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Novelty Preference In Rats: Global Cerebral Ischemia Versus Cytotoxic Lesions Of The

Hippocampus

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A Thesis in
The Department of Psychology

Presented in Partial Fulfillment of the Requirements
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ABSTRACT

Novelty Preference In Rats: Global Cerebral Ischemia And Cytotoxic Lesions Of The

Hippocampus

Stephane Gaskin

Rats have a natural tendency to spend more time exploring novel objects than familiar

objects, and this preference can be used as an index of object-recognition memory. Rats

also show an exploratory preference for objects in locations where they have not

previously encountered objects (an index of place memory), and for familiar objects in

contexts different from those in which the objects were originally encountered (an index

of context memory). In Experiment 1, rats were subjected to either 10 minutes of global-

cerebral-ischemia (ischemia) --which resulted in significant neuropathology in the

hippocampus with no observable damage to other areas-- or sham ischemia. Rats were

then tested on all three versions of the novelty-preference paradigm (NPP), with retention

intervals, between the familiarization and test phases, of either 5 minutes or 24 hours.

Both ischemic and sham rats displayed a novelty preference on all three trial types when

the retention interval was 5 minutes. Rats that received sham surgery, but not ischemic

rats, displayed this preference on OBJECT and PLACE trials with a 24-hour interval.

Rats that received sham surgeries displayed a novelty preference. Neither group

discriminated between objects on CONTEXT trials with a 24-hour interval. In

Experiment 2, rats with cytotoxic lesions of the hippocampal formation were tested on

the OBJECT version of the NPP with retention intervals of either 15 minutes or 24 hours.

Both sham and rats with hippocampal ablation discriminated between novel and familiar

objects in both delay conditions. The combined findings of these two experiments

suggest that object-recognition deficits following ischemia are not due to hippocampal

damage.

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INTRODUCTION

Global-cerebral-ischemia (ischemia) refers to an interruption of blood supply to the brain. This condition commonly results from transient cardiac arrest and may result in significant memory deficits (Beuret et al., 1993; Grubb et al., 2000; Grubb, O'Carroll, Cobbe, Sirel, & Fox, 1996). Postmortem examinations of the brains of several ischemia survivors revealed neuropathology mostly in the hippocampus (HPC) (Volpe, Pulsinelli, & Davis, 1985; Zola-Morgan, Squire, & Amaral, 1986).

Several animal models have been developed to examine the neuropathology and behavioral consequences of ischemia (Eklof & Siesjo, 1972; Marshall et al., 1975; Pulsinelli & Brierley, 1979). Similarly to humans, rats often show neuropathology primarily in the HPC following relatively brief periods of ischemia, and may display significant memory deficits (Block & Schwarz, 1998; Milani, Uemura, Oliveira, Lepri, & Xavier, 1998; Nunn et al., 1994; Wood, Mumby, Pinel, & Phillips, 1993). However, significant damage may be observed in the striatum, thalamus and cortex if the ischemia is extended beyond 15 minutes (Nakano, Kogure, & Fujikura, 1990; Pulsinelli, Brierley, & Plum, 1982).

The importance of the HPC in learning and memory is well documented (Eichenbaum, 1999; Mumby, Astur, Weisend, & Sutherland, 1999; Nadel & MacDonald, 1980; Olton & Papas, 1979). Therefore, the observation that short periods of ischemia seems to cause damage restricted to the HPC has prompted some researchers to propose that the memory deficits following ischemia are due to HPC damage (Zola-Morgan et al., 1986). However, the HPC may not play an essential role in all types of memories. For

example, rats with HPC-ablations perform normally on tasks held to assess object-recognition memory. (Aggleton, Neave, Nagle, & Hunt, 1995; Kesner, Bolland, & Dakis, 1993; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Rothblat & Kromer, 1991). These results suggest that the HPC does not play an essential role in this type of memory. In contrast, rats subjected to ischemia display marked deficits on tasks held to assess object-recognition memory (Mumby et al., 1996; Wood et al., 1993). In object-recognition memory tasks, animals are required to discriminate between an object they have seen and an object they have never seen before.

In most studies, object-recognition memory has been assessed with the delayed non-matching-to-sample task (DNMS). In the present study, the effects of ischemia and those of HPC-ablation on object-recognition memory were examined using the novelty-preference task. This task takes advantage of rats' propensity to explore novelty (Ennaceur & Delacour, 1988). Cell counts in rats subjected to 10 minutes of ischemia revealed significant damage to the CA1, CA2 and CA3 regions of the HPC. The main behavioral findings were that these rats displayed significant object-recognition memory impairments. In contrast, rats with ablations of the HPC showed no object-recognition memory deficits. These results are a replication of previous DNMS findings in which ischemia, but not HPC-ablation, resulted in object-recognition memory deficits. In addition, these findings are extended to the use of the novelty-preference task which permits assessment of object-recognition memory with retention delays that are much longer than those that can be used with the DNMS task.

The remainder of this introduction comprises of four sections. The first section describes the neuropathology and memory deficits in two ischemia patients. The second

section discusses models of ischemia and includes a brief description of their use in animal studies. The third section deals with the mnemonic effects of HPC-ablations in animals. The fourth section provides a description of the task used in this study, and the rationale for this study. The fifth section provides a brief outline of the experimental design and hypotheses for the experiments presented in this thesis.

Ischemia-Induced Hippocampal Damage and Memory Impairments in Humans

Survivors of ischemia may experience a wide range of neurological symptoms including sensory and motor deficits, visual deficits, apraxia and neglect (Block, F., 1998). In the following cases, memory impairments were the only significant neurological symptom.

<u>Patient R.B.</u>

Zola-Morgan, Squire & Amaral (1986) describe the case of a patient (R.B) who suffered ischemia resulting from bypass surgery. Complications during surgery resulted in significant loss of blood and several episodes in which his blood pressure fell well below normal.

Neuropsychological testing of R.B. revealed anterograde-memory impairments. For example, he was impaired on tests of verbal and non-verbal memory function. He also performed poorly on tests of 2-choice recognition and cued recall. Tests of retrograde amnesia revealed that R.B. had little if any loss of information acquired before the ischemic events.

Thionin-staining of sections of R.B's brain, revealed damage mostly confined to the CA1 field of the HPC, with complete bilateral cell loss in this region. However, damage to other areas was apparent. For example, a small patch of gliosis within the internal medullary lamina, and a prominent lesion of the lateral medullary lamina of the thalamus were observed. Examinations of the cortex revealed an infarct in the right postcentral gyrus as well as in area 5. Also, small foci of cell loss were distributed through the cortex.

Patient G.D.

Patient G.D. (Rempel-Clower, Zola, Squire, & Amaral, 1996) was subjected to ischemia in the course of parathyroidectomy surgery. During surgery G.D. experienced cardiac arrhythmia resulting in a significant drop in heart rate and blood pressure.

Neuropsychological testing revealed that G.D. suffered significant anterograde memory deficits. He performed poorly on tests of diagram recall, word recall, and face recognition. In contrast to R.B, G.D. performed poorly on tests of retrograde memory. For example, he was impaired on tasks that test memory for public events and places learned prior to his surgery. `

Brain sections of G.D, stained by a modified Heidenhain-fiber procedure, revealed extensive bilateral cell loss in the CA1 field of the HPC. A slight loss of cells was also observed in layers 3 and 5 of the left entorhinal cortex, a small region of gliosis was also observed in the right ventromedial portion of the amygdala. The left thalamus showed a slight decrease in cell density.

Although the memory deficits observed in R.B. and G.D. are attributed to HPC-damage, brain damage was observed in other areas. It is possible that neuropathology

outside the HPC contributed to their memory impairments. For example, damage to either the thalamus or rhinal cortex, which was observed in both patients, has been shown to cause memory deficits in rats (Cho & Kesner, 1996; Mumby & Pinel, 1994) and monkeys (Meunier, Hadfield, Bachevalier, & Murray, 1996; Vnek, Gleason, Kromer, & Rothblat, 1995).

Animal Models of Ischemia

Several methods have been devised in order to assess the behavioral deficits and the neuropathology that results from ischemia. The most commonly used methods in rats are the 2-vessel occlusion (2-VO) and the 4-vessel occlusion methods (4-VO). The 2-VO method consists of the transient occlusion of both common carotid arteries combined with hypotension induced by bleeding (Eklof & Siesjo, 1972). The 4-VO method consists of the transient occlusion of both common carotid arteries combined with the permanent occlusion of the vertebral arteries (Pulsinelli & Brierley, 1979).

In the monkey, ischemia can be achieved by the occlusion of the posterior cerebral arteries (Bachevalier & Mishkin, 1989). Ischemia may also be achieved in monkeys by the non-invasive compression of both carotid arteries combined with pharmacologically induced hypotension. (Zola-Morgan, Squire, Rempel, Clower, & Amaral, 1992). The brain damage induced by the application of these methods is related to the duration of the ischemia (Pulsinelli et al., 1982). The loss of neurons in the CA1 cell-fields of the HPC have been detected after 10 minutes of ischemia. (Busto, Globus, Neary, & Ginsberg, 1994). Longer periods of ischemia have been shown to result in the loss of neurons in the striatum, thalamus, and cortex (Grisham & Granger, 1989).

Histological methods that go beyond the detection of the viability of neurons show neuropathology in other brain areas (Corbett & Nurse, 1998). However, most behavioral studies examining the mnemonic effects of ischemia rely on visual cell counts in order to determine the extent of brain damage produced by ischemia.

Mechanisms of Brain Damage in Ischemia

Several mechanisms of cellular death have been suggested to result from ischemia. There is evidence that a massive release of excitatory amino acids and an intracellular overload of calcium and free radicals may result in excitotoxicity of neurons (Oliver et al., 1990). An increase in nitric oxide concentrations (Elibol et al., 2001), and the production of reactive oxygen species (ROS) (Capani et al., 2001) are among other factors that may lead to neuronal death.

Ischemia leads to two types of neuronal death, necrotic and apoptotic. Necrotic cell death is likely due to swelling and damage of the mitochondria, followed by a dissolution of organelles and rupture of the plasma membrane, resulting in the leakage of cellular contents into the extra-cellular space (Trump & Berezesky, 1992). Apoptotic cell death may be triggered by an overactivation of N-methyl–D-aspartate (NMDA) receptors and may take place over several hours to several days. Glutamate can lead to apoptosis through the production of caspases by activating apoptotic genes (Banasiak, Xia, & Haddad, 2000). Thus, ischemia might not produce its maximal neuronal damage until days or weeks following the ischemic event. Therefore, the observed effects of ischemia on memory may be a function of the time that separates ischemia from behavioral testing.

The HPC, Ischemia, and Spatial Memory

Experiments with rats have shown that relatively short periods of ischemia may

cause deficits on both spatial (Olsen, Scheel-Kruger, Moller, & Jensen, 1994; Onifer &

Low, 1990; Schwartz, Wishart, Ijaz, & Shuaib, 1998) and object-recognition memory

tasks (Mumby et al., 1996; Wood et al., 1993). The brief periods of ischemia used in

these experiments are thought to produce damage mainly restricted to the HPC. Because

of this, the deficits observed on these tasks following ischemia are attributed to damage

to the HPC. However, considerable controversy exist as to whether this is the case. A first

step at resolving this issue might consist of comparing the effects of ischemia and

ablations of the HPC on spatial and object-recognition memory tasks.

The HPC is thought to be important for the storage of the spatial layout of

external cues within the environment. This information may be useful to efficiently

navigate in the environment (O'Keefe & Nadel, 1978). It has been known for decades

that lesions of the HPC result in impairments on spatial-memory tasks. In some of the

first demonstrations of this, rats with ablations of the HPC were impaired in finding the

location of a submerged platform in a water maze (Morris, Garrud & O'keefe, 1982) and

the location of food on a radial arm maze (Olton & Papas, 1979). Ever since, numerous

studies have demonstrated that ablations of the HPC produce significant impairments on

spatial-memory tasks (Barnes, 1988; Nadel, 1991). The HPC is also thought to play a role

in contextual-memory. Demonstrations of the role played by the HPC in this type of

memory are provided by studies on contextual fear conditioning (Anagnostaras, Gale, &

Fanselow, 2001; Maren, Aharonov, & Fanselow, 1997; Winocur & Gilbert, 1984) . In the

basic procedure, animals are exposed to a novel conditioning context. While in this

context animals are given a mild shock. As a result of this context/shock pairing, normal animals display a freezing response to the presentations of the context alone. This type of memory, which requires the animal to form relationships between the contextual stimuli and the shock is impaired by HPC-lesions (Maren & Fanselow, 1997).

