8-arm radial maze consists of a central starting area, which provides entry into eight arms (i.e. runways) that radiate away from the center. At the end of each arm is a food well, and certain arms are consistently baited with a food reward, while others are not. Food-restricted rats are then given repeated opportunities to search for food in the maze. The most efficient strategy is to only visit the baited runways, which normal rats will do after a given number of trials, suggesting they have learnt their location. Since there are no cues inside the maze to guide their search, animals have to rely on extra-maze cues to learn which arms are bated and which are not. A reference memory error is made when an animal visits a non-baited arm. A working memory error is made when the animal enters a baited arm that had already been visited on that trial. It has been shown that animals subjected to global cerebral ischemia are impaired on both reference, and working memory in the radial arm maze (Davis, Tribuna, Pulsinelli, & Volpe, 1986; Kiyota, Miyamoto, & Nagaoka, 1991).

The Morris water maze exploits rats' naturally strong ability to swim, and encompasses certain working advantages over the radial arm maze. Food restriction is unnecessary in water maze testing, and neither are habituation trials. Furthermore, the use of water eliminates possible complications due to odor traces. On the standard version of the water-maze test (i.e. fixed-platform navigation), the animal is placed into the circular pool at various starting locations and is given an opportunity to find a submerged escape

platform, which remains in one position throughout training. Although rats are naturally strong swimmers, they dislike water and are therefore motivated to find the platform, thereby reducing their time spent in water. Since the water is rendered opaque by milk powder or nontoxic paint, the animals cannot see the platform. Furthermore, since there are no cues inside the pool to guide the animals' search, and since reliance on egocentric strategies is devalued by the use of various starting locations, the animals must rely on extra-maze cues to learn the position of the platform. Increased latency to find the platform after a given number of training trials is taken as impairment in spatial learning. Reference memory impairment can be observed on probe trials, during which the platform is removed from the pool and the time allocated for its search in its previous location is measured. A common adaptation of this procedure to test working memory is known as a learning-set, in which the platform is moved to a different position each day. Finding the platform on the second daily swim places a heavy demand on working memory because the animals need to remember where they had previously found the platform on a single occasion (i.e. the first daily swim). It has been shown that animals subjected to global cerebral ischemia are impaired in spatial learning (Block & Schwarz, 1998; Green et al., 1995; Hagan & Beaughard, 1990; Jaspers, Block, Heim, & Sontag, 1990; Nelson et al., 1997a; Nunn et al., 1994; Olsen, Scheel-Krüger, Møller, & Jensen, 1994), reference memory (Block & Schwarz, 1998; Jaspers et al., 1990; Nelson et al., 1997a; Nunn et al., 1994), and working memory (Auer, Jensen, & Whishaw, 1989; Green et al., 1992; Green et al., 1995; Nelson et al, 1997b) in the water maze.

It is widely accepted that the HPC is important for processing and storage of spatial information, and numerous studies have demonstrated that lesions of the HPC

disrupt performance on spatial learning and memory tasks (Nadel, 1991; Smith & Mizumori, 2006). Given that the HPC is particularly vulnerable to cell death following global cerebral ischemia, and given that ischemic animals display impairments on tests of spatial learning and memory, many investigators have reasonably attributed ischemia-induced memory deficits to HPC damage. This assumption is further supported by correlational evidence from several studies; the severity of ischemia-induced HPC cell loss has been found to correlate with the number of reference memory and working memory errors in the radial-arm maze (Kiyota et al., 1991), as well as the severity of spatial-learning (Block & Schwarz, 1996; Briones & Therrien, 2000; Nelson et al., 1997a), reference memory (Nelson et al., 1997a), and working memory (Nelson et al., 1997b) impairments in the water maze.

There has been very little consideration given to the possibility that some aspects of memory impairment following global cerebral ischemia are due to damage outside the HPC, but some ensuing impairments, such as object-recognition deficits suggest this may be so. Object-recognition is defined as the ability to discriminate the familiarity of objects previously encountered. The two widely used methods for assessment of object-recognition memory in rats are the delayed-nonmatching-to-sample (DNMS) task (Mumby, Pinel, & Wood, 1990), and the novel-object preference (NOP) test (Ennaceur & Delacour, 1988).

In the DNMS task, the rat is first trained to displace objects in order to receive a food reward. Once it acquires this ability, the rat is presented with a sample object covering a baited food well. Following a retention interval, the rat is again presented with the sample object along with a novel object, both positioned over adjacent empty food

wells. This time, the rat is rewarded for displacing the novel object. Successful performance on this task is thought to require object-recognition memory because the rat has to discriminate between an object it has seen before, and one it has not.

The NOP test relies on rats' natural propensity towards novelty; the rat is presented with two identical sample objects inside an open-field arena and allowed to explore for several minutes. The rat is then removed for a retention interval, after which it is returned to the arena with two new objects; one is identical to the sample, and the other is novel. If the retention interval is not too long, normal rats spend more time investigating the novel object during the test, indicating that they recognize the sample object. This test is a convenient way to assess object-recognition memory because it takes advantage of a spontaneous preference, so appetitive motivation, training, and learning of a nonmatching rule are not required.

It has been demonstrated that global ischemia impairs rats' performance on tests of object-recognition memory (Gaskin, et al., 2002; Gulinello, Lebesgue, Jover-Mengual, Zukin, & Etgen, 2006; Mumby et al., 1996; Plamondon, Davignon, Khan, & Charron, 2008; Plamondon, Morin, & Charron, 2006; Wood et al. 1993), whereas HPC ablation typically does not (Forwood et al., 2005; Gaskin et al., 2002; Kesner et al., 1993; Mumby, 2001; Mumby et al., 1996; Rothblat & Kromer, 1991). Comparable findings of impaired object-recognition after global ischemia, but not after HPC ablation, have also been reported in monkeys (Bachevalier & Mishkin, 1989).

1.6 Object-recognition Impairment: The Search for Extra-hippocampal Damage

It seems peculiar that partial (i.e. ischemic) damage to the HPC leads to impairment of object-recognition, but complete ablation of the same structure does not.

One possibility is that extra-hippocampal damage is responsible for this impairment - a notion that has largely been neglected in ischemia literature. This notion, however, is further supported by recent findings that chronic estradiol treatment attenuates object-recognition deficits in rats without any evident neuroprotection in the HPC (Gulinello et al., 2006).

A major goal of the present study was to determine whether ischemia-induced impairment of object-recognition memory is related to damage in the PRh, a temporal lobe structure that is necessary for object-recognition. Rats with PRh lesions have been shown to be impaired on both, the DNMS task (Mumby & Pinel, 1994), and NOP test of object-recognition (Aggleton et al., 1997; Mumby, Piterkin, Lecluse, & Lehmann, 2007). In addition, electrophysiological studies have shown that a large number of PRh neurons display diminished responses following presentation of previously encountered objects compared to novel objects, which is thought to support familiarity discrimination (Brown & Aggleton, 2001; Brown & Bashir, 2002), and it has been recently demonstrated that viral transduction blocking of mechanisms that support long-term depression in the PRh impairs object-recognition in rats (Griffiths et al., 2008).

Only two previous studies that reported object-recognition deficits following global ischemia in rats included assessment of neuropathology in the PRh (Mumby et al. 1996; Wood et al., 1993). Although no significant damage in the PRh was detected, the histological methods used in those studies relied exclusively on estimation of neuronal-density from Nissl-stained tissue. It is reasonable to suspect that ischemia may cause damage that is sufficient to impair brain function and behavioral performance yet insufficient to induce cell death and thus be detected by conventional histological

methods (Bachevalier & Meunier, 1996; Corbett & Nurse, 1998). For this reason, immunohistochemical staining for an astrocyte marker GFAP was included in the present study. GFAP is found in microfilaments of glial astrocytes. When damage occurs in the adult brain, astrocytes become reactive, proliferating near the site of damage, thus upregulating the expression of GFAP (Norenberg, 1994; Panickar & Norenberg, 2005). Elevated GFAP expression following global ischemia has been previously described in the HPC (Ordy et al., 1993; Langdon, Granter-Button, & Corbett, 2008), but no study to date has examined this glial response in the PRh following global cerebral ischemia.

1.7 Experimental Design

Rats' object-recognition memory was assessed using the NOP test following transient global cerebral ischemia or sham-ischemia. The rats were also trained on object-discrimination tasks in order to determine whether ischemia produced a general impairment in the ability to learn about objects. Additionally, rats' allocentric-spatial memory was assessed in the water maze in order to confirm the functional effectiveness of the ischemia in the event that no impairments were observed on the object-recognition or object-discrimination tasks. Neuropathology continues to evolve for months following global ischemia (Colbourne & Corbett, 1995; Corbett & Nurse, 1998), therefore it is essential to assess neuropathology at a time-point close to when behavioral data are collected. Accordingly, all behavioral testing was carried out over a 10-day period, and brains were removed 48 hrs later for histopothological assessment using conventional neuronal-density measures and immunohistochemical staining for GFAP . Each day began with the object-discrimination training in the morning, followed by NOP testing in the late afternoon, and water-maze testing in the late afternoon. All rats in the present

study were also tested in mid-afternoons on a shock-probe fear-conditioning task, and a subset of the rats (eight ischemic and 8 sham-ischemic rats) received a predator-odor avoidance test on different days, as part of a different experiment (Piterkin et al., under review); fear-learning tests were conducted in different rooms from those used for the present experiments.

EXPERIMENT 1

Rats were trained on a simple object-discrimination task and a 6-pair concurrent-object-discrimination task in order to assess whether global cerebral ischemia produces a general impairment in the ability to learn about objects. The simple object-discrimination task assessed rats' ability to learn an association between a particular object and a food reward. The 6-pair concurrent-object-discriminations task assessed the ability to learn six object-discrimination problems concurrently, under conditions of high retroactive and proactive interference. Although it has been shown that HPC lesions can slow acquisition of simple object-discrimination learning (Mumby, Pinel, Kornecook, Shen, & Redila, 1995), it has been demonstrated that with pre-surgery training, neither HPC lesions (Wible, Shiber, & Olton, 1992), nor global cerebral ischemia (Wood et al., 1993) lead to impairment of simple object-discriminations. Since the rats in the present study were familiarized with the procedural aspects of the task before surgery, it was expected that global ischemia would not impair simple object-discrimination learning. Performance on the multiple concurrent object-discrimination task has not been previously assessed in rats following global cerebral ischemia, but HPC lesions with (Wible et al., 1992) and without (Mumby et al., 1995) pre-surgery training produce impairments on this task. It

was therefore expected that rats subjected to global cerebral ischemia would be impaired in learning multiple concurrent object-discriminations.

Materials and Methods

Subjects

The subjects were 48 male Long-Evans rats (Charles River, St. Constant, Quebec), 10-12 weeks old at the beginning of the experiment. They were housed in pairs until surgery, after which they were housed individually. The rats had continuous access to water and were on a restricted feeding schedule (20-25 g of food per day). The colony room was on a 12:12 light-dark cycle, with light onset at 8:00 p.m. All procedures described below were replicated with six squads of eight rats; four ischemic (ISC) rats and four control (SHAM) rats per squad. All procedures were approved by the Concordia University Animal Care and Use Committee, and were in accordance with the guidelines of the Canadian Council on Animal Care.