Davis et al. (1986) assessed the mnemonic effects of ischemia on a task using the 8-arm radial maze (Morris et al., 1982). Half the arms of the maze were baited and normal rats learned that, once visited, an arm no longer contained a food reward, thus seldom revisited it during the same trial. Normal rats also rarely visited arms that had never been baited. Revisiting a previously visited arm constituted a working-memory error whereas visiting an arm that had never been baited constituted a reference-memory error. Successful performance on this task is thought to require spatial learning, because rats are required to associate a food reward with its location relative to distal spatial cues. Davis et al. (1986) found that rats subjected to ischemia made significantly more spatial working memory errors than control animals. Davis et al. (1986) pointed out that these results were consistent with the findings that rats with damage to the HPC are impaired on spatial-memory tasks, and concluded that the spatial-memory deficits observed in rats subjected to ischemia were due to HPC damage. However, although the histological analysis revealed damage mostly to the CA1 field of the HPC, damage was also observed in the dorsolateral caudate nucleus, thalamus and anterior neocortex. This pattern of brain damage puts into question the interpretation that the spatial-memory deficits observed by Davis et al. (1986) are solely due to damage to the HPC. For example, damage to the thalamus has been associated with spatial-memory deficits in rats (Langlais, Mandel, &

Mair, 1992; Mair & Lacourse, 1992; Mumby, Cameli, & Glenn, 1999) and monkeys

(Isseroff, Rosvold, Galkin, & Goldman-Rakic, 1982).

The Morris water maze (Morris et al., 1982) is extensively used for the

assessment of spatial-memory. In a task using this apparatus, rats must learn to find a

submerged platform in a pool of water. The water is made opaque with either powdered-

milk or non-toxic white paint. Thus, rats have to learn the location of the submerged

platform relative to distal cues. Several measures are used as indices of spatial learning

on this task, including latency to reach the platform and time spent in the correct quadrant

on probe trials. On a probe trial, the platform is removed and the percentage of time a rat

spends in the quadrant in which the platform was previously located is recorded. When a

rat spends significantly more time in the previous platform quadrant than expected by

chance, it is inferred that the rat remembers were the platform was situated.

Olsen et al. (1994) found that rats subjected to ischemia displayed significant

impairments in the Morris water maze. Rats subjected to ischemia displayed longer

escape latencies and were impaired relative to controls on probe trials. The histological

analysis revealed brain damage restricted to the CA1 field of the HPC with only a few

rats in which damage extended to the CA2 and CA3 fields.

In another study, Milani et al. (1998) found ischemia-induced spatial-memory

deficits, on a task that exploits rats' natural propensity to prefer darker areas to more

illuminated areas. The task was administered on a circular platform with 18 equally-

spaced holes located along its perimeter. One of the holes had a black wooden drawer

inserted below it, serving as a goal a box. Rats were required to learn the position of this

goal box in relation to distal cues. On a probe trial, rats were required to learn that the

goal box was moved to a new location. Milani et al. (1998) found that control rats spent significantly more time than ischemic rats in the area in which the goal box was previously situated. Histological analysis in this study revealed that most damage occurred in the CA1 field of the HPC and in the presubiculum area. However, minor damage was also observed in the CA2, CA3 and CA4 cell fields of the HPC, and caudate-putamen. These results were consistent with a previous report from Morris et al. (1982) in which rats with HPC-ablations persisted in searching for a platform in its original location on a probe trial. However, Milani et al (1998) suggested that the impairments shown by ischemic rats in this study may have partly been due to caudate-putamen-damage, as damage to this area has been shown to cause place acquisition deficits in the water maze (Devan, McDonald, & White, 1999).

Olsen, Scheel-Kruger and Milani et al. (1998), found that the degree of impairments in rats subjected to ischemia was significantly correlated with the extent of cell loss in the CA1 field of the HPC, but not in other areas. Such correlations were found in several other studies. For example, Block & Schwarz (1997, 1998) found that impairments in the Morris water maze were significantly correlated with the extent of cell loss in the CA1 region of the HPC. However, no correlation was found between these impairments and damage to the CA2, CA3, dentate gyrus or striatum. Nevertheless, other studies have not found a correlation between behavioral impairments on spatial-memory task and the extent of cell loss in the HPC. For example, Nunn et al. (1994) found that impairments in the Morris water maze did not correlate with CA1 cell loss in the HPC.

Altogether, the results from the above studies are consistent with the idea that spatial-memory impairments following ischemia are due to damage to the HPC.

However, not all analyses show a correlation between spatial-memory deficits and extent of cell loss in the HPC. This lack of correlation may be due to the inclusion of neurons that appear normal, but in which components necessary for the cells' normal functioning have been damaged by ischemia. Therefore, extraction of a correlation coefficient based strictly on the number of viable neurons can result in an underestimation of the relationship between the functional deficits of neurons and the behavioral deficits observed in animals. In addition, a significant correlation may not imply cause and effect because the degree of deficits might also correlate with undetected damage in other brain regions.

<u>Ischemia and Object-Recognition Memory</u>

Several experiments have been conducted in order to assess the effects of ischemia on object-recognition memory in monkeys (Alvarez, Zola-Morgan, & Squire, 1995; Bachevalier & Mishkin, 1989; Zola-Morgan et al., 1992) Some of these experiments focused on attempting to resolve the issue as to whether the object-recognition memory impairments observed following ischemia are solely due to damage to the HPC (Mumby et al., 1996; Zola-Morgan et al., 1992). Object-recognition memory in animals is mostly tested using the DNMS task. Trials on this task consist of two phases, a sample phase and a test phase. On the sample phase, subjects learn to displace a sample object covering a baited food well. On the test phase, after a delay interval, the sample and a new object are presented each covering a different food well, and subjects are rewarded for displacing the new object. Successful performance on this task is held to require object-recognition memory because subjects must discriminate between an object they have seen before and an object they have never encountered.

Zola-Morgan et al. (1992) proposed that if object-recognition memory deficits after ischemia were due to damage outside of the HPC, then monkeys subjected to ischemia should perform at levels equal to, or worse, than monkeys with HPC ablation, including collateral damage to other brain areas. Zola-Morgan et al. (1992) designated this group of monkeys HPC +. However, if damage produced by ischemia is restricted to the HPC, then the deficits in those monkeys should be less severe than monkeys with HPC+ lesions.

Zola-Morgan et al. (1992) found that monkeys subjected to ischemia were as impaired on the DNMS task as monkeys with HPC+ ablation and concluded that HPC damage alone was sufficient to result in the object-recognition deficits observed on the DNMS task. Nevertheless, there is no evidence that the brain damage in extrahippocampal regions in monkeys with HPC+ ablation was equivalent to that in monkeys subjected to ischemia.

In another study, Alvarez et al. (1995) found that monkeys with HPC ablation were impaired on the DNMS task. However, 8 out of 16 monkeys in this experiment sustained considerable damage to the entorhinal cortex and 5 monkeys sustained slight to moderate damage to the parahippocampal gyrus. Lesions circumscribed to these areas have been found to produce object-recognition deficits by themselves (Meunier et al, 1993).

Bachevalier and Mishkin (1989) compared the DNMS performance of monkeys subjected to ischemia versus ablations of the HPC. They found that monkeys subjected to ischemia had test scores that were significantly lower than control animals across retention delays ranging from 60-200 seconds. The brain damage observed in these

animals ranged from little observable damage in one animal, to massive unilateral damage to the occipitotemporal cortex and HPC in another. However, for most animals damage was observed to be mostly confined to the HPC, with variable damage to the entorhinal cortex and parahippocampal gyrus. A correlational analysis revealed that the degree of deficits was positively related to the extent of cell loss in the HPC.

In contrast, monkeys with ablation of the HPC had significantly better test scores than monkeys subjected to ischemia across the same retention delays. These results are not consistent with the view that object-recognition memory deficits following ischemia are due to damage to the HPC, because if damage to the HPC alone was responsible for the object-recognition deficits following ischemia, then partial damage to the HPC induced by ischemia should not have resulted in more severe deficits than complete ablation of the HPC.

Bachevalier and Mishkin (1989) put forward two hypotheses in an attempt to explain those paradoxical findings. According to Bachevalier and Mishkin's (1989) first hypothesis, ischemia might produce undetected damage in areas outside of the HPC that are important for normal object-recognition memory. This idea would be consistent with the findings that ischemia can cause significant spatial-memory deficits in rats with no observable brain damage (Jaspers, Block, Heim, & Sontag, 1990). These findings suggest that the effects of ischemia may not always be detectable by histological analyses that rely on visual cell counts, and that ischemia may result in covert damage in other brain areas important for learning and memory. In addition, neurons at the focus of epileptic seizures appear to be normal while exhibiting abnormal electrophysiological activity (Corbett & Nurse, 1998).

Mumby et al. (1996) proposed that if ischemia produces object-recognition memory deficits through direct damage to brain areas outside of the HPC, then the effects of ischemia on object-recognition memory should not change in rats that previously received HPC ablation. In a test of this idea, Mumby et al. (1996) found that rats were not impaired on the DNMS task if given HPC-ablation prior to being subjected to ischemia. This result is not consistent with Bachevalier and Mishkin's (1989) first hypothesis that ischemia produces object-recognition memory deficits by producing damage in brain areas outside of the HPC.

According to Bachevalier and Mishkin's (1989) second hypothesis, the HPC is rendered dysfunctional by ischemia and might disrupt the activity of intact neuronal circuitry efferent to the HPC. Mumby et al. (1996) proposed that if ischemia produces object-recognition memory deficits by disrupting neuronal circuitry efferent to the HPC, then ablation of the partially damaged HPC following ischemia should result in the attenuation of the ischemia–induced object-recognition memory deficits. In a test of this idea, Mumby et al.(1996) found that ablations of the HPC several days after ischemia did not ameliorate the ischemic rats' deficits observed on the DNMS task. This result is not in accordance with Bachevalier and Mishkin's (1989) second hypothesis that ischemia partially damages the HPC, and that a partially damaged HPC disrupts information processing in other brain regions that are important for object-recognition memory.

Alternatively, Mumby et al. (1996) hypothesized that the object-recognition memory deficits observed following ischemia are due to ischemia -induced seizures in the HPC which in turn causes damage in other brain areas important for learning and memory. According to this hypothesis, it may be possible to remove the HPC soon

enough after ischemia to prevent seizures in the HPC to produce damage in other brain areas. In a test of this hypothesis, Mumby et al. (1996) found that deficits on the DNMS task were attenuated if the HPC was ablated 1 hour after ischemia. This result supports Mumby et al.'s (1996) hypothesis that seizures in the HPC may cause damage in other brain areas important for learning and memory. Furthermore, this result may provide an explanation for all of the above findings concerning the effects of ischemia on object-recognition memory. It is also possible that extra-hippocampal damage following ischemia is partly accountable for the effects of ischemia on spatial-memory.

Whether object-recognition memory deficits following ischemia are solely due to HPC damage remains unresolved by studies with monkeys. Nevertheless, a great majority of studies in the rat (Mumby, 2001) revealed that ischemia produces severe object-recognition memory deficits whereas ablation of the HPC does not.

Although many studies have investigated the effects of ischemia on object-recognition memory in monkeys, very few studies assessed the effects of ischemia on object-recognition memory in rats. In one of these studies, Wood et al. (1993) tested the effects of ischemia in rats on a DNMS task developed by Mumby, Pinel & Wood (1990). They found that ischemia resulted in significant impairments on that task. Rats subjected to ischemia took significantly more trials to master the task. In addition, rats subjected to ischemia displayed a significant impairment in choice-accuracy with retention delays up to 300 seconds. Most of the damage produced by ischemia was observed in the CA1 region of the HPC and minor damage was found in the dentate-gyrus. No observable damage was found in any of the other regions examined, including the striatum, enthorhinal cortex, perirhinal cortex and mamillary nuclei. There was a positive

correlation between the extent of CA1 cell loss in the HPC and the severity of deficits observed on the DNMS task. These results are consistent with the findings with monkeys (Bachevalier & Mishkin, 1989; Zola-Morgan et al., 1992) and humans (Zola-Morgan et al., 1986) in which object-recognition memory was impaired by ischemia.

Studies that show positive correlations between the extents of ischemia induced HPC-damage and the severity of object-recognition memory deficits, may lead to the conclusion that object-recognition memory deficits following ischemia are caused by damage to the HPC. However, as with the findings on spatial-memory, correlational analyses may not provide a complete picture of the effects of ischemia on object-recognition memory. For example, the observed object-recognition memory deficits may also correlate with unobserved damage to areas outside of the HPC. Other findings cast doubt on this notion. Mumby (1996) found that rats subjected to ischemia were more impaired in the DNMS task than rats with ablations of the HPC. These results were consistent with findings in monkeys (Bachevalier et al.,1989) and suggested that damage to the HPC cannot solely account for the deficits observed in the DNMS task following ischemia

Ablation of the HPC and Object-Recognition Memory

The DNMS task, the HPC and Object-Recognition Memory

So far, studies in monkeys have failed to demonstrate that object–recognition-memory deficits can be caused by damage restricted to the HPC. The main reason for this failure stems from the difficulty of producing lesions of the HPC in monkeys, without damaging overlying cortical areas. Experimental ablations of the HPC in monkeys, often involve

collateral damage to cortical areas, including the parahippocampal gyrus and entorhinal cortex (Alvarez et al., 1995; Squire, 1992; Zola-Morgan et al., 1992). This results in uncertainty as to whether object-recognition memory deficits following ablations of the HPC are due to damage to the HPC itself or damage to adjacent cortical areas.

The effects of HPC ablation on object-recognition memory in rats has been tested on several versions of the DNMS task. (Cassaday & Rawlins, 1995, 1997; Mumby et al., 1996; Wiig & Bilkey, 1995). A majority of studies suggest that the HPC is not essential for object-recognition memory. For example, Aggleton, Hunt & Rawlins (1986) tested the DNMS performance of rats with ablations of the HPC, and found that they performed as well as controls at retention delays of up to 60 seconds. In contrast, the rats with HPC-ablation were impaired on a spatial-memory task in which they had to learn the location of a food reward on a T-maze. On another rat version of the DNMS task, Mumby et al. (1992) found that rats with ablation of the HPC were not impaired relative to pre-surgery performance with retention delays up to 600 seconds. It is important to note that 4 out of 11 rats in the group with HPC lesions displayed severe impairments on the task. However, these rats sustained unintended various amounts of damage to the perirhinal cortex, an area known to be involved in object-recognition memory (Ennaceur & Aggleton, 1997; Mumby, Glenn, Nesbitt, & Kyriazis, 2002) and to the lateral geniculate nucleus, an area known to be involved in vision (Wang & Shou, 2000). Thus, it is possible that the impairments observed in these rats were due to damage in these areas.

Relatively few studies report DNMS deficits following HPC-ablation. In one of them, Clark, West, Zola-Morgan & Squire (2001) suggested that HPC-ablation impaired performance on the DNMS task. Nevertheless, two of the five rats in the rats in the HPC-

ablation group sustained damage to the perhirinal cortex. Furthermore, once these rats were eliminated only three rats were left constituting the group with HPC ablation.

In another study, Wiig and Bilkey (1995), report that rats with fornix lesions were impaired on the DNMS task. Fornix lesions are often equated with HPC lesions because lesions of the fornix results in the HPC being deprived of its' reciprocal connections to the mid-diencephalic region (Calabrese, Markowitsch, Harders, Scholz, & Gehlen, 1995), and of its' cholinergic input from the medial forebrain (Ridley, Thornley, Baker, & Fine, 1991; Wall, Wolfe, & Kromer, 1994). Furthermore, numerous studies have shown that fornix lesions produce effects similar to HPC lesions on spatial-memory tasks (E. A. Gaffan & Eacott, 1997; Murray, Davidson, Gaffan, Olton, & Suomi, 1989; Walker & Olton, 1979, 1984). However, Wiig and Bilkey (1995) suggested that these impairments might have been due to unintended damage to the septal and medial septal areas. This interpretation would be consistent with the finding that medial-septal lesions disrupt electrophysiological activity in the perirhinal cortex (Dickson, Trepel & Bland, 1994).

Altogether most studies show that performance on the DNMS task is not impaired in animals with HPC ablation. In both rats and monkeys, studies in which animals did display significant impairments on the DNMS task following HPC ablation also sustained damage to other brain areas (Alvarez et al., 1995; Wiig & Bilkey, 1995; Zola-Morgan et al., 1992). Because damage to some of those areas is known to impair object-recognition memory, the HPC damage cannot solely be held accountable for the DNMS deficits observed in those studies.

The Novelty-Preference Task, the HPC and Object-Recognition Memory

The effects of ablating the HPC on object-recognition memory have also been investigated using the novelty-preference task. In this task, rats are familiarized to a pair of identical objects in an open field. Following a retention interval, rats are placed back into the open field, only this time the familiar object is present along with a novel object. Normal rats spend more time exploring the novel object than the sample. This is taken as an index of object-recognition memory because in order to show a novelty preference, rats have to recognize the sample object as being familiar. Noted advantages associated with the use of the novelty-preference task include the fact that performance does not include the retention of a rule, and is not influenced by changes in responsivity due to a food reward. Importantly, in contrast to the DNMS task, the novelty-preference task does not necessitate extensive training, which may last several days. In addition to object-recognition memory, the novelty-preference- paradigm can be adapted to assess memory for places and contexts (Dix & Aggleton, 1999; Ennaceur & Delacour, 1988; Mumby, Gaskin et al., 2002).

HPC ablation has also failed to produce deficits on the object-recognition version of the novelty-preference task (Ennaceur & Aggleton, 1994; Ennaceur, Neave, & Aggleton, 1996, 1997; Warburton & Aggleton, 1999). However, in one study, Clark, Zola-Morgan & Squire (2000) tested rats on the novel-object preference paradigm at retention delays of 10 seconds, 1 minute, 10 minutes, 1 hour and 24 hours. They tested the effects of two types of hippocampal lesions: radio-frequency (RF) and ibotenic-acid (IBO). They found that rats with HPC ablation could not discriminate between a novel and familiar object, when averaging the results of the 10-minute, 1-hour and 24-hour

conditions. However, a closer look at the data reveals that the rats with HPC lesions were not impaired in the 24-hour condition while impaired in the 10-minutes and 1-hour conditions.

In summary, a great majority of studies with rats revealed that ablations of the HPC do not result in deficits in object-recognition memory tasks. However, ischemia has been shown to produce severe deficits on tests of object-recognition memory. With a few exceptions, these findings were consistent on two tasks held to assess object-recognition memory, the DNMS task and the novelty-preference task. However, use of the DNMS task does not permit testing with long retention delays. For example, even intact animals cannot perform on this task if the retention delay lasts more than a few minutes. In contrast to the DNMS task, the novelty-preference task permits the assessment of object-recognition memory with retention delays of up to 24 hours. So far, only one study assessed object-recognition memory with such a retention delay using the novelty-preference task (Clark, Zola, & Squire, 2000). To further clarify the issue of whether ischemia-induced object-recognition memory deficits are due to HPC damage, the present study compared the effects of ischemia to the effects of HPC ablation on object-recognition memory, using the novelty-preference task with retention delays of 15 minutes and 24 hours.

Experimental Design and Hypotheses

In Experiment 1, rats subjected to ischemia were tested on the novelty-preference task, with retention delays of 5 minutes and 24 hours. Previous studies have shown that ischemia induces both object-recognition (Mumby et al., 1996; Wood et al., 1993) and spatial-memory (Davis, Tribuna, Pulsinelli, & Volpe, 1986) deficits in rats. Also, rats with HPC-damage have been shown to be impaired on tasks of contextual-memory (Anagnostaras et al., 2001; Maren et al., 1997; Winocur & Gilbert, 1984). Thus, it was hypothesized that ischemia would produce brain damage resulting in deficits in the object, place and context versions of the novelty-preference task at both retention delays.

In Experiment 2, rats that received N-methyl-d-aspartate (NMDA) lesions of the HPC were tested on the object-recognition memory version of the novelty-preference task with retention delays of 15 minutes and 24 hours. Because a great majority of studies demonstrated that the HPC is not essential for object-recognition memory at retention delays of up to 10 minutes (Cassaday & Rawlins, 1997; Kesner et al., 1993; Mumby et al., 1996), it was hypothesized that damage to the HPC produced by NMDA lesions would not result in object-recognition memory deficits at the 15-minute retention delay. This study was the first to compare the performance of rats with HPC-ablation with that of rats subjected to ischemia with a 24-hour retention delay, using the same materials and behavioural procedures.

EXPERIMENT 1

INTRODUCTION

Ischemia has been shown to impair object-recognition memory in rats (Mumby et al., 1996; Wood et al., 1993). However, the effects of ischemia on object-recognition memory in rats have mostly been tested using the DNMS task. There are limitations to the use of the DNMS task: First, rats require extensive training in order to learn the non-matching rule. Consequently, several weeks are often dedicated to the initial phase of an experiment. Second, rats must be food restricted because a food reward is used as a reinforcer following the displacement of the correct object. This latter consideration complicates the interpretation of the results of the task because of the possible interactions between the motivational effects of hunger, memory and the brain damage induced by ischemia. Third, rats do not show reliable retention at intervals longer than a few minutes.

In contrast, the novelty-preference task permits the assessment of object-recognition memory without the disadvantages, noted above, of the DNMS task. Neither food deprivation or extensive training is required and normal rats perform well with retention delays of several hours. In this experiment, we assessed the effects of ischemia on object-recognition memory using the novelty-preference task. It was hypothesized that 10 minutes of ischemia, a duration that causes observable cell-loss primarily to the HPC with little observable cell-loss in other structures, would result in deficits on the object version of the novelty preference task. It was therefore expected that rats would be

impaired on the novelty-preference task, consistent with the findings that ischemia results in deficits on the DNMS task. Because the novelty-preference task can be adapted to test spatial and contextual-memory and because ischemia has been shown to cause spatial-memory impairments in rats, I also tested the effects of ischemia on memory for places and contexts using the novelty-preference task.

METHOD

Subjects

Seventeen male Long-Evans rats weighing between 250-300 g were used in this study. The animals were experimentally naïve and were housed in plastic shoebox cages under 12-12 h light/dark cycle. Animals had *ad libitum* access to food and water throughout the experiment. All animals were tested during the light phase of their cycle and testing began 21 days following ischemia.

Surgery

Rats were either subjected to the 4-VO (Group ISC, n = 9) method of ischemia or received sham surgeries (Group SHAM, n = 8). Rats were initially anesthetized with 5% isoflurane and were subsequently maintained with 2-3% isoflurane and a balance of oxygen was delivered via a closely fitting facemask. In the ISC group, both vertebral arteries were permanently occluded by electrocauterization. Following a 24-hour recovery period, rats were again anesthetized and both common carotid arteries were

exposed, and occluded for 10 minutes, using microvascular clamps. The clamps were then removed allowing for immediate reperfusion. Body temperature was regulated, by a thermoprobe coupled with a heating pad, and kept constant at 37 degrees Celsius for the duration of the occlusion. For sham surgeries, the vertebral arteries were permanently occluded by electocautery. Twenty-four hours later both common carotid arteries were exposed but not occluded. The rats were then permitted to recover for a period of 21 days, after which they were submitted to testing. During the recovery period, the rats were kept under daily observation. Any rat displaying motor deficits, which could prevent exploratory behavior, was excluded from the study.

Apparatus

Testing for the Object, Place and Context versions of the task were conducted in 2 open field arenas (60 cm x 70 cm x 70 cm), constructed with grey PVC plastic. Each arena was situated in a different room, proving two different contexts. Both rooms differed from the other by their constellation of extramaze cues. The cues were positioned such as they can easily be detected from inside the arena. One arena had green cardboard strips lining two of its walls in order to provide further a salient feature distinguishing the two arenas from each other. All other features of the arenas remained constant between contexts. A stainless steel tray covered with wood shavings served as the floor of the apparatus. The floor could easily be removed through a slot at the bottom of one of the walls in order to permit the changing of wood shavings between trials. Place and Context trials differed in that during testing on Place trials, an object was seen in a different place

but within the same context as seen in the sample phase; whereas on Context trials an object was seen in the same place however in a different context than when seen in the sample phase.

For the place version, an oval arena was used and its location cycled between six distinctive rooms. Cycling of the rooms ensured that objects labeled, as being in a novel location had in fact never been seen there on previous trials. The oval shaped arena ensured that no association could be made between the objects and the arenas used in the previous versions of the task.

Forty-six objects were used as stimuli, and were either made of metal, glass, porcelain or glazed ceramic. The objects varied in height (4.5 -15 cm) and width (4-10 cm). The objects were washed with a solution of diluted bleach after every trial. A glass jar was attached, with epoxy, at the bottom of each object. The objects were then fixed on the stainless-steel tray on which inverted jar lids had been screwed into place. The position of the objects for both the object and context trials remained the same throughout the experiment in both the sample and test phases. The standard position for the objects in these trials was 27 cm from opposing corners of the arena. For the Place trials, objects in the sample phase were position 10 cm from each corner radius of the oval. For the test phase, one of these objects was moved to 10 cm from any of the other corners of the oval. A video camera was positioned over both type of arenas and the sample and test phases were recorded for later analysis.

Procedure

Figure 1 illustrates the Object, Place and Context versions of the novelty-preference paradigm. On Object trials, the rat was placed in the arena with two identical sample objects. The rat was allowed to explore for 5 minutes. The rat was removed for a retention interval. The objects were then replaced with two new objects. One of the objects was an identical copy of the sample objects and the other was novel. The rat was then returned to the arena and allowed to explore for 3 minutes.