Surgery

Rats received either 15-min global cerebral ischemia ($n = 24$) or sham-ischemia surgery ($n = 24$). Transient global cerebral ischemia was induced using a modified version of the four-vessel occlusion procedure (Pulsinelli & Brierley, 1979). The surgical procedure was performed over two days. On the first day, the rats were deeply anesthetized using isoflurane. The areas of incision were shaved and treated with iodine. The rats were secured in a stereotaxic device, and an incision (rostral/caudal) was made on the back of the neck at the level of the first vertebrae. Muscle tissue was gently separated to expose the first cervical vertebra, and the vertebral arteries were permanently

occluded via the alar foramina using an electro-cauterizer (Macan, Model MV-8). The incision was closed using wound staples, and the area was treated with antibacterial powder. The rats were removed from the stereotaxic device, placed on their back, and an incision (rostral/caudal) was made at the level of the clavicle. The common carotid arteries were located and gently separated from the vagus nerve and the adjoining adipose tissue. The arteries were encircled with dental floss ligatures, and the incision was closed using wound staples. The area was treated with antibacterial powder; the rats were taken off anesthetic and returned to their home cages for the 24-hr recovery period. On the second day, the rats were briefly anesthetized using isoflurane and placed on their back on top of a regulated heating pad. Body temperature was regulated by a thermoprobe and kept constant at 37 °C. The incision at the level of the clavicle was reopened, and the common carotid arteries were located using the dental floss ligatures. The ligatures were removed, the rats were taken off anesthetic, and global cerebral ischemia was induced by occlusion of the common carotid arteries for 15 min using microvascular clamps. Following occlusion, the arterial clamps were detached, the incision was resealed using wound staples, and the area was treated with antibacterial powder. The rats were returned to their home cages and closely monitored for three days. SHAM rats underwent the same two-day procedure with the exception of the carotid artery occlusion. Food restriction was suspended for seven days following surgery for all animals.

All rats were permitted to recover for 14 days before postsurgery testing commenced. Five of the ISC rats were excluded from postsurgery testing because their ischemia was considered to be partial, based on the observation that they did not remain

completely unconscious during the entire 15-min period of carotid-artery clamping. The data for Group ISC, therefore, are from 19 rats.

Apparatus

The apparatus for the object-discrimination task consisted of an elevated runway, separated from identical goal areas at each end by opaque guillotine doors (see Figure 1). Each goal area contained two food wells into which food pellets (45 mg Bio-Serv, Inc., Frenchtown, N. J.) could be delivered by hand through plastic tubes that were mounted on the outside of the apparatus. A short divider wall protruded from the center of the end wall to separate the two food wells. The stimuli were objects of various shapes, sizes, textures, and colors, each made of a similar plastic material. There were five copies of each object, which were used on different trials within sessions. Each object was large enough to cover a food well but small enough and light enough to be easily displaced by a rat. The objects and the apparatus were cleaned with 70% ethanol between animals to remove extraneous scents acquired during displacement by the rats or handling by the experimenter.

Behavioral Procedures

Presurgery habituation and shaping. Prior to surgery, the rats were habituated to the object-discrimination apparatus, and trained to gain access to food wells by displacing objects from on top of them. This was done over five daily sessions; each session lasted approximately 20 minutes. On the first day, all food wells were continually baited with food pellets, and the rats were allowed to freely explore the apparatus with both doors raised. On the second day, the rats were shaped to walk back and forth between the goal areas by alternatively delivering food pellets to the opposite ends of the apparatus. On the

Figure 1. A drawing representation of the apparatus used for object-discrimination testing.

third day, the rats were introduced to the guillotine doors. When the rat approached a food well, it received a pellet, following which the door leading to the goal area on the opposite end of the apparatus was lowered. Once the rat approached the lowered door, that door was raised providing access to the goal area behind it. When the food well in that goal area was approached, the rat received a pellet, at which time the door leading to the opposite end of the apparatus was lowered. This procedure was repeated for the duration of the session. On the fourth day, the rats were shaped to displace an object from on top of the food wells. The general procedure was the same as on the third day, except that when the rat entered the goal area, one of the food wells was baited and occluded by an object. The rats quickly learnt to displace the object because they could smell the pellet in the food well underneath it. On the fifth day, a pellet was delivered to a food well only after the rat displaced the object from on top of it.

Postsurgery rehabituation. The rats were rehabituated to the object-discrimination apparatus, while the experimenter reshaped each rats' tendency to approach the food wells and retrieve pellets from them. The objective was to bring all rats to a similar level of familiarity and competence with the procedural aspects of the task, and to eliminate any side preferences (i.e. preference for either the left or the right food well at either goal area) before the actual discrimination training began. This was done over two daily sessions, and each session lasted approximately 20 minutes.

Object-discrimination (single problem). The object-discrimination task assessed rats' ability to learn an association between a particular object and a food reward. On each trial, a different copy of the same pair of objects was presented; selection of one of them (S+) was rewarded with a food pellet, whereas selection of the other object was

never rewarded (S-). On the first day, half of the rats were trained on a single object-discrimination problem, and the other half were trained on the same problem the following day. The rat was placed in the center of the apparatus; one door was lowered, and the other was raised. When the rat approached the lowered door, that door was raised providing access to the goal area, which had the S+ and S- objects each placed over one of the food wells. The position of S+ (left or right) was randomized across trials. When the rat displaced one of the objects, the door leading to the goal area on the opposite end of the apparatus was lowered. If the rat displaced the S+, a food pellet was delivered to that food well; if the rat displaced the S-, it was not rewarded. For the initial 10 trials, if the rat first chose S-, it was permitted to displace the S+ and receive a reward before the experimenter removed the objects. These correction trials were not included in the analysis, and correction was not permitted for the remainder of the object-discrimination training. The S+ and S- were then positioned in the goal area at the opposite end of the apparatus in preparation for the next trial, which began when the rat approached the lowered door leading to that goal area. Rats were trained to a learning criterion: ten consecutive correct trials in a single daily session. The session ended when the rat either reached the criterion, or else received a maximum of 70 trials without reaching it. The same object-pair was used for each rat, with the particular object serving as S+ and S- counterbalanced within groups.

Six-pair concurrent object discriminations. The concurrent-discriminations task assessed the ability to learn several object-discrimination problems concurrently, under conditions of high retroactive and proactive interference. Six different object-pairs (one S+ and the other S- within each pair) were presented several times per session. The rats

were trained on the 6 concurrent-discrimination problems over six days. They received five trials with each problem per day, in a mixed sequence (Pair 1, Pair 2, Pair 3, Pair 4, Pair 5, Pair 6, Pair 1, Pair 2, and so on). Thus, there was a total of 30 trials per day, and a total of 30 trials with each object-problem over the six days.

Histological Procedures

Two days after the last day of testing, all rats received an overdose of pentobarbital and were transcardially perfused with 250 ml of 4% formaldehyde in 0.1 M phosphate buffer (PB) (Sigma, Canada). Brains were removed and post-fixed in 30% sucrose in 4% formaldehyde made with 0.1 M PB for 48 hours, and stored at -80 °C. The brains were sliced into 20 μm and 40 μm sections throughout the entire anterior-posterior extent of the HPC.

Cell counts. The 20 μm sections were mounted on gel-coated microscope slides and stained with cresyl violet. These specimens were used to quantify the extent of neuronal loss in the HPC and PRh. Sections were visualized using a Leica DMR-HC microscope mounted with a Hitachi 3CCD camera (model # HV-C20). Images were captured at 400x magnification using Scion Image 1.66 software (NIH) and stored on a Macintosh G4 computer.

HPC neurons were visually quantified bilaterally at three coronal planes. Cells were counted along a 150 μm line in the CA1, CA3, and the dentate gyrus (DG), and within a 150 x 150 μm square in the hilus. For the anterior plane (-3.6 mm relative to bregma), the CA1 pyramidal neurons were counted at the dorsal-most extent of the CA1 cell line, where it forms an arch. The CA3 was sampled at the lateral portion of the CA3 cell line, immediately prior to where it extends parallel to the alveus. Granule cells of the

DG were counted in the lateral end of its lower blade, and hilar neurons were counted immediately dorsal to that location. For the middle plane (-4.8 mm relative to bregma), the CA1 pyramidal neurons were counted in the dorsal extent of the CA1 line, anterior to the hippocampal fissure. The CA3 was sampled in the middle portion of its dorsal/ventral extent. The granule cells of the DG were counted in the lateral end of its upper blade, and hilar neurons were quantified in the polymorph layer of the DG. For the posterior plane (-6.04 mm relative to bregma), the CA1 pyramidal neurons were counted in the ventral extent of the CA1 line, at the level of the rhinal fissure. The CA3 was sampled in the middle of its dorsal/ventral extent. The granule cells of the DG were counted in the lateral end of its lower blade, and hilar neurons were quantified in the polymorph layer of the DG. Cell counts were totaled across the left and right hemispheres, and expressed as the mean number of neurons per selection (cells/150μm line, or cells/150x150μm square) for each of the subfields, at each coronal section examined.

Neurons in the PRh were visually quantified bilaterally at three coronal planes (-2.56 mm, -4.3 mm, and -6.3 mm relative to bregma). At each coronal section, cells were counted within a 100 x 100 μm square medial to the rhinal fissure. Cell counts were totaled across the left and right hemispheres, and expressed as the mean number of neurons per selection (cells/100x100μm square), at each coronal section examined.

Immunohistochemistry (GFAP). The 40 μm sections were first rinsed for 3-10 min in Tris-buffered saline (TBS, Sigma) and then incubated for 24 hrs at 4 °C in primary antibody (1:400, anti-GFAP monoclonal, no. G-A-5, IgG1, Sigma, Canada), diluted in 0.3% Triton X-100 PB (Sigma, Canada), 3% normal horse serum (NHS, Vector Laboratories, USA), and stored in TBS for 48 hrs at 4 °C. Sections were then washed in

TBS, (3-5 min), and incubated in secondary antibody (1:200 anti-mouse IgG H+L, Vector Laboratories) for 1 hr at room temperature. The secondary antibody was diluted in 1.5% NHS and TBS. After incubation in the secondary antibody, sections were again washed in TBS 3 times (3-5 min) and processed with avidin-biotin complex (ABC Elite kit, Vector Laboratories) for 30 min at room temperature. After a further wash in TBS, (3-5 min) staining was visualized by immersing sections in diaminobenzidine-2, 3 (DAB) using a DAB-peroxidase (Vector Laboratories) with the nickel chloride enhancement kit omitted, thus giving a light brown stain for GFAP. Sections were mounted on gel-coated slides and dehydrated in graded ethanol baths, rinsed in xylene (Fisher, Canada) and cover-slipped using Permount (Fisher, Canada).

Sections were visualized using a Leica DMR-HC microscope mounted with a Hitachi 3CCD camera (model # HV-C20). Images were captured at 400x magnification using Scion Image 1.66 software (NIH) and stored on a Macintosh G4 computer. To quantify the amount of GFAP immunohistostaining, a rectangular selection (300 x 225 μm) was made within the CA1, CA3, hilus, and the DG of the HPC at three coronal planes (-3.6 mm, -4.8 mm, and -6.04 mm relative to bregma), and within the PRh at three coronal planes (-2.56 mm, -4.3 mm, and -6.3 mm posterior from bregma). Regions of interest were located in the same manner as described for cell counts. Analyses were performed on grayscale images using the Scion Image 1.66 "Density Slice" function, which separates the image into an area of interest (immunohistochemical stain) and background (lack of immunohistochemical stain) as a function of gray level. Threshold values were adjusted for individual images based on the relative intensity of the stain.

Density counts were totaled across the left and right hemispheres and expressed as a mean number per region of interest, at each coronal section examined.

Results

Behavioral Results

One SHAM rat and one ISC rat spent long periods of time immobile in the center of the apparatus, and were therefore excluded from the object-discrimination and concurrent-discriminations training. The remaining ISC and SHAM rats acquired the single discrimination problem at a similar rate. Nine out of 23 SHAM rats and 6 out of 18 ISC rats reached the performance criterion of 10 correct trials in fewer than the maximum of 70 trials. The relationship between group membership and reaching criterion was not statistically significant, $X^2 = 0.15$, $df = 1$, $p > .05$. The median number of trials to reach criterion by the rats that did so was 46 for the SHAM group and 42 for the ISC group. The groups also made a similar number of errors in reaching the criterion or the maximum 70 trials; SHAM median errors = 24, and ISC median errors = 29.

The groups also learned the concurrent-discrimination problems at a similar rate. Figure 2A shows the percentage of trials that were correct on each of the six training sessions. A 2 x 6 mixed-factorial ANOVA revealed a significant effect of Session, $F(5, 200) = 105.4$, $p < .01$, a nonsignificant effect of Group, $F(1, 40) = 0.15$, $p > .05$, and a nonsignificant Group x Session interaction, $F(5, 200) = 0.73$, $p > .05$.