On Place trials, the rat was placed in the arena with two identical objects. The rat was allowed to explore for 5 minutes. The rat was removed from the arena for a retention interval, and one of the objects was moved to a new location. The rat was returned to the arena and allowed to explore for 3 minutes.

On Context trials, the rat was placed into the arena with two identical objects and allowed to explore for 5 minutes. The rat was then removed and carried into another arena situated in a different room and was allowed to explore another pair of objects for 5 minutes. The rat was then removed for a retention interval. The rat was then placed in one of the two contexts with a copy of each object.

The rats were habituated to both arenas by allowing them explore them in the presence of two identical objects which were not used in the study. Rats were placed in the arenas on 3 consecutive days. On day 1 rats were placed in the arenas in pairs for a period of 15 minutes. On day two and 3 single rats were permitted to explore the arenas for 15 minutes.

Testing began one week following habituation. All rats were tested with a 5-

minute and 24-hour retention delay. For the 5-minute delay, rats received one trial type

A

Sample OBJECT **Test**

PLACE

B

CONTEXT

C **Sample 1** **Sample 2** **Test**

Context A Context B Context A or B
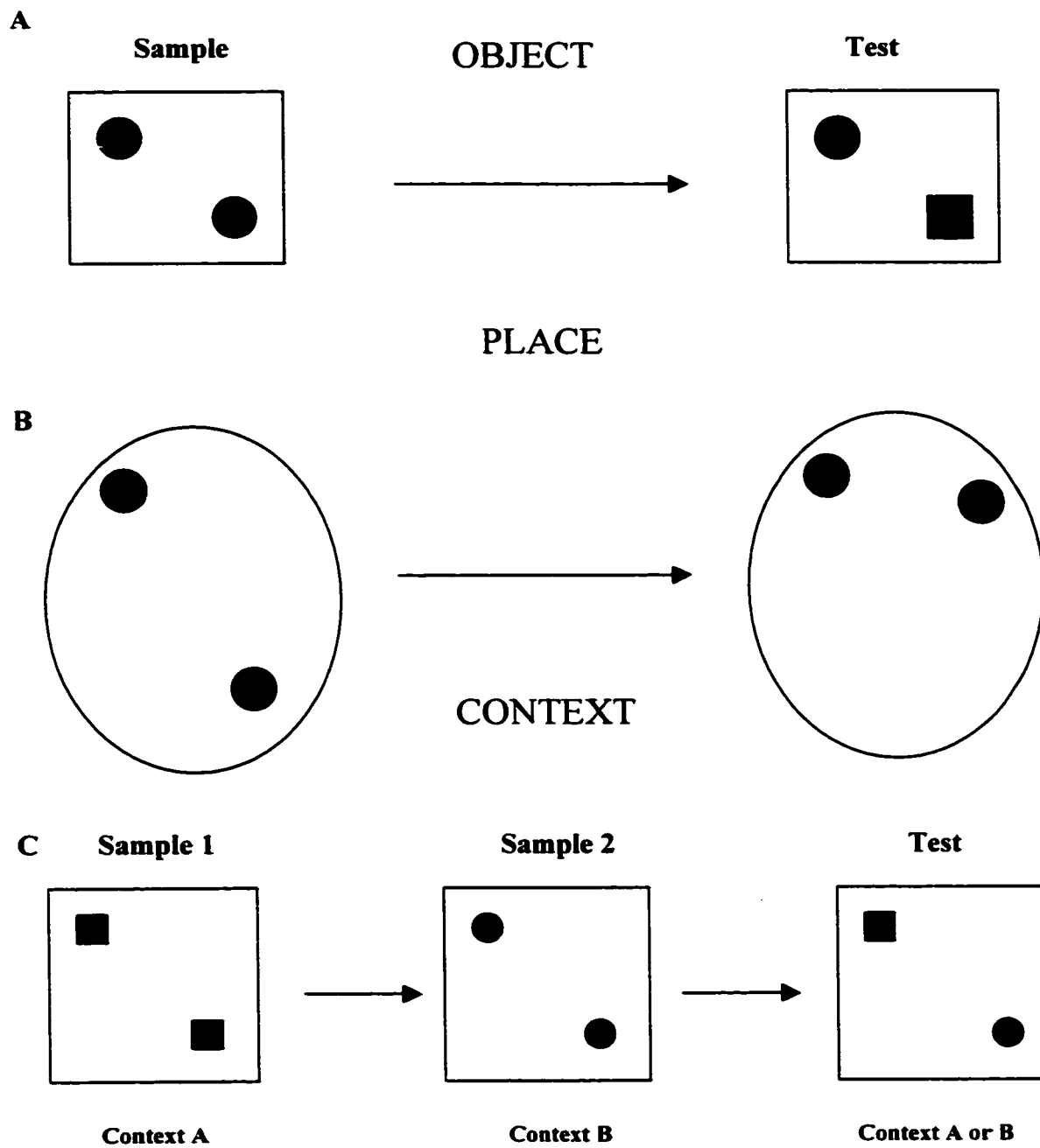
Figure 1. Schematic representation of the sample and test conditions of the Object (A), Place

(B) and Context (C) version of the novelty-preference task.

per day, on three successive days. Each rat was given one type of trial per day. This procedure was repeated over three consecutive weeks. Thus, each rat received all trial types on three occasions. Testing with the 24-hour retention delay began one week following the last day of testing with the 5-minute delay.

For the 24-hour delay, rats received three trials of each type with the sample phase given on one day and the test phase given on the following day. This procedure was repeated over three consecutive weeks. The objects used in each trial were randomly determined and were used only for one trial per rat. The experimenter was blind as to the group-identity of each rat.

In the Object version of the task, half of the rats in each group received their trials in room A and the other half in room B. Place trials, were conducted in a different room every day. During the test-phase, objects could be moved to either of four novel locations. A different novel location was chosen on each of the three place trials for each rat. In the context version, half the rats of each group were tested in room "A" and the other half in room "B".

The main dependent measure was the exploration ratio, that is, the proportion of total object-exploration that was spent exploring the novel object (t_{novel} /[t_{novel}+ t_{sample}]) during each minute of the 3-minute test phase. This minute-by-minute assessment makes it possible too see how exploratory preferences change over the retention test. Previous studies found that preference for the novel object on the Object version is robust only during the first one or two minutes of a retention test and diminishes thereafter, presumably because both objects become equally familiar as they are explored (Dix & Aggleton, 1999). A rat was considered to be exploring an object when its head was

oriented within 45 degrees of an object and within four centimeters of it. A rat was also

considered engaged in the exploration of an object while rearing if at least one forepaw

was touching the object.

Histological Procedures

Following behavioral testing rats received an overdose of sodium-pentobarbital

(100mg/kg, ip) and were perfused transcardially with 10% paraformaldehyde (PFA) in

0.05% phosphate buffer. The brain were then removed and fixed in 10% PFA. Coronal

sections, 14 um thick, were cut through the rostrocaudal extent of the forebrain, and

every tenth section was mounted and stained with 1 % cresyl violet. Neurons in the CA1,

CA2, CA3 and dentate-gyrus, of the HPC were quantified by visual counting of viable

neurons using a light microscope. These cell counts were compared with those in control

rats. Neurons in the four sub-fields of the HPC were quantified in three different coronal

sections (-2.5mm, -4.5mm, -5.8mm relative to bregma). These neuronal counts were then

average across the left and right HPC and were expressed as the mean number of neurons

per 500 um for each section of the HPC.

RESULTS AND SUMMARY

Histological Results

Figure 2 shows photomicrographs of the HPC of a rat subjected to 10 minutes of

ischemia in relation to the HPC of a control. Figure 3 shows the mean number of neurons

per 500 um for each of the cell-fields at all three coronal sections combined (bregma

–2.5mm,-4.5mm,-5.8mm). Independent sample t-tests (one-tailed) revealed a significant

difference in the number of viable neurons between the SHAM and ISC rats in the CA1

(t[14] = -5.71, p < .000), CA2 (t[14] = -5.80, p < .000), CA3 (t[14] = -3.83, p < .001) and

dentate gyrus (t[14] = -1.87, p < .041) regions of the HPC.

Figures 4 shows the mean number of neurons per 500 um at each of the three

coronal sections for ISC and SHAM rats. Independent sample t-test were conducted in

order to determine if a significant difference existed between the number of viable

neurons in the HPC of the SHAM and ISC rats at each of the 3 coronal section.

Bregma -2.5 mm

The number of viable neurons in ISC rats was significantly lower than that of

SHAM animals in the CA1 (t[14] = -4.999, p < .000), CA 2 (t[14] = -4.203, p < .000),

and CA 3 cell fields (t[14] = -2.650 p < .009). However no significant difference was

found between the number of viable neurons in the dentate gyrus (t[14], = -1.612, p <

.063) of SHAM and ISC animals.

Bregma –4.5 mm

The number of viable neurons in the HPC of the SHAM and ISC rats was

significantly different. Rats in the ISC had significantly fewer viable neurons relative to

SHAM animals in the CA1 (t [14], = -4.909, p< .000), CA2 (t [14], = -2.978, p < .005),

CA3 (t [14], = -2.901, p < .006) and D.G. (t [14], -2.009 = p < .032).

Bregma –5.8

Analysis of the mean number of viable neurons revealed that ISC rats had

significantly less neurons than SHAM animals in the CA1 (t [14], = -4.211, p < .000),

CA2 (t [14], = -7.470, p < .000), and CA3 cell fields (t [14], = -2.701, p < .008) but not in the D.G. (t [14], = .016, p < .493).

Correlational analyses were performed in order to determine whether a relationship existed between the extent of cell-loss in the ISC rats and their exploration ratios. These analyses revealed no correlation between the mean number of viable neurons for any of the cell fields and exploration ratios on any of the versions of the task, whether the analysis used the mean number of the viable neurons in the cell-fields at each coronal section, or the mean number of viable neurons in each of the cell fields at all planes of section combined.
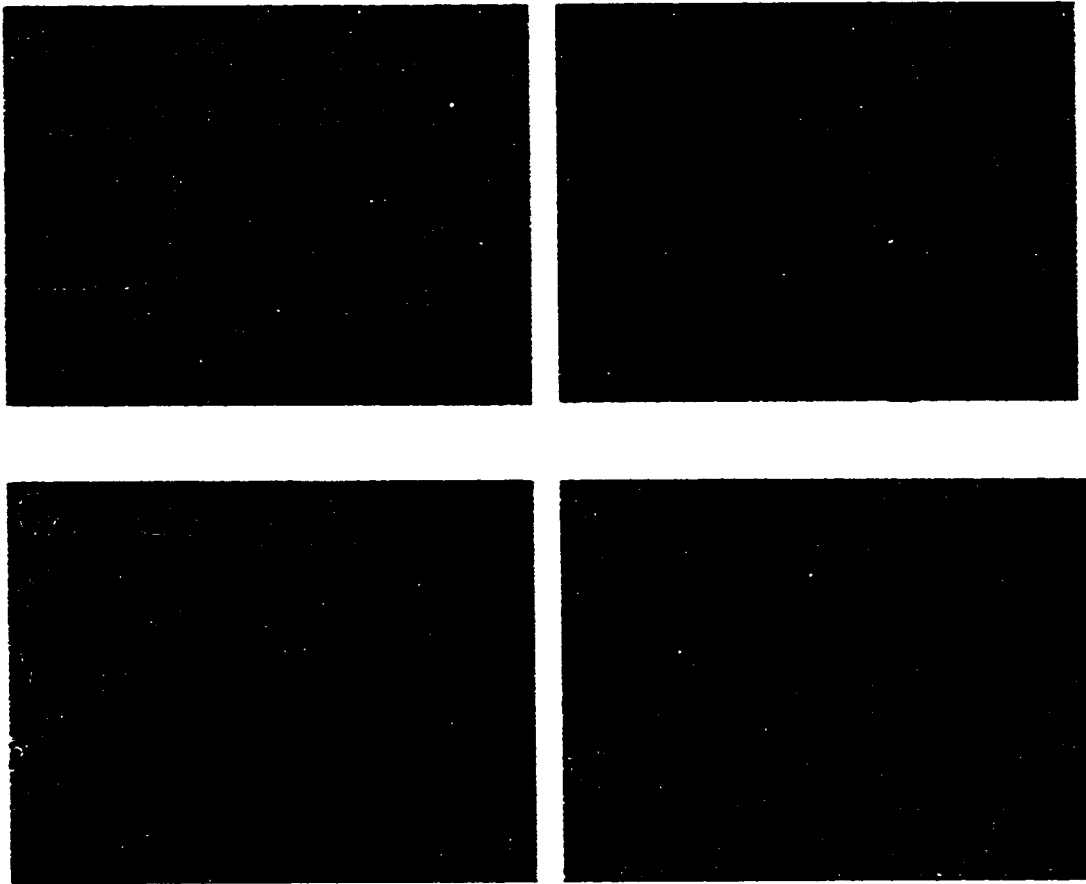
Figure 2. Top panels: Photomicrographs of the hippocampal formation from

(A) a rat that received sham-ischemia, illustrating the normal cell density and

distribution, and (B) a rat that received ischemia, illustrating the degeneration

of CA 1 pyramidal neurons. Bottom panels: photomicrographs of part of the

CA1 cell-fields from (C) a rat that received sham-ischemia, and (D) a rat that

received ischemia, illustrating the decrease in the number of viable CA1

pyramidal cells following ischemia.

All 3 Coronal Sections Combined
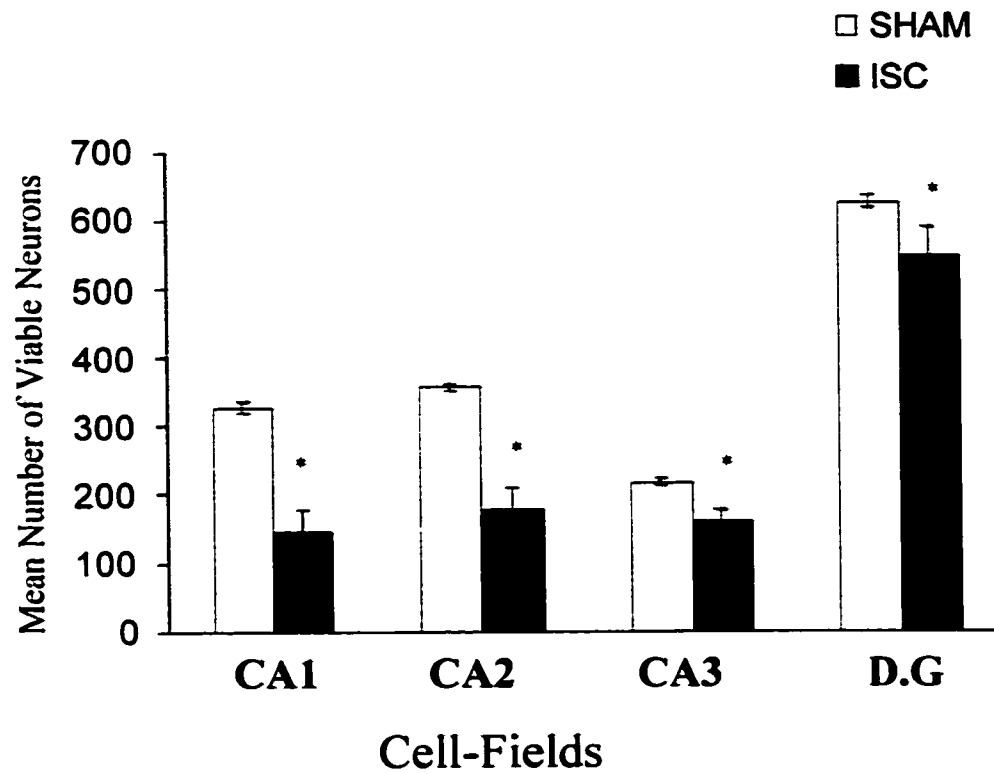
Figure 3. Mean number of viable neurons in each of the cell-fields

(D.G. = dentate-gyrus) across the 3 coronal sections (-2.5 mm, -4.5 mm

and –5.8 mm relative to bregma) combined (Error bars indicate

the standard error of the mean).

* Between group significance (t-tests, p < .05)

A

-2.5 mm

B

-4.5 mm

C

-5.8 mm

Mean Number of Viable Neurons
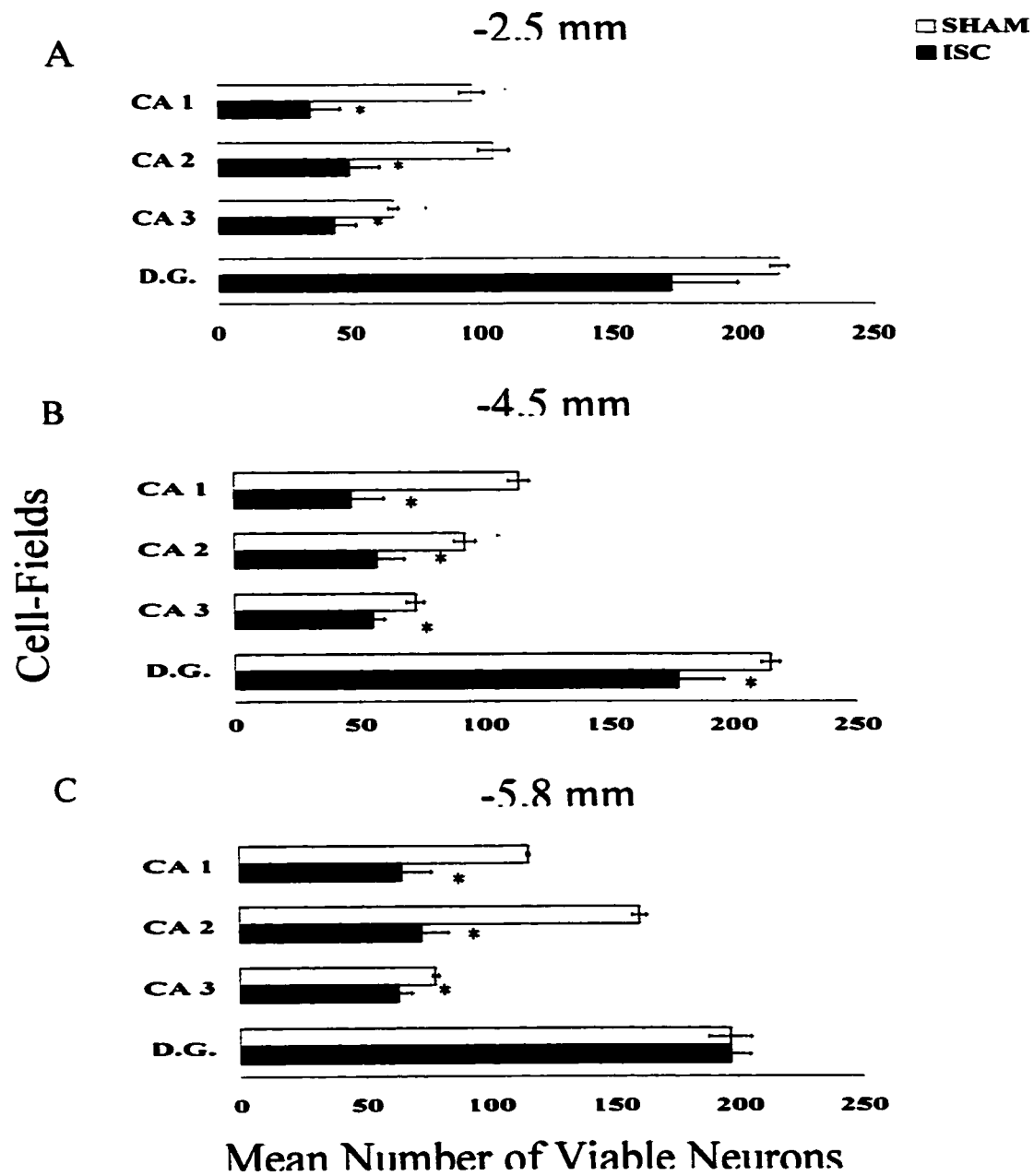
Figure 4. Mean number of viable neurons in each of the cell-fields for each of the 3 coronal sections: -2.5 mm (A), -4.5mm (B) and –5.8 (C) relative to bregma (Error bars indicate the standard error of the mean).

* Between group significance (t-tests, $p < .05$)

Behavioral Results

Figures 5 and 6 show the time spent exploring objects during the familiarization and test phases of each trial type with the 5-minute and the 24-hour retention delay. Time spent exploring the objects in the sample phases during all three trial types did not change as a function of group, as revealed by a non-significant Group x Trial-Type interaction for the 5-minute (F [2, 30] =. 828, p < .447) and 24-hour retention delay (F [2,28] = .683, p <. 578). Independent sample t-tests (one-tail) revealed no significant differences in exploration time between the groups in the Object (t [15] = -657, p < .059), Place (t [15] = -. 629, p < .269) or Context versions (t [15] = -. 329, p < .134) for the 5-minute retention delay. Or for the Object (t[14] = .851, p < .204), Place (t [14] = .868, p < .200) or Context versions (t[14] = -.058, p < .477) for the 24-hour retention delay. Figures 7, 8 and 9 show the mean exploration ratios during the test phases of the Object, Place and Context versions of the task for the 5-minute retention delay. Data are shown for the first, second, and third minutes of the test phase. The data for the first two minutes combined and for the entire three minutes is also shown.

One-sample t-tests (one tail) were performed on the exploration ratios of both groups in order to determine if they were significantly different from chance. With the 5-minute retention interval, exploration ratios based on the first 2-minutes of the retention test were significantly above chance in all three versions types for both the SHAM and ISC groups. With the 24-hour retention interval, SHAM rats showed significantly above-chance exploration ratios in the Object and Place versions of the task. In contrast, rats in

the ISC group did not perform at above-chance levels in the Object (t [6] = .804, p >

.226) or Place (t [7] = .709, p > .250) versions. Neither SHAM (t [7] = -591, p > .286) nor

ISC (t [7] = .057, p > .478) rats displayed significantly above-chance exploration ratios in

the Context version.

SAMPLE PHASES (5-minute retention delay)

□ SHAM
■ ISC
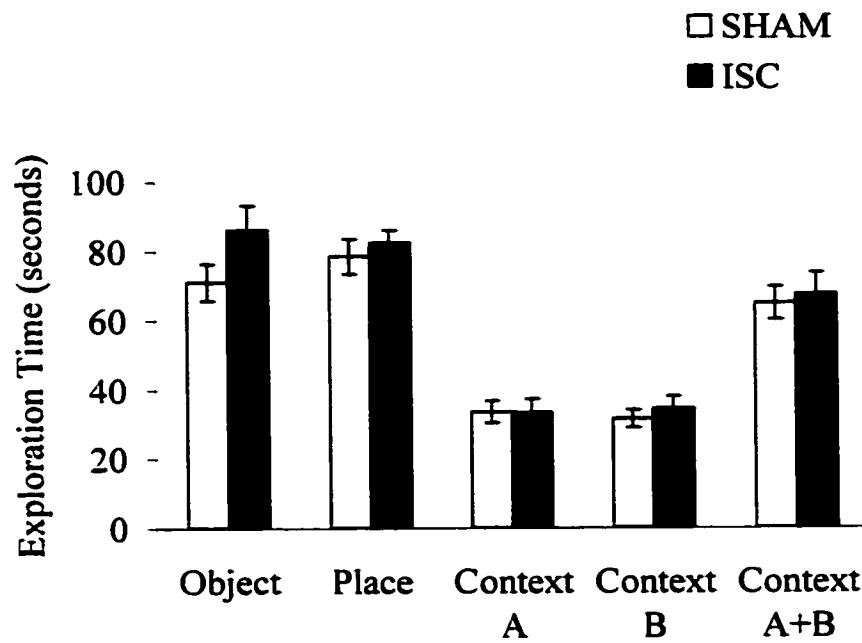
Figure 5. Time spent exploring the objects during the sample

phases of the Object, Place and Context versions of the novelty-

preference task with the 5-minute retention delay. (Errors bars

represent the standard error of the mean

SAMPLE PHASES (24-hour retention delay)
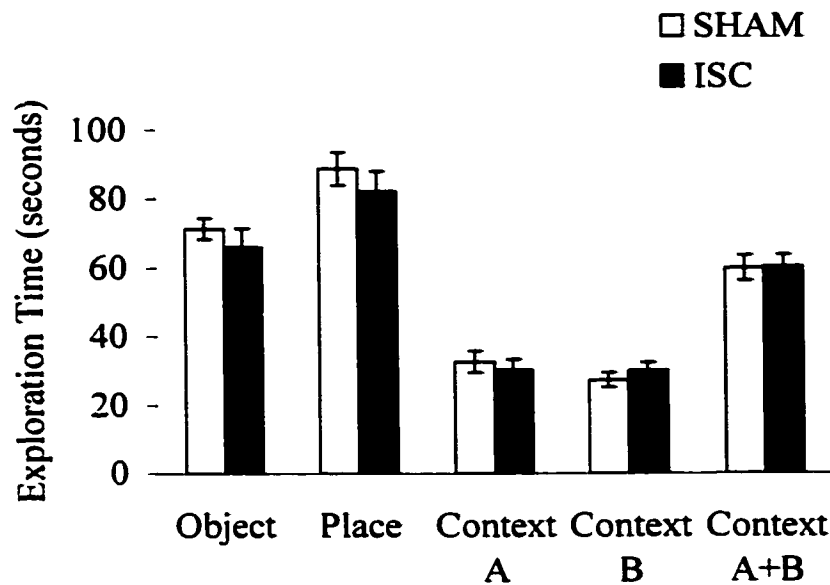
Figure 6. Time spent exploring the objects during the sample phases of the Object, Place and Context versions of the novelty-preference task with the 24-hour retention delay. (Errors bars represent the standard error of the mean).