To assess 24-hr retention of the concurrent-discrimination problems, the accuracy of choices on the first daily trial with each problem was determined. Figure 2B shows the results. A 2 x 5 mixed-factorial ANOVA performed on the data from Sessions 2 through

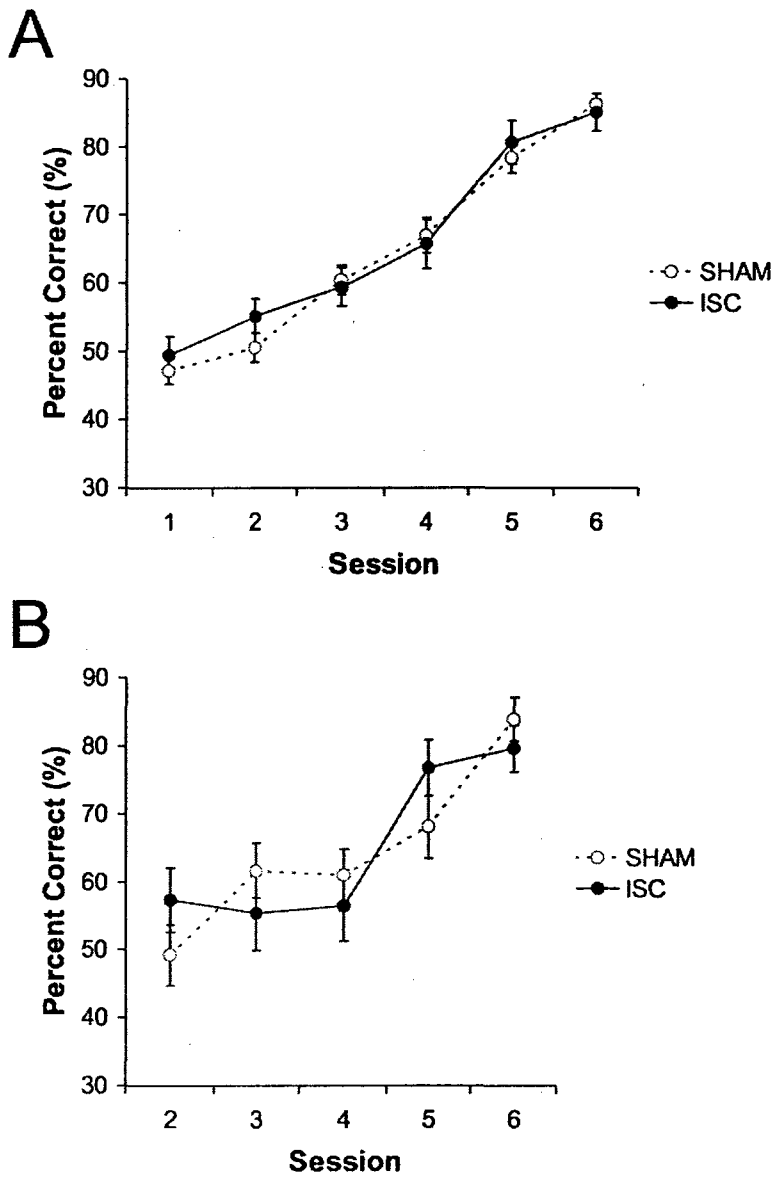

Figure 2. Performance on the six-pair concurrent object discriminations task. A: Mean percent correct on each daily session of the concurrent-discriminations task. B: Mean percent correct on the first daily trial of each problem on the concurrent-discriminations task. Error bars represent SEMs.

6 revealed a significant main effect of Session, $F(4, 160) = 18.65, p < .01$, but a nonsignificant effect of Group, $F(1, 40) = 0, p > .05$, and a nonsignificant Group x Session interaction, $F(4,160) = 1.49, p > .05$, indicating normal 24-hr retention of the concurrent-discrimination problems in the ISC rats.

Histological Results

Figure 3A shows photomicrographs of cresyl-violet stained coronal sections of the HPC of an ISC rat relative to a SHAM; Figure 3B shows photomicrographs of GFAP expression in the HPC of an ISC rat relative to a SHAM.

HPC cell counts. Figure 4 shows the mean number of cells per 150 μm in the CA1, CA3, hilus, and DG of ISC and SHAM rats at the anterior (-3.6 mm relative to bregma), middle (-4.8 mm relative to bregma), and posterior (-6.04 mm relative to bregma) planes examined. Planed comparisons using independent-samples *t*-tests (two-tailed) revealed that the ISC rats had on average significantly fewer cells than SHAM rats in the anterior CA1, $t(41) = 14.85, p < .01$, anterior hilus, $t(41) = 2.53, p = .02$, middle CA1, $t(41) = 13.52, p < .01$, posterior CA1, $t(41) = 3.76, p < .01$, posterior CA3, $t(41) = 4.69, p < .01$, and posterior hilus, $t(41) = 4.40, p < .01$. Means, standard deviations, and the results of all comparisons are presented in Appendix A (Table 1).

HPC GFAP expression. Figure 5 shows mean density count values per 300 x 225 μm in the CA1, CA3, hilus, and DG of ISC and SHAM rats at the anterior (-3.6 mm relative to bregma), middle (-4.8 mm relative to bregma), and posterior (-6.04 mm relative to bregma) planes examined. Planed comparisons using independent-samples *t*-tests (two-tailed) revealed that the ISC rats had on average significantly more GFAP expression than SHAM rats in the anterior CA1, $t(41) = 9.85, p < .01$, anterior CA3, $t(41)$

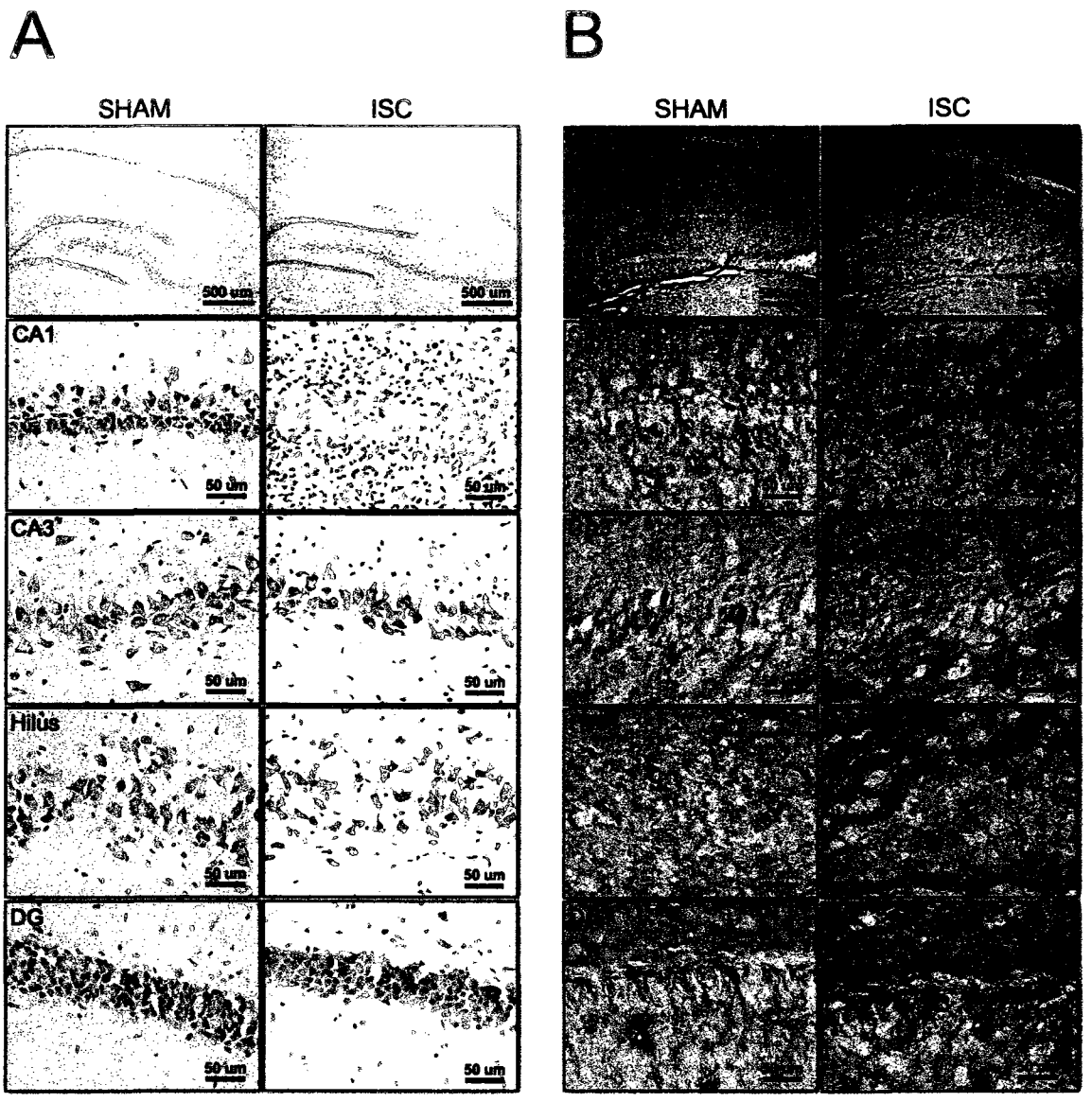

Figure 3. Representative photomicrographs of the HPC from a SHAM rat and an ISC rat. A: Cresyl-violet stained coronal sections of the HPC of an ISC rat relative to a SHAM. B: Photomicrographs of GFAP expression in the HPC of an ISC rat relative to a SHAM.

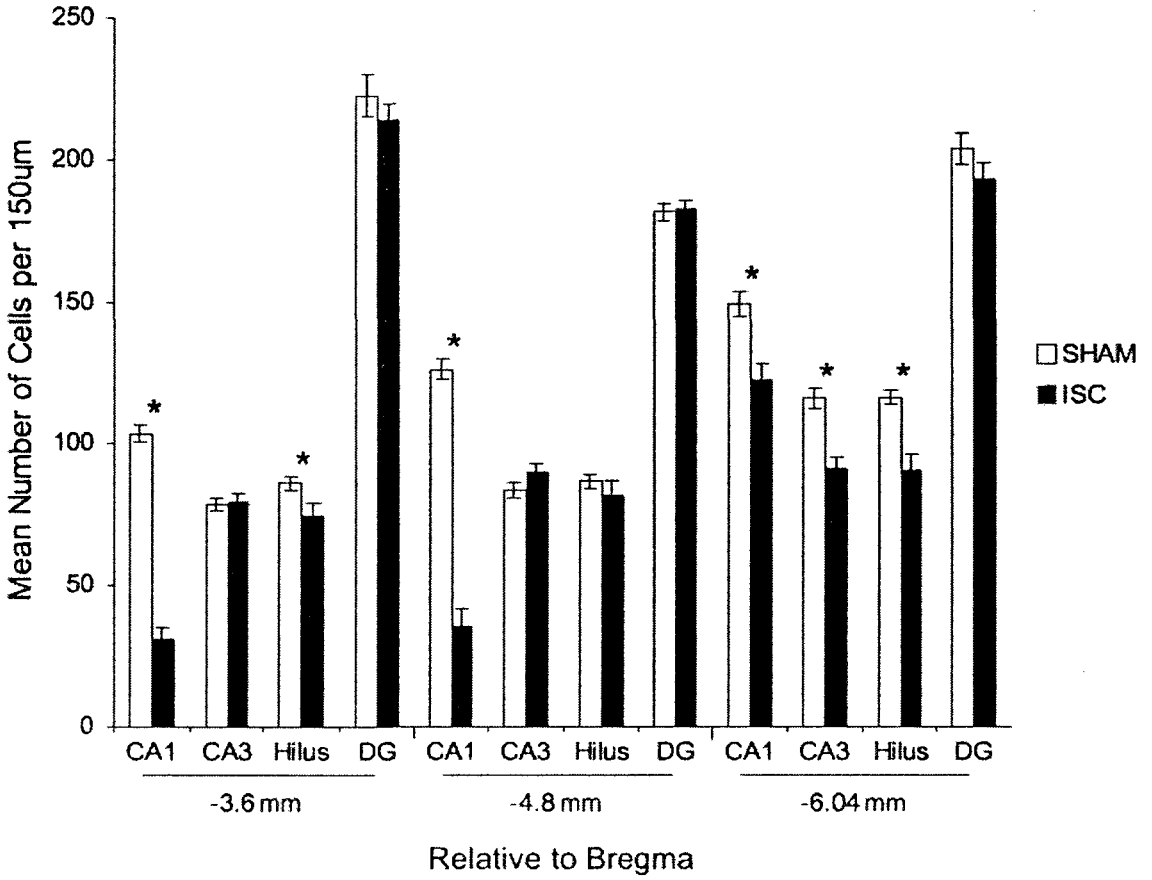

Figure 4. Mean number of cells per 150 µm in each of the HPC cell-fields at each coronal section examined. Error bars represent SEMs. Asterisks denote statistically significant between-group differences (t-tests, $p < .05$).

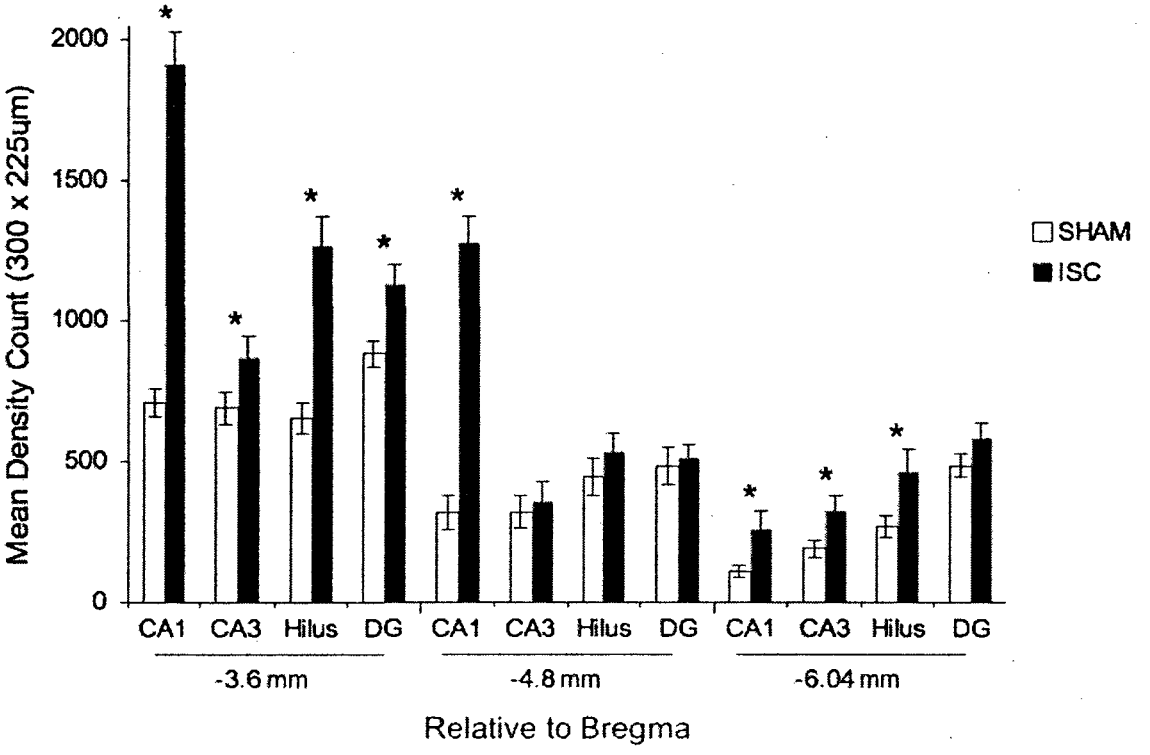

Figure 5. Mean GFAP density count per 300 x 225 µm in each of the HPC cell-fields at each coronal section examined. Error bars represent SEMs. Asterisks denote statistically significant between-group differences (t-tests, $p < .05$).

= 2.02, $p = .05$, anterior hilus, $t(41) = 5.48$, $p < .01$, anterior DG, $t(41) = 2.99$, $p = .01$, middle CA1, $t(41) = 8.93$, $p < .01$, posterior CA1, $t(40) = 2.16$, $p = .04$, posterior CA3, $t(40) = 2.15$, $p = .04$, and posterior hilus, $t(40) = 2.33$, $p = .03$. Means, standard deviations, and the results of all comparisons are presented in Appendix A (Table 2).

Figure 6A shows photomicrographs of cresyl-violet stained coronal sections of the PRh of an ISC rat relative to a SHAM, and Figure 6B shows photomicrographs of GFAP expression in the PRh of an ISC rat relative to a SHAM.

PRh cell counts. Figure 7 shows the mean number of cells per 100 x 100 μm in the PRh of ISC and SHAM rats at the anterior (-2.56 mm relative to bregma), middle (-4.3 mm relative to bregma), and posterior (-6.3 mm relative to bregma) planes examined. Planed comparisons using independent-samples *t*-tests (two-tailed) revealed that the ISC rats had on average significantly fewer cells than SHAM rats in the anterior PRh, $t(41) = 3.78$, $p < .01$, middle PRh, $t(41) = 2.33$, $p = .03$, and posterior PRh, $t(41) = 3.74$, $p < .01$.

PRh GFAP expression. Figure 8 shows mean density count values per 300 x 225 μm in PRh of ISC and SHAM rats at the anterior (-2.56 mm relative to bregma), middle (-4.3 mm relative to bregma), and posterior (-6.3 mm relative to bregma) planes examined. Planed comparisons using independent-samples *t*-tests (two-tailed) revealed that the ISC rats had on average significantly more GFAP expression than SHAM rats in the posterior PRh, $t(41) = 3.34$, $p < .01$. Means, standard deviations, and the results of all comparisons are presented in Appendix A (Table 4).

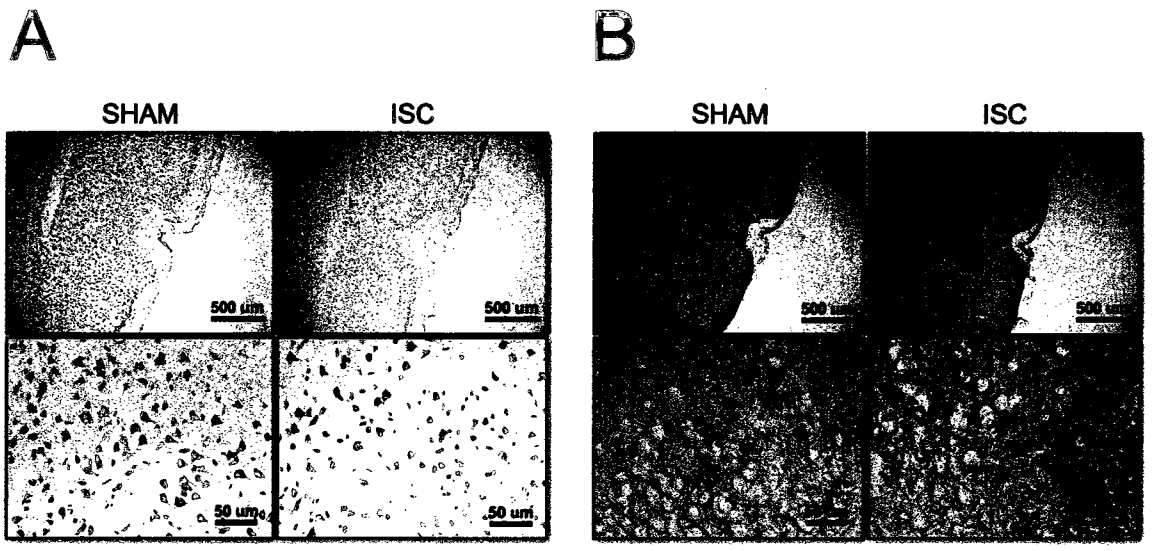

Figure 6. Representative photomicrographs of the PRh from a SHAM rat and an ISC rat. A: Cresyl-violet stained coronal sections of the PRh of an ISC rat relative to a SHAM. B: Photomicrographs of GFAP expression in the PRh of an ISC rat relative to a SHAM.

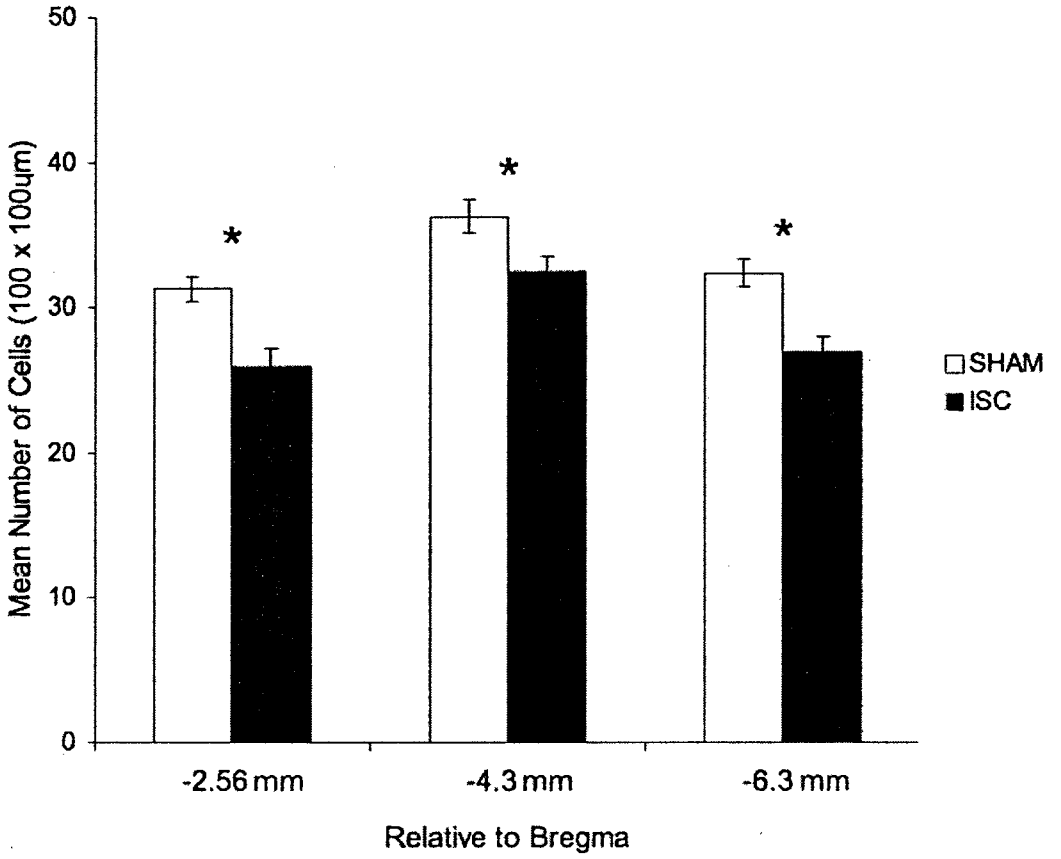

Figure 7. Mean number of cells per 100 x 100 µm in the PRh at each coronal section examined. Error bars represent SEMs. Asterisks denote statistically significant between-group differences (t-tests, $p < .05$).