OBJECT (5-minute retention delay)
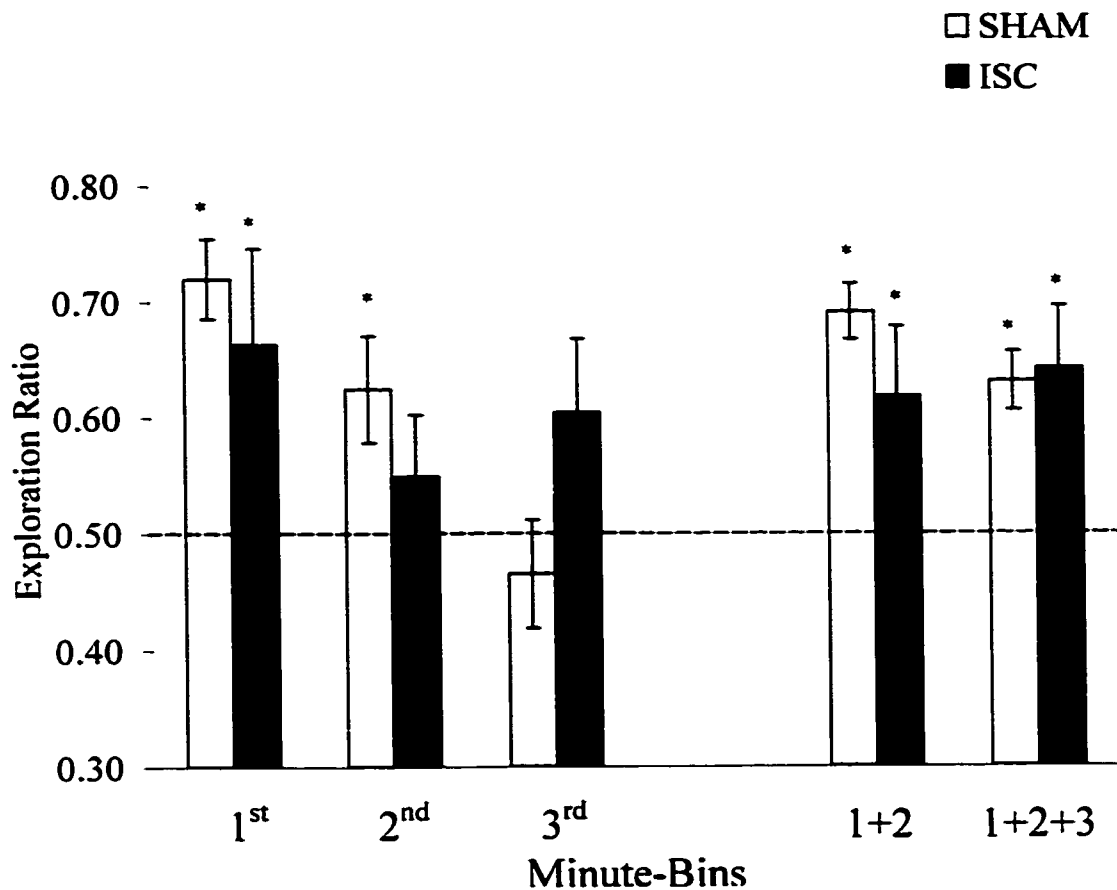
Figure 7. Mean exploration ratio for SHAM and ISC rats in each

of the 3 minutes, first 2 minutes and all 3 minutes combined on the

Object version of the novelty-preference task with a 5-minute

retention delay. (Error bars represent the standard error of the mean)

* Significant difference from chance (t-tests, $p < .05$)

PLACE (5-minute retention delay)
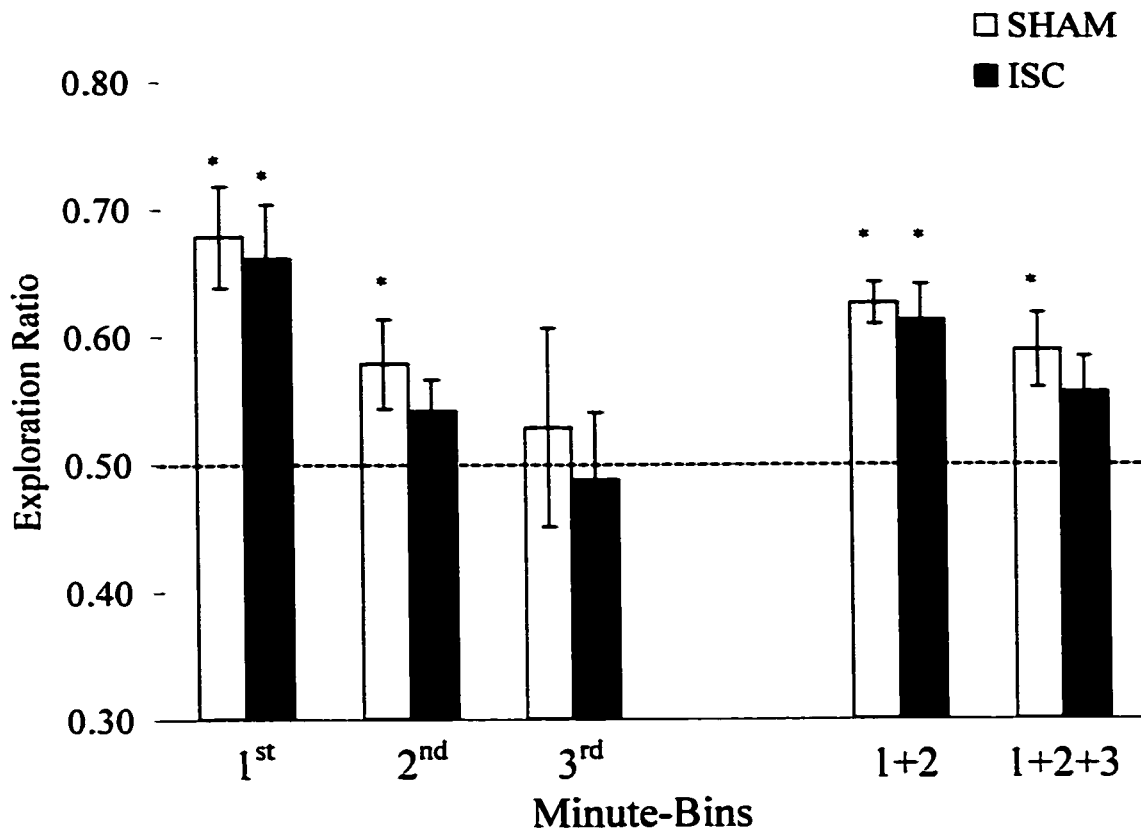
Figure 8. Mean exploration ratio for SHAM and ISC rats in each of the 3

minutes, first 2 minutes and all 3 minutes combined on the Place version of

the novelty-preference paradigm with the 5-minute retention delay (Error bars

represent the standard error of the mean).

* Significant difference from chance (t-tests, p < .05)

CONTEXT (5-minute retention delay)
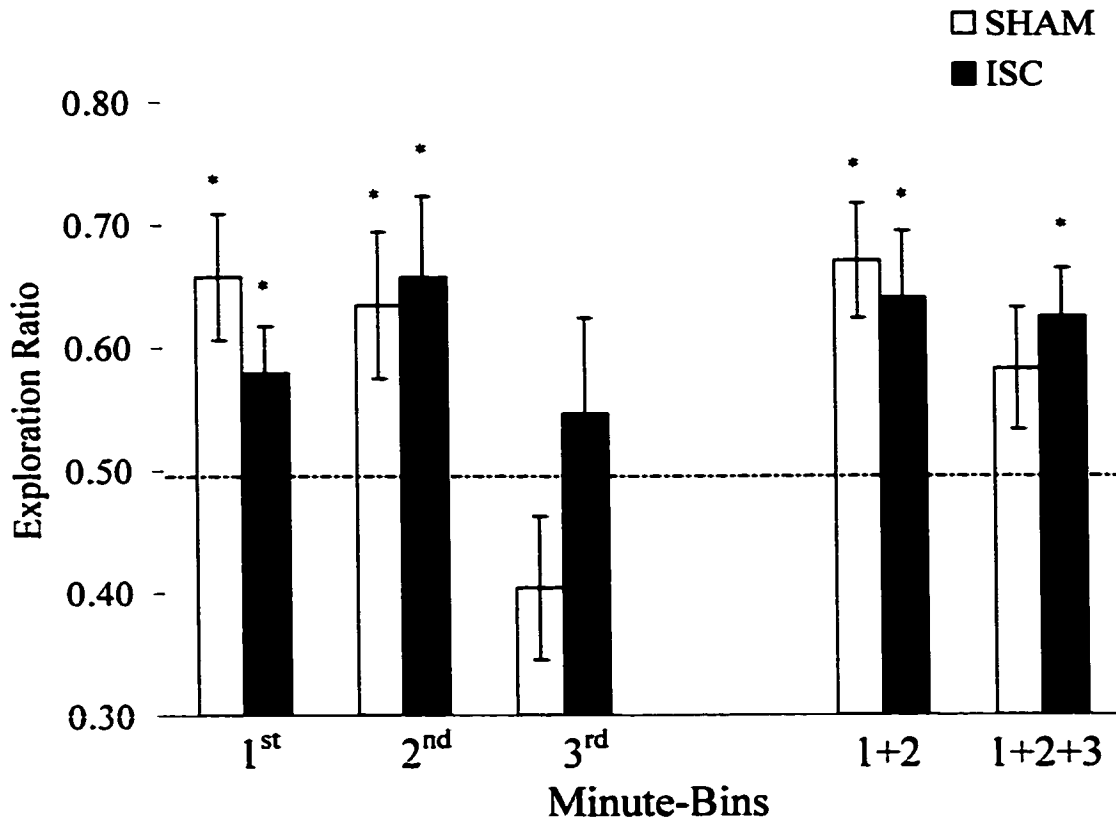
Figure 9. Mean exploration ratio for SHAM and ISC rats in each of the

3 minutes, first 2 minutes and all 3 minutes combined on the Context version

of the novelty-preference task with the 5-minute retention delay (Error bars

represent the standard Error of the mean).

* Significant difference from chance (t-tests, p < .05)

Mixed-design ANOVAs were performed on the ratios for each trial type to compare the exploratory behavior of SHAM animals to that of ISC animals. However, the purpose of these ANOVA's was not to compare memory for objects, places and contexts between the groups. These analyses revealed a significant effect of Delay on the Context version (F [1,14] = 12.426, p < .003), a marginally significant effect of Delay on the Object version (F [1,13] = 4.372, p < .057), and a non-significant effect of Delay on the Place version (F [1,14] = 3.201 p < .095).

Tests of between subject effects revealed no significant effect of group for any of the versions: Object (F [1,13] = 1.571, p < .232), Place (F [1,14] = 3.527 p < .081) or Context (F [1,14] = .104 p < .752). The same pattern of significance was found when all 3 minutes were taken into consideration for the Object (F [1,13] = 4.742, p< .048), Place, (F [1,14] = 1.679, p < .216) and Context (F [1,14] = 4.760, p < .047).

Within-subjects ANOVAs with repeated measures on Minute-Bins performed on the minute-by-minute data for each group at both retention delays revealed a significant linear trend for the SHAM animals on the Object (F [1,7] = 19.264 p < .003) and Context versions (F [1,7] = 12.888, p < .008) but not on the Place (F [1,7] = .2821, p < .138) version. For the animals in the ISC condition a significant linear trend was only found on the Place version (F [1,8] = 7.494, p < .026) in the 5-minute condition. In the 24-hour condition a significant linear trend was found for the SHAM animals on the Place [1,7] = 16.386, p < .006) and context versions (F [1,7] = 8.488, p < .004) but not on the Object version (F [1,7] = .247 p < .317). No significant linear trend was observed on any of the versions for the animals in the ISC condition.

Minute-by-minute data revealed that SHAM animals had exploration ratios that were above chance during the first and second minutes in the three types of trials with the 5-minute retention delay. The ISC animals showed significantly above-chance exploration ratios during the first minute of the Object and Place trials and during the second minute of the Context trial. With the 24-hour retention delay (Figures 10, 11and 12) SHAM animals had significantly above-chance exploration ratio during the first, second and third minutes of the Object version of the task and during the first and second minutes of the Place version. Animals in the ISC condition displayed significantly above-chance ratios during the first minutes of the Object and Place versions of the task.