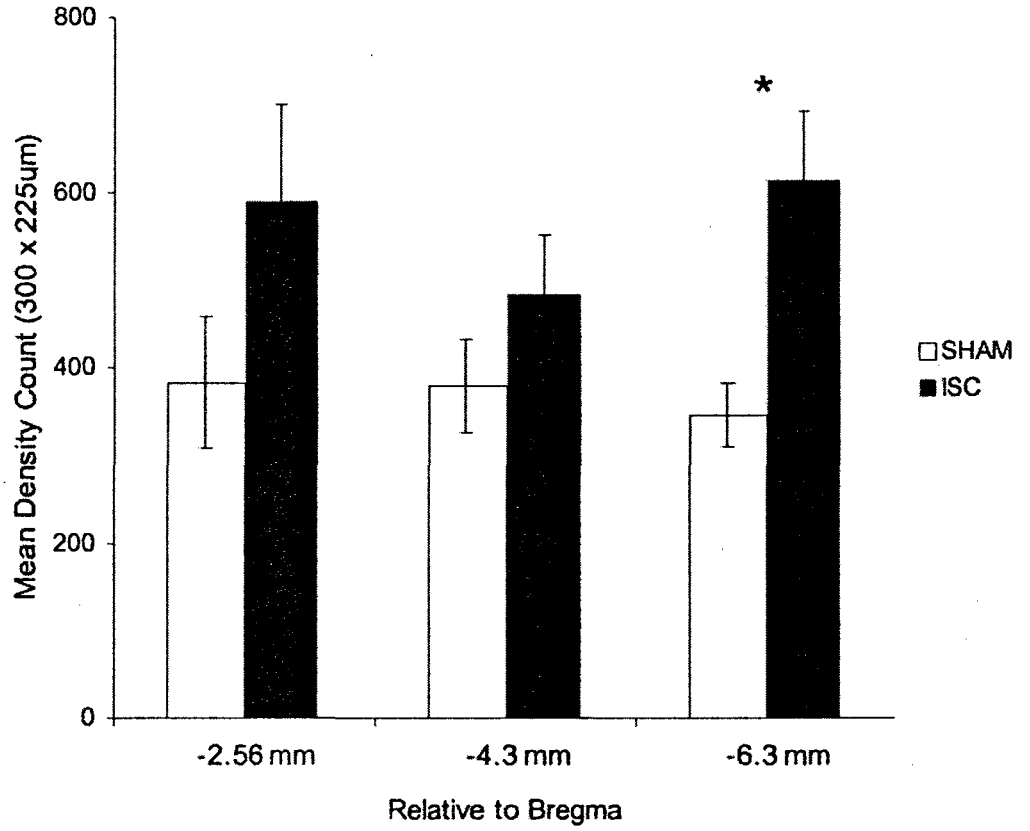

Figure 8. Mean GFAP density count per 300 x 225 μm in the PRh at each coronal section examined. Error bars represent SEMs. Asterisks denote statistically significant between-group differences (t-tests, $p < .05$).

Summary

The ISC rats learned the single object-discrimination problem at a normal rate, which is consistent with previous reports that global ischemia does not lead to impairment in learning single-pair object-reward associations (Wood et al., 1993). Based on previous findings that HPC lesions in rats produce impairments on the multiple concurrent object-discriminations task (Mumby et al., 1995; Wible et al., 1992), it was predicted that global ischemia would also lead to impairments. This hypothesis was not supported; the ISC rats in the present study learned six concurrent object-discrimination problems at a normal rate, and showed no evidence of a deficit in 24-hr retention of the discrimination problems. The results of the present study extend previous findings of intact single-pair object-discrimination learning in rats (Wood et al., 1993) to conditions that involve significant proactive and retroactive interference among individual objects and their relations to reward.

Histopathological assessment revealed significant cell loss in the HPC of ISC rats, which is in agreement with the widely accepted notion that global cerebral ischemia leads to HPC cell death. The ISC rats displayed increased GFAP expression in the HPC, which is also consistent with previous findings (Ordy et al., 1993; Langdon et al., 2008). The ISC rats in the present study also displayed elevated GFAP expression in the PRh. To the author's knowledge, this is the first report of increased glial response in this structure following global cerebral ischemia. Additionally, ISC rats in the present study suffered significant cell loss in the PRh, which was unexpected given that both previous studies that included PRh neural density assessment but failed to detect significant damage, induced global cerebral ischemia for 20-min (Mumby et al. 1996; Wood et al., 1993),

whereas a 15-min duration was used in the present experiments. There are several differences between the present study and those of Mumby et al. (1996), and Wood et al. (1993), that may account for this discrepancy. The previous studies used the 2VO + hypotension procedure to induce global ischemia, whereas the 4VO procedure was used in the present study. Both previous experiments used Wistar rats, whereas Long-Evans rats were used in the present experiments. Additionally, rats' temperature during ischemia induction was kept at 35 °C in the earlier studies, whereas the rats in the present study were kept at 37 °C throughout occlusion. Temperature variation (Colbourne, Nurse, Corbett, 1993), different rat strains, different animal suppliers, and even different shipments of rats from the same supplier (Pulsineli & Buchan, 1988) have been known to contribute to variability in ischemia induction and outcome. Thus, any one or a combination of these factors may explain the discrepancy between the earlier studies and the present study in terms of PRh pathology, or the lack of.

EXPERIMENT 2

Rats' object-recognition memory was assessed using the NOP test following global cerebral ischemia or sham-ischemia. The NOP test relies on rats' natural propensity towards novelty, and is therefore a convenient way to assess object-recognition memory since appetitive motivation, extensive training, and learning of a nonmatching rule are not required as they are with the DNMS task. Based on previous reports of impaired object-recognition memory following global cerebral ischemia in rats (Gaskin, et al., 2002; Gulinello et al., 2006; Mumby et al., 1996; Plamondon et al., 2008;

Plamondon et al., 2006; Wood et al. 1993), it was expected that ISC rats in the present experiment would display impairments in object-recognition.

Materials and Methods

Subjects

The subjects were the same as those used in Experiment 1.

Surgery

The surgical procedures were the same as described in Experiment 1.

Apparatus

The open-field arena used for NOP testing was 60 cm x 70 cm x 70 cm high, and was constructed of gray PVC plastic. The floor was covered with wood shavings. A video camera positioned over the arena recorded all sessions for later analysis. The NOP test stimuli were 12 different objects made of glass, plastic, or glazed ceramic, varying in height between approximately 6 cm and 12 cm, and in width between 6 cm and 10 cm. Attached with epoxy to the bottom of each object was a small glass jar (6 cm high), and attached to the floor of the arena were two inverted jar lids, each positioned 27 cm from opposing corners of the arena. Objects were fixed in place by screwing the jars into the lids. There were four copies of each object, which were used interchangeably. The objects were cleaned after each use with 70% ethanol.

Behavioral Procedures

Presurgery habituation. Prior to surgery, the rats were habituated to the open-field arena. Each rat was placed alone in the arena for 10 min on two consecutive days. The arena did not contain objects.

Postsurgery rehabituation. Each rat was placed alone in the arena for a single 5-min rehabituation session. The arena did not contain objects.

Novel-object preference. Each rat was placed in an open-field arena with two identical sample objects and allowed to explore for 5 minutes. After a retention interval, it was returned to the arena, which contained two new objects - one was identical to the sample and the other was novel. The amount of time spent investigating each of the objects during the sample-exposure phase and the test phase was recorded. The main dependent measure was the investigation ratio - the proportion of total object investigation during the test phase that was spent investigating the novel object $[t^{novel}/(t^{novel} + t^{sample})]$. A rat was considered to be investigating an object when its head was oriented within 45 degrees of the object and within 4 cm of it. Rearing with the head oriented upward was also included if at least one forepaw was on the object. Climbing over the objects or sitting on them was not included. Rats received three NOP trials with a 15-min retention interval (on Days 1, 4, and 7) and three trials with a 24-hr retention interval (Days 2 and 3; 5 and 6; 8 and 9). For all NOP trials, the sample and test phases lasted for 5 min. The experimenter who scored the videotapes was blind to the treatment of individual rats.

Results

Behavioral Results

Figure 9A shows the mean investigation ratios for the NOP test. The ratios are based on the first minute of each test phase. Rats that did not spend at least 1 sec exploring both objects during the test were excluded from the analyses. One-sample *t*-

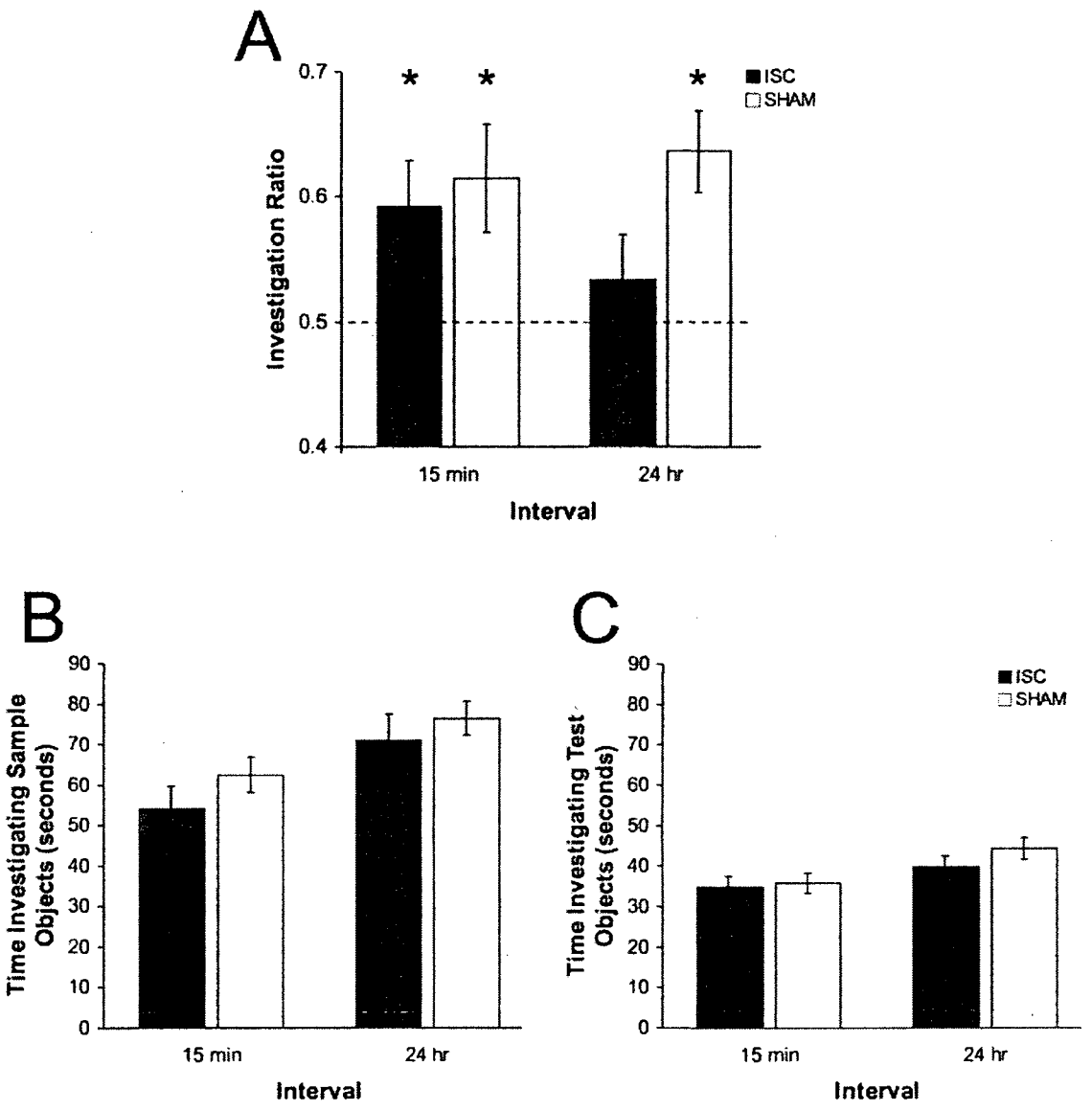

Figure 9. Performance on the NOP test. A: Investigation ratios for each condition, based on the first minute of the test phase. Asterisks denote ratios that are significantly different from chance ($p < .05$). B: Time spent investigating the sample objects during the sample-exposure phase in each condition. C: Overall time spent investigating objects during the test phase in each condition. Error bars represent SEMs.

tests (two-tailed) revealed that both SHAM rats, $t(22) = 2.67, p = .01$, and ISC rats, $t(15) = 2.51, p = .02$, had investigation ratios that were significantly higher than chance at the 15-min retention interval. At the 24-hr retention interval, the SHAM rats had ratios that were significantly above chance, $t(22) = 4.19, p < .01$, while the ISC rats did not, $t(17) = 0.92, p > .05$. Furthermore, the 24-hr ratios were significantly higher in SHAM rats than in ISC rats, $t(39) = 2.11, p < .05$.

Figure 9B shows the overall time spent investigating objects during the sample-exposure phase. A 2 x 2 mixed-factorial ANOVA revealed a significant main effect of Interval, $F(1, 41) = 10.70, p < .01$, suggesting that rats in both groups spent more time exploring sample objects in the sample-exposure phase of the 24-hr condition than the 15-min condition. There was no significant main effect of Group, $F(1, 41) = 0.42, p > .05$, and no significant Group x Interval interaction, $F(1, 41) = 0.14, p > .05$.

Figure 9C shows the overall time spent investigating objects during the test-phase. A 2 x 2 mixed-factorial ANOVA revealed a significant main effect of Interval, $F(1, 41) = 6.36, p = .02$, suggesting that rats in both groups spent more time exploring objects in the test phase of the 24-hr condition than the 15-min condition. There was no significant main effect of Group, $F(1, 41) = 0.74, p > .05$, and no significant Group x Interval interaction, $F(1, 41) = 0.51, p > .05$.

Correlational Analyses

Correlational analyses were used to determine whether any of the neuropathological measures (cell counts or GFAP expression), in either the HPC or PRh, were related to performance on the NOP test. Only ISC rats were included in these analyses. Because the ischemic rats performed as well as the control rats on NOP trials

with a 15-min delay, only the scores from NOP trials using a 24-hr retention delay were used in the correlational analysis. Additionally, only those regions in which statistically significant neuropathology was detected by planned comparisons (reported in Experiment 1) were included. A significant correlation, $r = +.47$, $p = .05$, was found between cell counts in the posterior PRh and performance on the NOP test. Results of all corelational analyses are presented in Appendix B.

Summary

Global cerebral ischemia produced delay-dependent deficits on the NOP test. The SHAM rats and ISC rats displayed a novel-object preference on trials with a 15-min retention interval, and scores in the two groups were not significantly different in that condition. The SHAM rats also displayed a novel-object preference on trials with a 24-hr retention interval, whereas the ISC rats did not, and the scores of ISC rats were significantly lower than those of SHAM rats. Although rats in both groups spent more time exploring objects during the sample-exposure and test phases of the 24-hr condition compared to the 15-min condition, there were no between-group differences in object exploration at either interval. This is important because it allows one to dismiss the possibility that ISC rats' deficits at the 24-hr interval were due to decreased tendency to explore objects. Furthermore, the fact that ISC rats displayed a novel-object preference at the 15-min interval allows one to rule out the possibility that their deficits at the 24-hr interval were due to some ischemia-induced disruption of the natural propensity to explore novelty.

The present finding that the ISC rats subjected to 15-min 4VO showed significant novelty-preference at the 15-min retention interval is inconsistent with Gulinello et al.

(2006), who reported NOP deficits with a 10-min retention interval in rats subjected to 10-min 4VO. There are several differences between the two studies, however, that make it difficult to compare the findings directly. The most notable difference is that the previous study used 3-min sample-expose, whereas 5-min sample-exposure was used in the present study. Our rats had more opportunity to explore the sample objects than the rats in the previous study, which is also evident in the average time ISC rats spent exploring the objects during the sample-exposure phase; approximately 16 sec in Gulinello et al. (2006), and an average of 58 sec in the present study. Therefore, our ISC rats likely benefited from better encoding of the sample objects during sample-exposure, which most likely provided them with an advantage during the test phase. In addition, Gulinello et al. (2006) used a 6-day recovery period between ischemia-induction and behavioral testing, whereas our rats were permitted to recover for 14 days. A somewhat brief recovery period may have contributed to ischemic rats' poorer performance in the previous study.

The significant correlation between the degree of cell loss in the PRh of ISC rats and their NOP performance at the 24-hr interval suggests that PRh damage may make a significant contribution to ischemia-induced object-recognition deficits. Although correlational data alone cannot demonstrate a causal role for PRh pathology in producing ISC rats' object-recognition deficits, they are entirely consistent with such a role. Additionally, the lack of significant correlations between ISC rats' NOP performance at the 24-hr interval and any measure of pathology anywhere in the HPC supports the notion that ischemia-induced object-recognition deficits are due to extra-hippocampal damage.

EXPERIMENT 3

Allocentric-spatial memory tests in the water maze were included in order to confirm the functional effectiveness of the ischemia in the event that no impairments were observed on the object-recognition or object-discrimination tasks. The fixed-platform place navigation task assessed the ability to learn the location of a submerged escape-platform relative to multiple extra-maze cues (i.e., allocentric-spatial memory). The delayed matching-to-place (DMTP) task assessed the rats' ability to remember where they recently found the hidden platform on a single occasion, and it therefore places a heavy demand on working memory, whereas the fixed-platform task assesses primarily reference memory. Based on previous findings that global cerebral ischemia leads to reference memory (Block & Schwarz, 1998; Jaspers et al., 1990; Nelson et al., 1997a; Nunn et al., 1994), and working memory (Auer et al., 1989; Green et al., 1992; Green et al., 1995; Nelson et al, 1997b) impairments in the water maze, it was expected that the ISC rats in the present study would also be impaired.

Materials and Methods

Subjects

The subjects were the same as those used in Experiments 1 and 2.

Surgery

The surgical procedures were the same as described in Experiment 1.

Apparatus

The water maze was 137 cm in diameter and 46 cm high, and filled with water (23 °C) to a depth of approximately 30 cm. The water was made opaque by adding instant

milk powder. A movable Plexiglas platform (10 cm x 10 cm x 28 cm) was submerged approximately 2 cm below the surface. The rats could not see the platform, but several extra-maze cues (e.g., posters, doors, shelves, a computer, etc.) were visible from within the pool, and the rats could learn the location of the platform relative to these distal cues. Figure 10 shows the sequence of platform locations used throughout water maze training and testing.

Behavioral Procedures

Fixed-platform place navigation. On each trial, the rat was placed into the pool, facing the wall, at one of the four cardinal compass points, N, E, S, or W. Each of the four starting positions was used twice per session in a pseudorandom sequence, which was the same for all rats. The platform was located in the center of the NW quadrant on every trial. A trial continued until the rat climbed onto the platform or until 60 s elapsed. The rat was left on the platform for 10 s; if it failed to find the platform within the 60-s maximum, it was guided to the platform and left there for 10 s. Rats received eight fixed-platform trials per day on Days 1 and 2, with the platform at location #1 (see Figure 10). The intertrial interval was 5 minutes. A single 20-sec probe trial with the platform removed was conducted following the last trial on Day 2.

Delayed matching-to-place (DMTP). This task assessed the rats' ability to remember where they recently found the hidden platform on a single occasion, and it therefore places a heavy demand on working-memory. During the learning phase of a DMTP trial, the rat searched for and found the hidden platform. After a delay, the rat was returned to the pool and allowed to find the platform again, in the same location. Decreases in escape-latency from the first swim to the second swim reflect the extent to

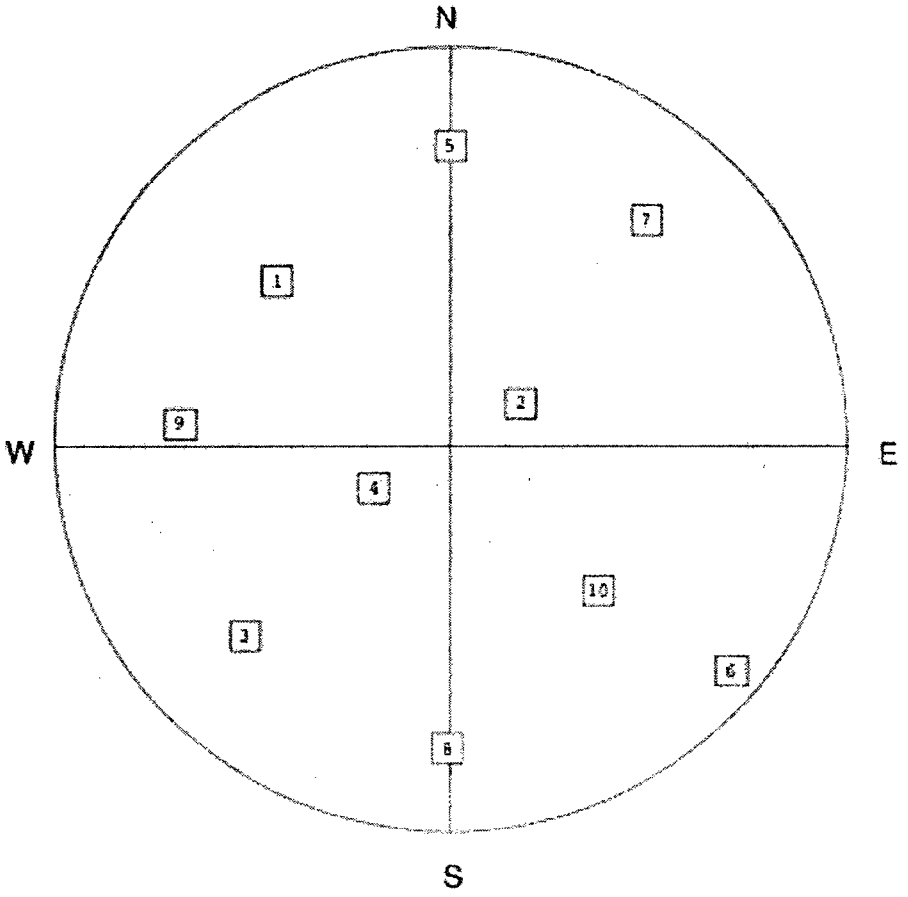

Figure 10. The sequence of 10 platform locations that were used for the water-maze fixed-platform and DMTP tasks.

which the rat remembered the platform location. The platform location changed on each trial.

After the two days of fixed-platform training, the rats were gradually introduced to the moving platform. On Day 3, the platform was in location #2 for all 8 trials. On Day 4, it was in location #3 for Trials 1-4, and in location #4 for Trials 5-8, and on Day 5, it was in location #5 for Trials 1-4, and in location #6 for Trials 5-8.

On Days 6 to 10, the rats received four DMTP trials per day (i.e., four pairs of swims), with the platform in a new location on each trial, starting with location #7, and continuing to cycle through the ten locations until the end of the experiment. The first and third trial each day had a 15-s interval between first and second swims; the second and fourth trial had a 60-s interval. The main dependent measure was the savings ratio - the escape latency on the second swim divided by the sum of escape latencies on first and second swims [$t^{2nd\ swim}/(t^{1st\ swim} + t^{2nd\ swim})$]. Lower ratios reflect better retention of the platform location.

After DMTP testing on Day 10, the platform was moved once more and all rats received six visible-platform trials, in order to probe for perceptual, motor, or navigational impairments that could potentially underlie deficits on the fixed-platform or DMTP tasks. The top 3 cm of the platform was wrapped with black electrical tape and the platform was raised so that it extended approximately 2 cm above the surface of the water. Each rat received six trials, with the visible platform in the same location for each trial, and with different start locations. The intertrial interval was 5 minutes.