OBJECT (24-hour retention delay)

Figure 10. Mean exploration ratio for SHAM and ISC rats in each of the 3 minutes, first 2 minutes and all 3 minutes combined on the Object version of the novelty-preference task with the 24-hour retention delay (Error bars represent the standard error of the mean).
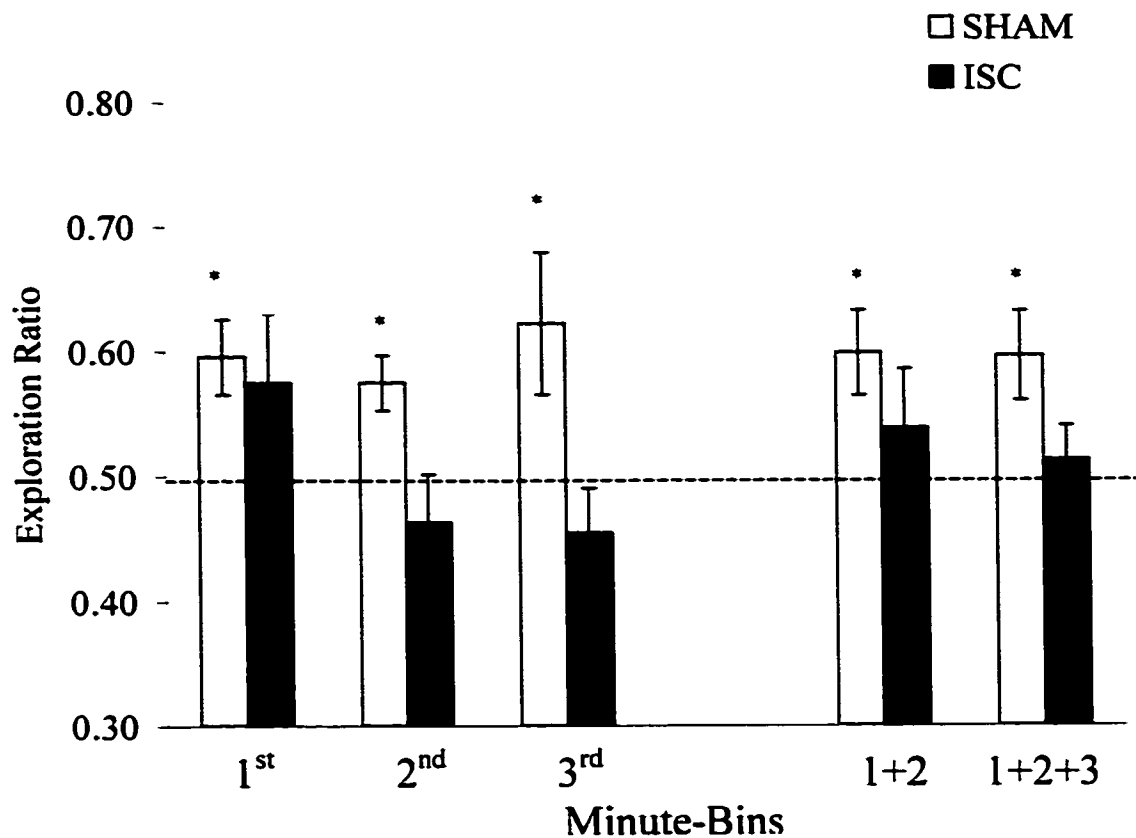
* Significant difference from chance (t-tests, p < .05)

PLACE (24-hour retention delay)
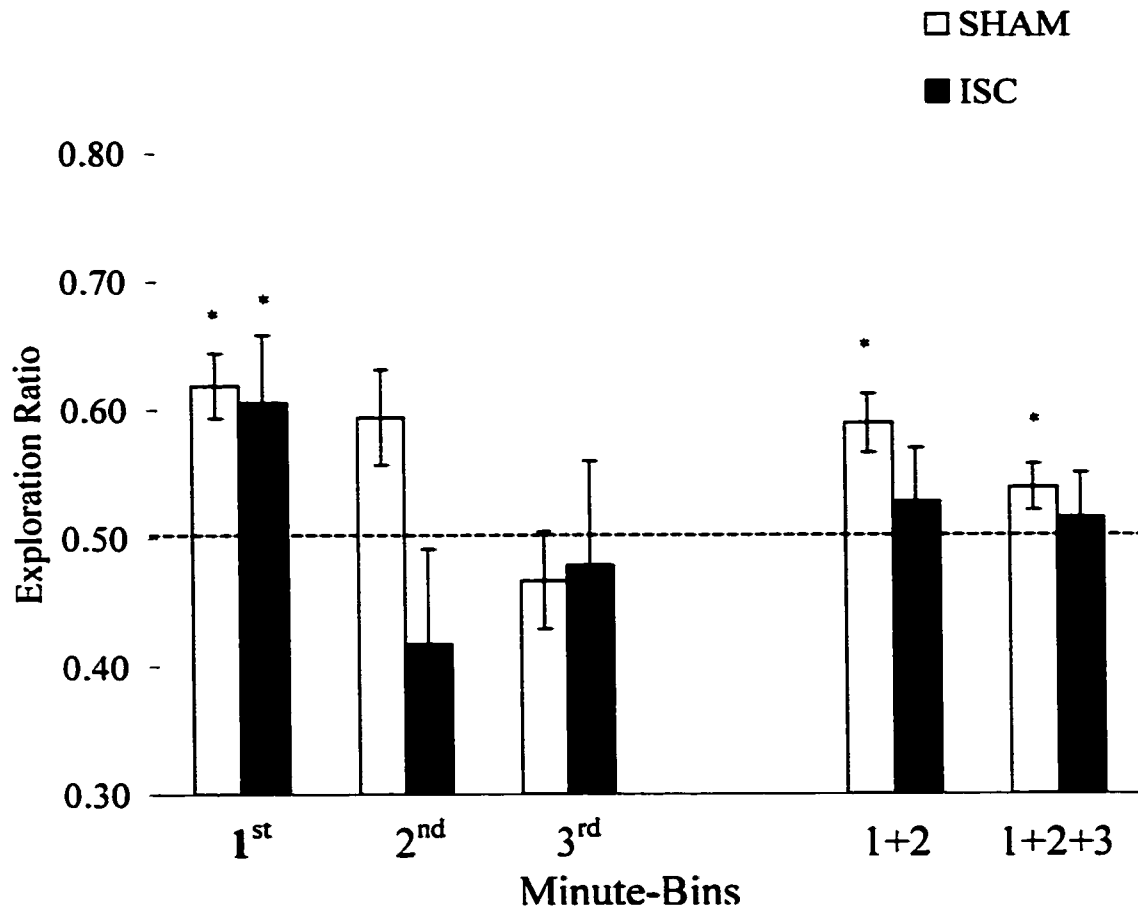
Figure 11. Mean exploration ratio for SHAM and ISC rats in each of the 3 minutes, first 2 minutes and all 3 minutes combined on the Place version of the novelty-preference task with a 24-hour retention delay (Error bars represent the standard error of the mean).

* Significant difference from chance (t-tests, p < .05)

CONTEXT (24-hour retention delay)
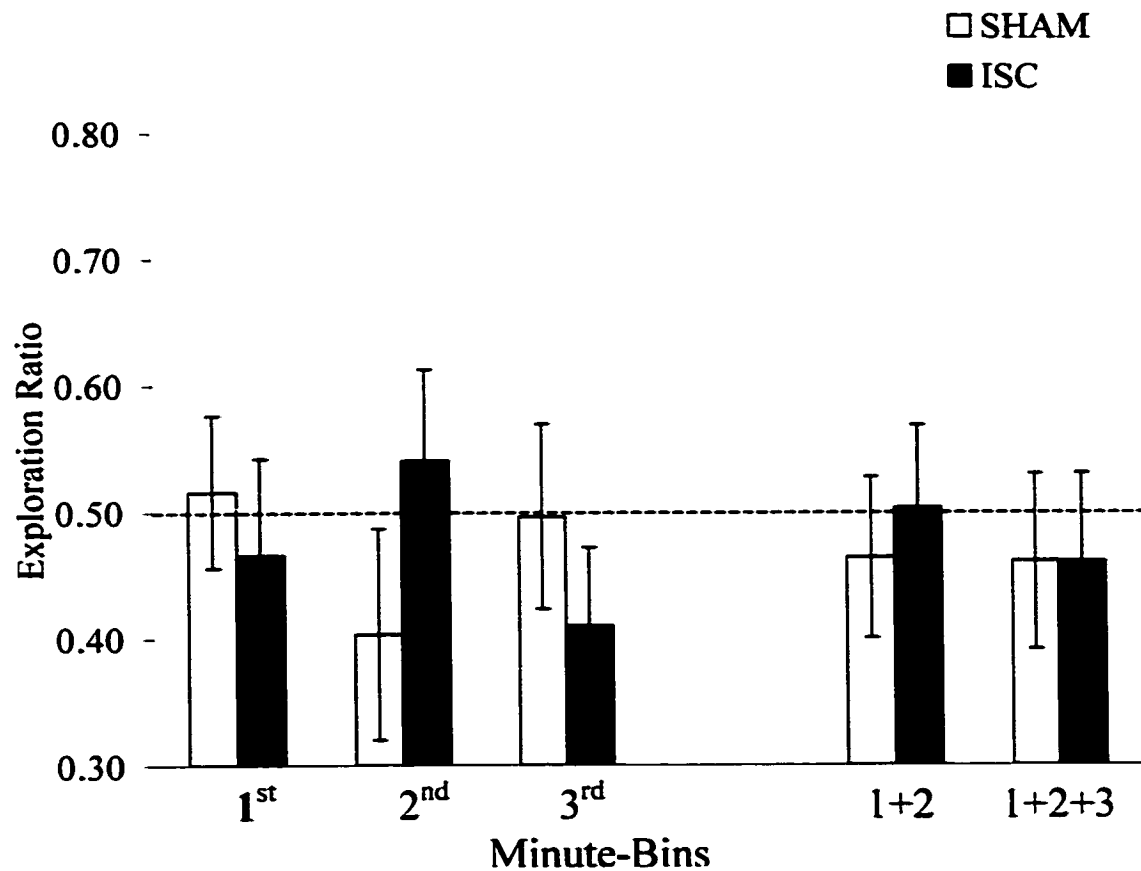
Figure 12. Mean exploration ratio for SHAM and ISC rats in each of the 3

minutes, first 2 minutes and all 3 minutes combined on the Context version

of the novelty-preference task with a 24-hour retention delay (Error bars

represent the standard error of the mean).

Summary

Histological analyses revealed that 10 minutes of ischemia resulted in significant cell-loss in the CA1, CA2 and CA3 neurons of the HPC. These results are consistent with previous studies in which it was demonstrated that the HPC is sensitive to a brief period of ischemia (Banasiak et al., 2000; Corbett & Nurse, 1998; Elibol et al., 2001; Pulsinelli & Brierley, 1979; Pulsinelli et al., 1982).

The main behavioral findings were that 10 minutes of ischemia caused deficits in the Object and Place versions of the novelty-preference task with a 24-hour retention interval. Neither SHAM nor ISC rats discriminated between novel and familiar contexts with the 24-hour interval. Ischemia did not result in deficits on any of the three versions of the task with the 5-minute retention interval.

These results are consistent with previous findings in which ischemia impaired object-recognition deficits (Mumby et al., 1996; Wood et al., 1993) and spatial memory (Davis et al., 1986). Damage to the HPC has also be found to result in contextual-memory tasks (Anagnostaras, Gale, & Fanselow, 2001; Fanselow, 2000; Maren, Ahronov, & Fanselow, 1997). However, the results for the context version with the 24-hour retention delay of the task are inconclusive because SHAM rats did not discriminate between the objects. This suggests that specific object-context associations could not be retrieved after a 24-hour period.

This pattern of results suggest that the memory systems affected by a relatively short period of ischemia are important in remembering objects, and where objects have been seen. The findings that rats in the ISC group discriminated in all three trial types, with a 5-minute retention delay, but were impaired with the 24-hour delay, suggest that

brain regions affected by ischemia may be important for the retention of information for periods of several hours long but not for a period of a few minutes or seconds. Alternative explanations for the observed deficits in ischemic rats, in both the Object and Place versions, include the possibility that the rats suffered visual impairments, or that ischemia affected the rats' propensity to explore novelty. However, these alternative explanations can be ruled out because ISC rats' exploration ratios were no different from SHAM animals with the 5-minute retention delay.

In summary, the effects of ischemia on object-recognition and spatial-memory using the novelty-preference task are consistent with the effects found in other studies in which a brief period of ischemia, sufficient to cause significant cell loss in the HPC, produced deficits on other object-recognition (Mumby et al., 1996; Wood et al., 1993) and spatial-memory (Davis et al., 1986) tasks.