Results

Behavioral Results

Fixed-platform place navigation. Figure 11A shows the mean escape-latencies (expressed as an average of two consecutive trials) during acquisition of the fixed-platform task. The SHAM and ISC groups had similar latencies throughout training, and both groups decreased to a similar level by the end of training on Day 2. A mixed-factorial ANOVA revealed a main effect, of Trial $F(7, 280) = 40.64$, $p < .01$. The main effect of Group, $F(1, 40) = 0.97$, $p > .05$, and the Group x Trial interaction, $F(7, 280) = 1.06$, $p > .05$, were not statistically significant. Deficits emerged on the probe trial (see Figure 11B), as the ISC rats spent significantly less time swimming in the target-quadrant, $t(38) = 2.21$, $p = .03$, and crossed the previous platform location significantly fewer times than did the SHAM rats (SHAM median = 3, mode = 3; ISC median = 2, mode = 1; Mann-Whitney U, $z = 2.02$, $p = .04$). Both groups learned the new platform location on Day 3 at a similar rate (see Figure 11C).

DMTP. A deficit reappeared in the ISC group on Days 4 and 5, when the platform was moved to a new location after every 4 trials. For each rat, a difference score was computed for each of the four platform locations used on Days 4 and 5. A difference score was the average latency on the first two trials minus the average latency on the last two trials for a particular location. Figure 12A shows the results of this analysis. A mixed-factorial ANOVA revealed a significant main effect of Group, $F(1,40) = 6.94$, $p < .01$, indicating that ISC rats had significantly lower difference scores overall, across platform locations. There was also a significant main effect of Location, $F(3,120) = 6.76$, $p < .01$, indicating that regardless of treatment, animals had significantly lower difference

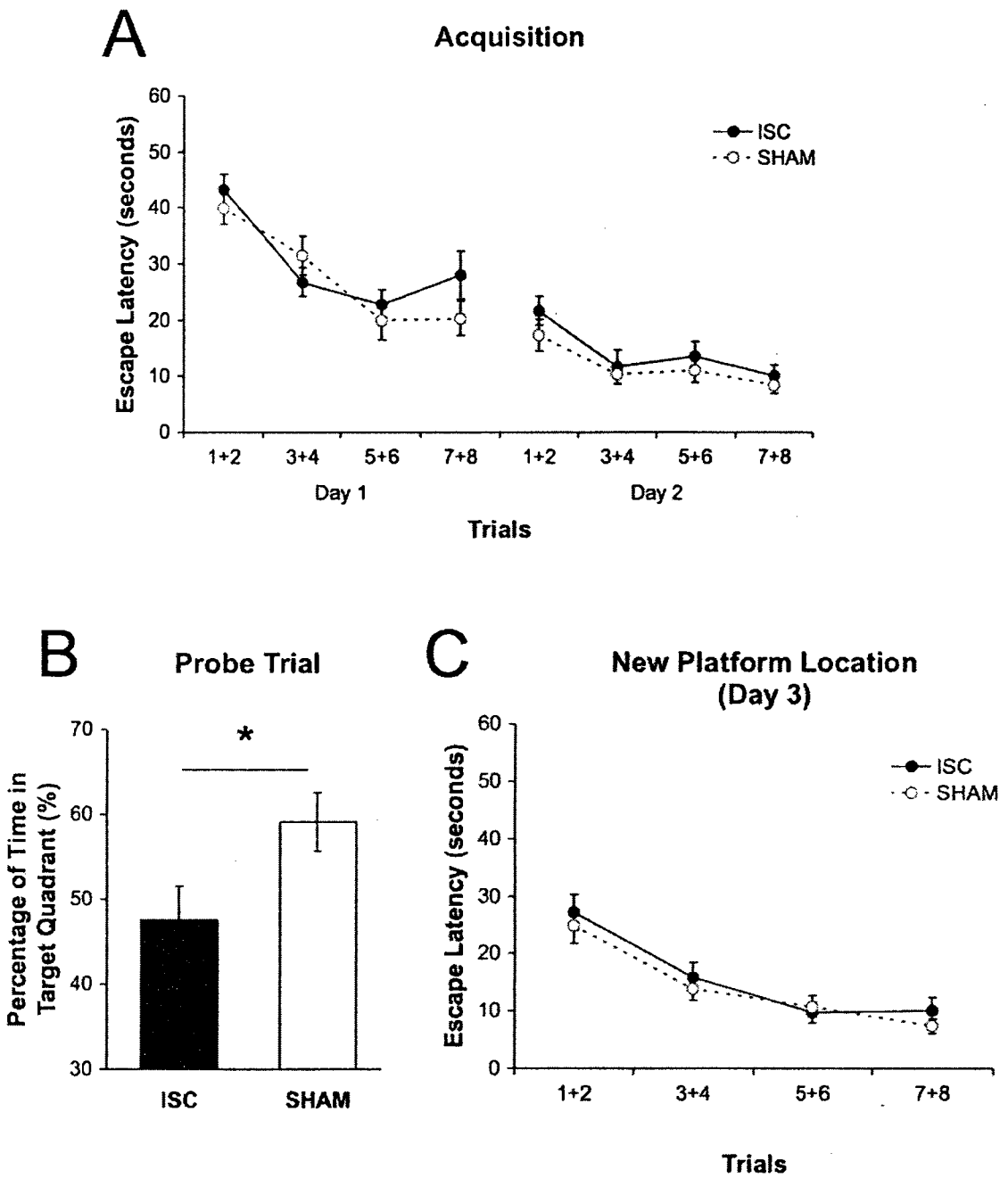

Figure 11. Performance on the fixed-platform water-maze task. A: Mean escape-latencies across training trials. B: Percentage of time spent swimming in the correct pool quadrant on the probe trial. Asterisk denotes statistically significant between-group difference (t-test, $p < .05$). C: Mean escape-latencies on Day 3, when the platform was moved to a new location. Error bars represent SEMs.

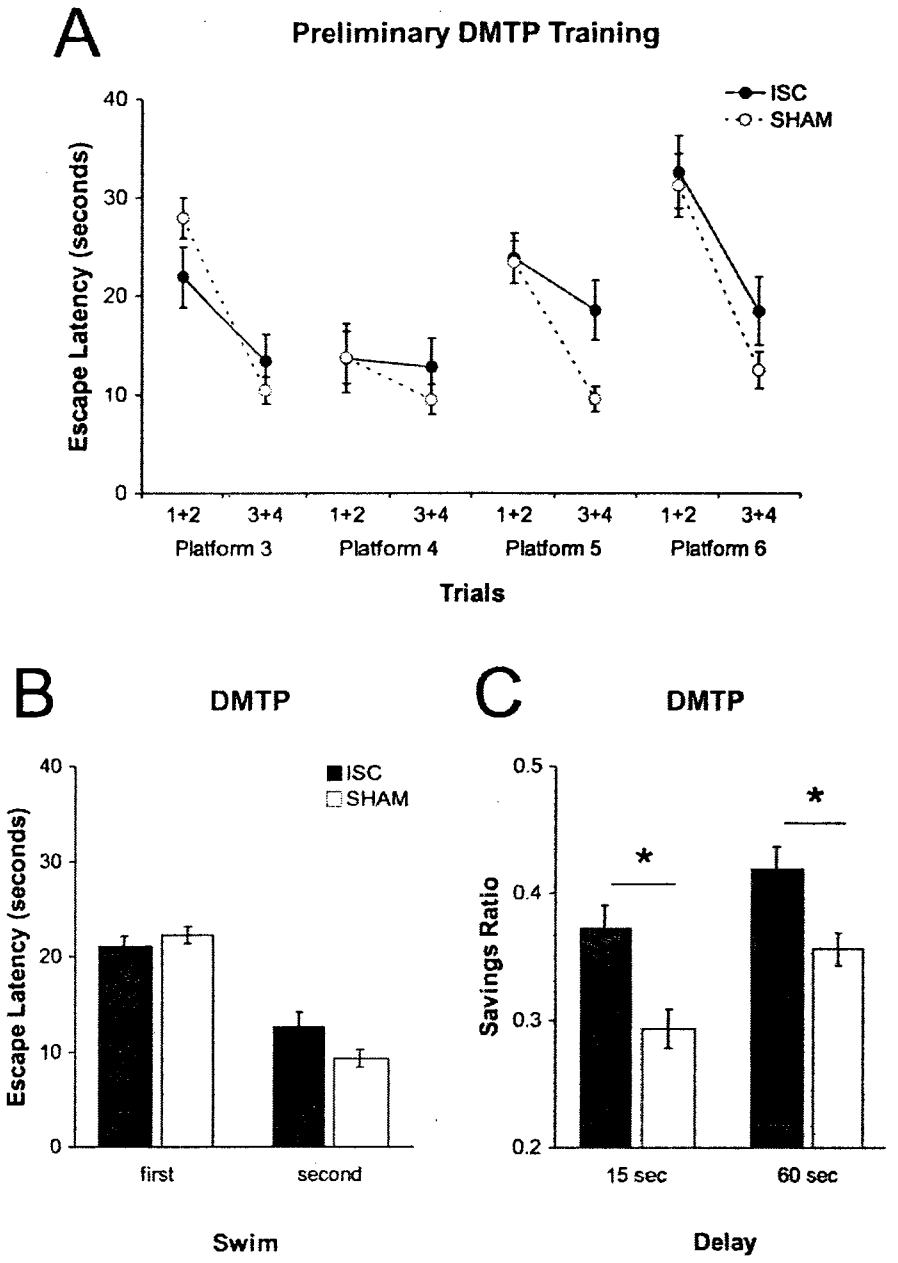

Figure 12. Performance on the DMTP task. A: Mean escape latencies during the preliminary phase of DMTP training, when the platform location changed after every 4 trials. (Days 4 and 5). Each data point represents a block of two trials. B: Mean first-swim and second-swim latencies on DMTP trials. C: Savings-ratios on DMTP trials with 15-s and 60-s retention intervals. Asterisks denote statistically significant between-group differences (t-tests, $p < .05$). Error bars represent SEMs.

scores at certain locations than others. The Group x Location interaction was not statistically significant, $F(3,120) = 0.39, p > .05$.

The ISC rats continued to display deficits during DMTP testing. Figure 12C shows the mean savings-ratios on DMTP trials. An ANOVA revealed significant main effects of Group, $F(1,40) = 16.15, p < .01$, and Delay, $F(1,40) = 17.73, p < .01$, and a nonsignificant Group x Delay interaction, $F(1, 40) = 0.42, p > .05$. SHAM rats had lower ratios (i.e., greater savings) than ISC rats at both retention delays: 15-sec, $t(40) = 3.41, p < .01$; 60-sec $t(40) = 3.04, p < .01$. Within-group comparisons indicated that ratios were significantly lower at 15-sec delays than at 60-sec delays, in SHAM rats, $t(23) = 4.24, p < .01$, but not ISC rats $t(17) = 2.05, p > .05$.

An ANOVA performed on mean escape-latencies on the six visible-platform trials conducted on the last day of water maze testing did not reveal a statistically significant main effect of Group, $F(1, 38) = 0.33, p > .05$, Trial, $F(5, 190) = 0.84, p > .05$, nor a significant Group x Trial interaction, $F(5, 190) = 1.32, p > .05$.

Correlational Analyses

Correlational analyses were used to determine whether any of the neuropathological measures (cell counts or GFAP expression), in either the HPC or PRh, were related to performance on the DMTP task. Only ISC rats were included in these analyses. Because the DMTP deficits in ISC rats were roughly equivalent after delays of either 15 s or 60 s, a combined score was calculated for each rat, which was simply the average savings-ratio over all of its DMTP trials. Additionally, only those regions in which statistically significant neuropathology was detected (reported in Experiment 1) were included in the analysis. ISC rats' DMTP scores correlated significantly with cell

counts in the posterior CA1, $r = -.62, p = .01$, and GFAP expression in the anterior DG, $r = +.57, p = .01$, posterior CA1, $r = +.57, p = .01$, and posterior PRh, $r = +.69, r < .01$. Results of all corelational analyses are presented in Appendix B.

Summary

Global cerebral ischemia did not significantly affect escape latencies on the fixed-platform water maze task, although evidence of a mild deficit could be seen on the probe trial at the end of fixed-platform training. Ischemia had a more profound effect on DMTP performance, which is consistent with previous findings that global ischemia impairs performance on allocentric-spatial working memory tasks, and to a lesser extent, reference memory tasks (Auer et al., 1989; Green et al., 1992). The DMTP deficits were delay-independent, as the ISC rats displayed deficits of similar severity after retention intervals of either 15 s or 60 s, although one cannot rule out the possibility that the ISC rats were capable of normal performance with retention intervals shorter than 15 s.

Significant correlation between ISC rats' DMTP scores and the degree of pathology in the HPC is consistent with previous reports that the severity of spatial-memory impairment following global cerebral ischemia is related to the extent of ischemia-induced HPC damage (Block & Schwarz, 1996; Briones & Therrien, 2000; Nelson et al., 1997a, Nelson et al., 1997b; Volpe et al., 1992). These findings are also consistent with the widely accepted notion that the HPC is important for processing and storage of spatial information.

The significant correlation between GFAP expression in the PRh and the ISC rats' DMTP scores was unexpected. Although several lesion studies have shown that PRh

damage can lead to spatial memory deficits, this appears to be the case primarily when performance on a spatial memory task requires the use of visually ambiguous or overlapping distal cues (Aggleton, Kyd, & Bilkey, 2004), which does not fit the description of the manner in which water maze testing was conducted in the present study. In fact, previous experiments conducted in our laboratory using the same pool and testing procedures have demonstrated that rats with PRh lesions are capable of normal DMTP performance on delays as long as 300 s (Glenn & Mumby, 1998). It is possible that the relationship between GFAP expression in the PRh and the ISC rats' DMTP scores may be mediated by damage elsewhere. Since significant correlations were found between pathology in the HPC and ISC rat' DMTP scores, additional correlations were computed to determine whether GFAP expression in the posterior PRh of ISC rats correlated with pathology in the HPC. The results of this analysis revealed that GFAP expression in the posterior PRh significantly correlated with cell counts in the posterior CA1, $r = -.51$, $p = .03$, and GFAP expression in the posterior CA1, $r = +.68$, $p < .01$, both of which were found to significantly correlate with ISC rats DMTP scores ($r = -.62$ and $r = +.57$, respectively). It should also be noted that cell counts in the posterior PRh, which significantly correlated with ISC rats' 24-hr NOP scores ($r = +.47$), did not significantly correlate with any measure of pathology anywhere in the HPC. In addition, ISC rats' DMTP scores did not significantly correlate with their 24-hr NOP ratios, $r = -.35$, $p = .15$.

GENERAL DISCUSSION

A major goal of the present study was to determine whether ischemia-induced impairment of object-recognition memory is related to damage in the PRh. ISC rats

performed normally on the NOP test of object-recognition memory after a 15-min retention interval, but displayed deficits after a 24-hr interval. NOP deficits were not significantly correlated with the severity of damage in the HPC, which is consistent with recent findings that treatments can attenuate object-recognition deficits in ischemic rats without any evident neuroprotection in the HPC (Gulinello et al., 2006), and the general notion that ischemia-induced object-recognition deficits are due to extra-hippocampal damage. The significant correlation between the degree of cell loss in the PRh of ISC rats and their NOP performance at the 24-hr interval suggests that PRh damage may make a significant contribution to ischemia-induced object-recognition deficits, which is in agreement with converging evidence from lesion and electrophysiological studies that the PRh is necessary for object-recognition (Aggleton et al., 1997; Brown & Aggleton, 2001; Brown & Bashir, 2002; Griffiths et al., 2008; Mumby & Pinel, 1994).

ISC rats learned a single object-discrimination problem and six concurrent object-discrimination problems at normal rates, and displayed normal 24-hr retention of the discriminations, which is consistent with previous reports that global ischemia can impair object-recognition memory while sparing the ability to learn object-reward associations (Wood et al., 1993). The data from the concurrent-discriminations task in the present study extend this finding to conditions that involve significant proactive and retroactive interference among individual objects and their relations to reward.

ISC rats were impaired on the DMTP water-maze task, and the DMTP deficits were significantly correlated with the degree of cell loss and GFAP expression in the HPC, which is consistent with the widely accepted notion that the HPC is important for processing and storage of spatial information. The ISC rats' DMTP deficits were also

found to significantly correlate with the degree of GFAP expression in the PRh. Although the finding that GFAP expression in the PRh significantly correlated with the severity of HPC pathology suggests that HPC damage may have mediated the relationship between PRh pathology and the degree of DMTP deficits, one cannot rule out the possibility that ischemia-induced PRh pathology directly contributed to ISC rats' DMTP performance. Just as there are reasons to suspect that extra-hippocampal damage underlies ischemia-induced object-recognition deficits, extra-hippocampal damage may also contribute to ischemia-induced spatial-memory deficits. Some treatments that significantly reduce hippocampal damage fail to prevent spatial learning and memory deficits (e.g., Benetoli et al., 2007), and some treatments attenuate spatial learning and memory deficits without protecting against hippocampal damage (Roberge, Bernard, Messier, & Plamondon, 2008). Findings such as these suggest that extra-hippocampal damage contributes to ischemia-induced impairments in spatial-memory.

In the present study, significant pathology following global cerebral ischemia was detected in anatomically distinct areas, but this does not permit one to draw firm conclusions about causal relationships between pathology in specific brain areas and the ischemia-induced NOP or DMTP deficits. Novel experimental approaches are necessary to determine those causal links. Presently, it is only possible to produce hypotheses based on correlations between task performance and the magnitude of changes detected in specific brain areas, and on comparisons of the present results with those of studies involving surgical lesions in specific areas and performance on similar memory tests. The widespread effects of global cerebral ischemia on forebrain structures make it difficult to discern whether impairments on the different memory tasks reflect the same underlying

functional impairment or different impairments that result from distinctive aspects of the brain damage. The latter possibility seems likely, however, given the lack of a significant correlation between ISC rats' 24-hr NOP scores and their DMTP scores, and the observation that ISC rats performed normally on the NOP test after a 15-min delay, despite displaying deficits on the DMTP task after a 15-s delay.

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APPENDIX A

Descriptive Statistics and Results of Between-Group Comparisons

Table 1

Descriptive Statistics and Results of Between-Group Planned Comparisons for Cell Counts in the HPC of SHAM and ISC rats

	SHAM	ISC	
Subfield	*M (SD)*	*M (SD)*	Independent-Samples *t*-test
-3.6 mm from Bregma			
CA1	103.71 (14.57)	31.21 (17.45)	$t(41) = 14.85, p < .01$
CA3	78.58 (10.42)	79.58 (13.02)	$t(41) = 0.28, p = .78$
Hilus	86.21 (11.17)	74.95 (17.88)	$t(41) = 2.53, p = .02$
DG	222.71 (37.50)	214.37 (22.37)	$t(41) = 0.86, p = .40$
-4.8 mm from Bregma			
CA1	126.33 (16.99)	35.53 (26.84)	$t(41) = 13.52, p < .01$
CA3	83.83 (13.30)	90.11 (13.73)	$t(41) = 1.51, p = .14$
Hilus	86.92 (11.90)	82.05 (21.20)	$t(41) = 0.95, p = .35$
DG	181.46 (15.03)	182.68 (12.48)	$t(41) = 0.29, p = .78$
-6.04 mm from Bregma			
CA1	149.25 (22.13)	123.00 (23.56)	$t(41) = 3.78, p < .01$
CA3	116.13 (17.43)	91.21 (17.13)	$t(41) = 4.69, p < .01$
Hilus	116.33 (12.70)	90.68 (24.82)	$t(41) = 4.40, p < .01$
DG	203.96 (26.89)	193.16 (24.51)	$t(41) = 1.36, p = .18$

Table 2

Descriptive Statistics and Results of Between-Group Planned Comparisons for GFAP Density Counts in the HPC of SHAM and ISC rats

	SHAM	ISC	
Subfield	*M (SD)*	*M (SD)*	Independent-Samples *t*-test
-3.6 mm from Bregma			
CA1	709.13 (248.01)	1886.21 (516.41)	$t(41) = 9.85, p < .01$
CA3	691.33 (272.97)	872.79 (315.53)	$t(41) = 2.02, p = .05$
Hilus	649.54 (269.47)	1266.53 (462.02)	$t(41) = 5.48, p < .01$
DG	885.17 (226.14)	1130.32 (311.44)	$t(41) = 2.99, p < .01$
-4.8 mm from Bregma			
CA1	320.08 (301.84)	1287.47 (408.62)	$t(41) = 8.93, p < .01$
CA3	321.96 (291.91)	357.05 (313.82)	$t(41) = 0.38, p = .71$
Hilus	445.25 (331.92)	534.68 (294.74)	$t(41) = 0.92, p = .36$
DG	485.33 (331.30)	514.53 (197.93)	$t(41) = 0.34, p = .74$
-6.04 mm from Bregma			
CA1	110.74 (98.58)	252.16 (294.87)	$t(40) = 2.16, p = .04$
CA3	190.35 (150.73)	322.84 (244.67)	$t(40) = 2.15, p = .04$
Hilus	269.52 (186.57)	465.05 (346.54)	$t(40) = 2.33, p = .03$
DG	485.00 (190.60)	583.58 (228.59)	$t(40) = 1.49, p = .14$

Table 3

Descriptive Statistics and Results of Between-Group Planned Comparisons for Cell Counts in the PRh of SHAM and ISC rats

	SHAM	ISC	
Relative to Bregma	*M (SD)*	*M (SD)*	Independent-Samples *t*-test
-2.56 mm	31.29 (4.06)	26.05 (5.03)	$t(41) = 3.78, p < .01$
-4.30 mm	36.29 (5.74)	32.53 (4.59)	$t(41) = 2.33, p = .03$
-6.30 mm	32.38 (4.77)	27.00 (4.56)	$t(41) = 3.74, p < .01$

Table 4

Descriptive Statistics and Results of Between-Group Planned Comparisons for GFAP Density Counts in the PRh of SHAM and ISC rats

	SHAM	ISC	
Relative to Bregma	*M (SD)*	*M (SD)*	Independent-Samples *t*-test
-2.56 mm	382.83 (364.12)	589.33 (468.33)	$t(41) = 1.61, p = .12$
-4.30 mm	379.38 (257.19)	483.58 (292.33)	$t(41) = 1.24, p = .22$
-6.30 mm	345.63 (117.18)	613.74 (340.54)	$t(41) = 3.34, p < .01$

APPENDIX B

Correlational Analyses

Table 1

Pearson r-values of Correlations between Cell Counts in HPC and PRh of ISC Rats and their Performance on the DMTP task and the NOP Test

Region	DMTP	NOP
HPC		
Anterior CA1	-.20	-.13
Anterior Hilus	-.18	+.10
Middle CA1	-.21	+.13
Posterior CA1	-.62*	+.28
Posterior CA3	+.24	-.04
Posterior Hilus	+.02	+.19
PRh		
Anterior	+.12	+.02
Middle	+.14	+.20
Posterior	-.13	+.47*

Note. Anterior HPC (-3.6 mm from bregma). Middle HPC (-4.8 mm from bregma). Posterior HPC (-6.04 mm from bregma). Anterior PRh (-2.56 mm from bregma). Middle PRh (-4.30 mm from bregma). Posterior PRh (-6.3 mm from bregma).

* $p < .05$.

Table 2

Pearson r-values of Correlations between GFAP Density Counts in HPC and PRh of ISC Rats and their Performance on the DMTP task and the NOP Test

Region	DMTP	NOP
HPC		
Anterior CA1	+.37	+.11
Anterior CA3	+.26	-.04
Anterior Hilus	+.42	-.12
Anterior DG	+.57*	-.11
Middle CA1	+.01	-.05
Posterior CA1	+.57*	-.09
Posterior CA3	+.16	+.18
Posterior Hilus	-.11	-.01
PRh		
Posterior	+.69*	-.27

Note. Anterior HPC (-3.6 mm from bregma). Middle HPC (-4.8 mm from bregma). Posterior HPC (-6.04 mm from bregma). Posterior PRh (-6.3 mm from bregma).

* $p < .05$.