EXPERIMENT 2

INTRODUCTION

The results of Experiment 1 are consistent with the possibility that ischemia-induced deficits in object-recognition are caused by HPC damage. If so, then removing the HPC should also produce such deficits.

Thus, the purpose of this experiment was to assess object-recognition memory in rats with excitotoxic lesions of the HPC (ABL) using the novelty-preference task. Because it has previously been found that HPC-ablation impairs performance on a variety of place-memory (Beracochea, Alaoui-Bouarraqui, & Jaffard, 1989; Gaffan & Harrison,

1989; Sutherland & Rodriguez, 1989) and contextual-memory tasks (Fanselow, 2000; Maren et al., 1997), this experiment focused solely on the Object-recognition version.

METHOD

Subjects

Forty-four Long-Evans rats weighing between 250-300 g were used in this study. All animals were previously tested in a retrograde test of object recognition memory. Rats were housed in plastic shoebox cages under 12-12 h light/dark cycle. Animals had ad-lib access to food and water throughout the experiment. All animals were tested during the light phase of their cycle and testing began 21 days following ischemia.

Surgery

Rats in the ABL (n = 22), were anesthetized under sodium pentobarbital (65 mg/kg) and received intrahippocampal injections of N-methyl-d-aspartate (NMDA) (Sigma, St Louis, MO.) at ten sites bilaterally (See Appendix A for stereotaxic coordinates). The amount injected at each site was .4ul. The flow rate used was .15ul/min over 2.5 minutes. The cannula was then left in place for an additional 2.5 minutes. SHAM (n = 22) rats had the scalp incised and holes were drilled in the skull at the proper coordinates. The cannula was lowered into each site; however, NMDA was not injected. Rats were then removed to a recovery room and received an injection of diazepam (2 –3

mg i.m; Hoffmann-La Roche, Mississauga, Ont.) in order to prevent seizures. The experiment began 2 weeks following surgery.

Procedure

Testing procedures were identical to those used in Experiment 1. The rat was placed in the arena with two identical sample objects. The rat was allowed to explore for 5 minutes. The rat was removed for the retention interval. The objects were then replaced with two new objects. One of the objects was an identical copy of the sample objects and the other was novel. The rat was then returned to the arena and allowed to explore for 5 minutes. All rats were tested with retention delays of 5 minutes and 24 hours.

Rats were habituated to the arenas by allowing them explore them in the presence of two identical objects, which were not used in the study. Rats were placed in each of the arenas on 3 consecutive days. On day 1 rats were placed in the arenas in pairs for a period of 15 minutes. On day two and 3 single rats were permitted to explore the arenas for 15 minutes.

Testing began one week following habituation. For the 5-minute delay, rats received one trial per day, on three successive days.

Testing with the 24-hour retention delay began one week following the last day of testing with the 5-minute delay. Objects used in every trial were randomly determined and were used only for one trial per rat. The experimenter was blind as to the group-identity of each rat. Therefore, whether a SHAM or an ABL rat entered a trial was

randomly determined. Criteria as well as the measure for object exploration was outlined in experiment 1.

One-sample t-tests (one tailed) were used to compare each group mean exploration ratio to what would be expected by chance. A group was considered to discriminate between the novel and sample object if it's mean ratio was significantly above .5. A mixed design ANOVA, with repeated measures on retention delay, was used in order to compare group performance across retention delays. Results

Histological Procedure

The histological procedure used in Experiment 2 is identical to that of Experiment 1, with the exception that the brain sections of 30 um were taken. and visual assessments of the extent of lesions rather than cell counts were performed.

RESULTS AND SUMMARY

Histological Results

Figure 13 shows the smallest and largest lesions for all three sections for the rats in the ABL group. All rats sustained substantial cell loss within the HPC (CA fields, dentate gyrus and subiculum). However, there was some unilateral sparing of the CA1 and CA2 fields of the HPC, as well as the fimbria, in six rats. Three rats had complete unilateral sparing of the dorsal–frontal HPC and two rats had sparing of the fimbria

bilaterally. There was complete unilateral sparing of the ventral HPC in four rats and bilateral sparing of the ventral HPC in three others, in which, sparing of the ventral HPC was complete. One had minor unilateral sparing of the dentate gyrus and CA1 area. The dentate gyrus was spared in one rat unilaterally. Various amounts sparing of the rostral-most part of the HPC was observed in all rats. It is important to note that the amount of HPC-damage was substantially more extensive than that which resulted from ischemia in Experiment 1.
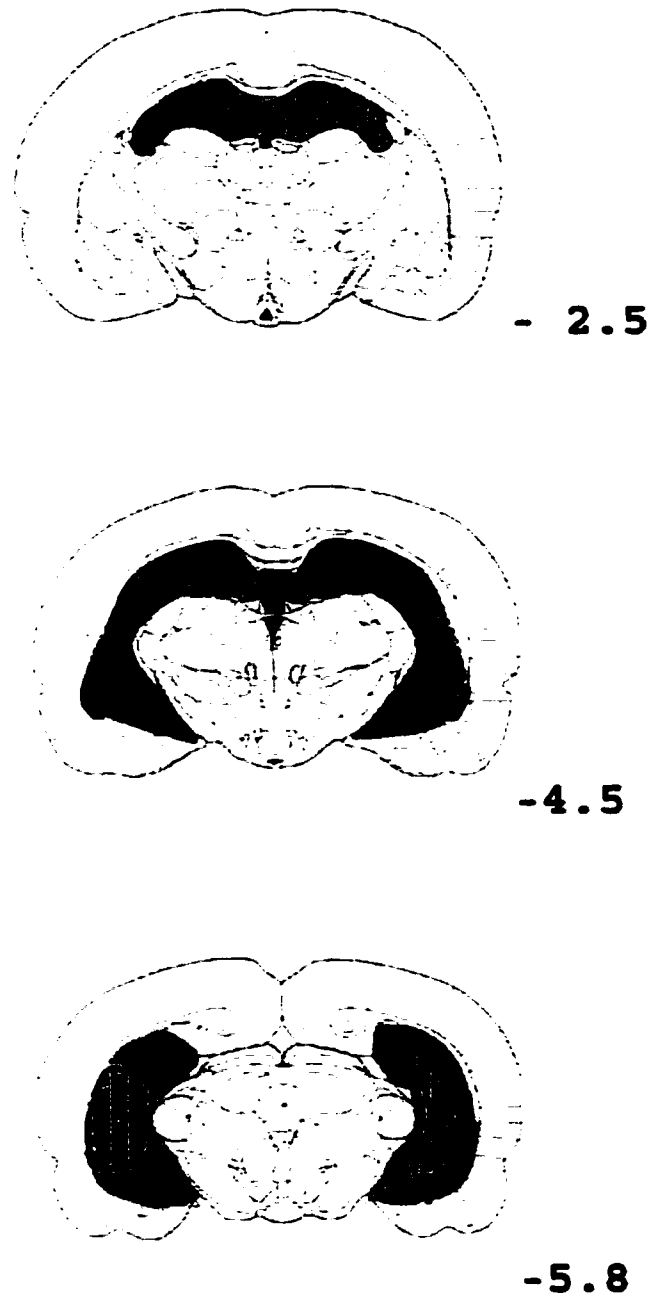
Figure 13. Extent of the smallest (hatched bars) and largest (grey) HPC-lesion.

(Numbers show distance [in mm] from Bregma.)

Behavioral Results

Figures 14 and 15 show the mean amount of time spent exploring the objects during the familiarization phase with the 15-minute and the 24-hour retention delay respectively. There were no significant differences in exploration time between the groups during the familiarization phase of the 15-minute (t[24] = .239 p < .465) and 24-hour (t[43] = .451 p < .298) conditions.

Figures 18 and 19 show the mean exploration ratios during the test phases of the Object, Place and Context versions of the task during the 15-minute and 24-hour retention delays respectively. Data are shown for the first and second minute of the test phases. The data for the first two minutes combined is also shown.

The main dependent measure was the exploration ratio based on the first 2 minutes of the test phase. One-sample t-tests (one tailed) were performed on the exploration ratios of both groups in order to determine if they were significantly different from chance. Exploration ratios based on the first 2 minutes of exploration were significantly above chance for both the SHAM (t [15] = 3.94 p< .0005) and HPL (t [23] = 3.778, p< .0005) animals with the 15-minute retention delay. Exploration ratios were also significantly above chance for the SHAM (t [21] = 4.655 p< .000) and HPL (t [22] = 4.29 p< .000).

Minute by minute data revealed that both SHAM and HPL animals had exploration ratios significantly above chance during the first and second minutes with both the 15-minute and 24 hour retention delay.

A mixed design ANOVA with repeated measures on Retention-Delay revealed no significant effect of Group (F[1,38], = .889, p < .352) or Retention-Delay (F[1,38] = .121, p < .729) and a non-significant Group x Retention-Delay interaction (F[1,38] = .027, p < .871).

SAMPLE PHASE EXPLORATION
(15-minute retention delay)

☐ SHAM
■ ABL
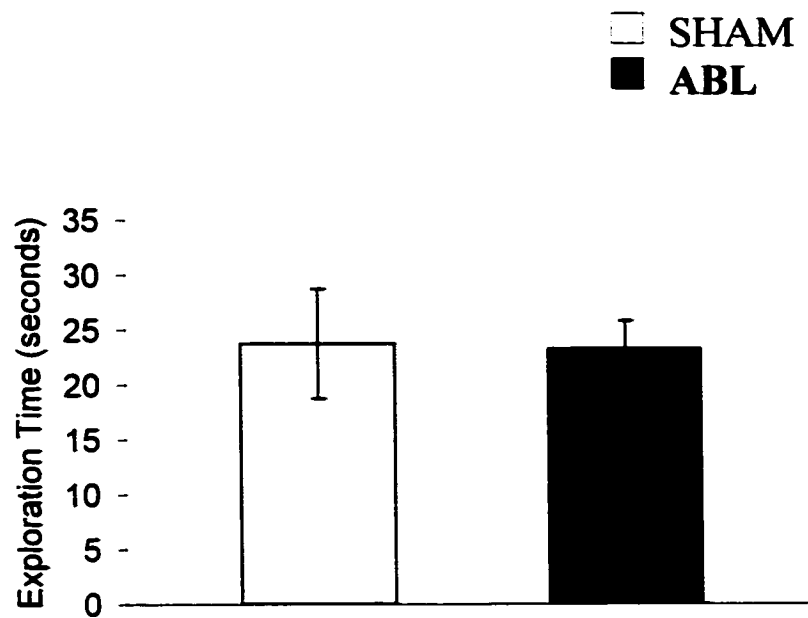
Figure 14 . Total time spent exploring the objects during

the sample phase of the Object version of the novelty-preference

task with the 15-minute retention delay (Error bars represent the

standard error of the mean).

SAMPLE PHASE EXPLORATION
(24-hour retention delay)

☐ SHAM
■ **ABL**
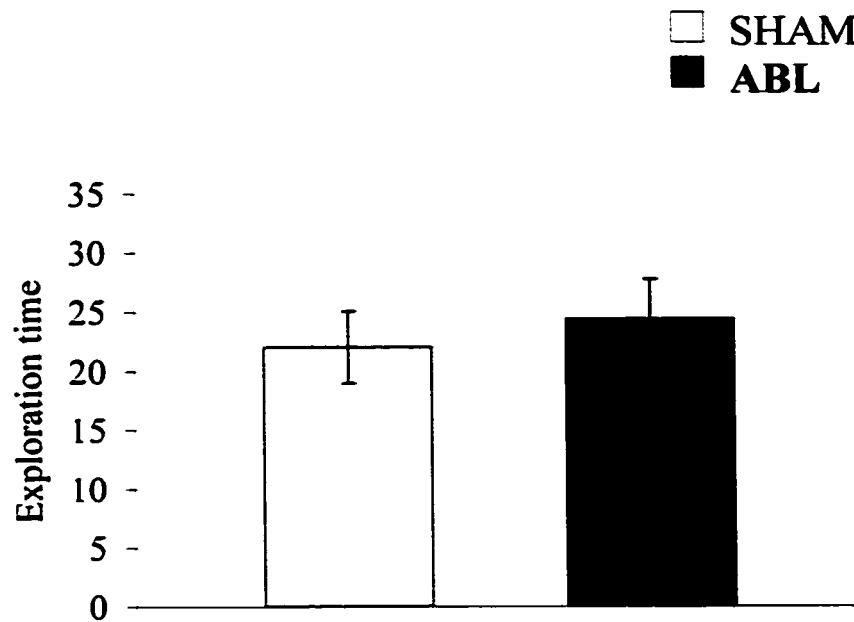
Figure 15. Total time spent exploring the objects during

the sample phase of the Object version of the novely-

preference task with the 24-hour retention delay.

(Error bars represent the standard error of the mean).

OBJECT (15-minute retention delay)
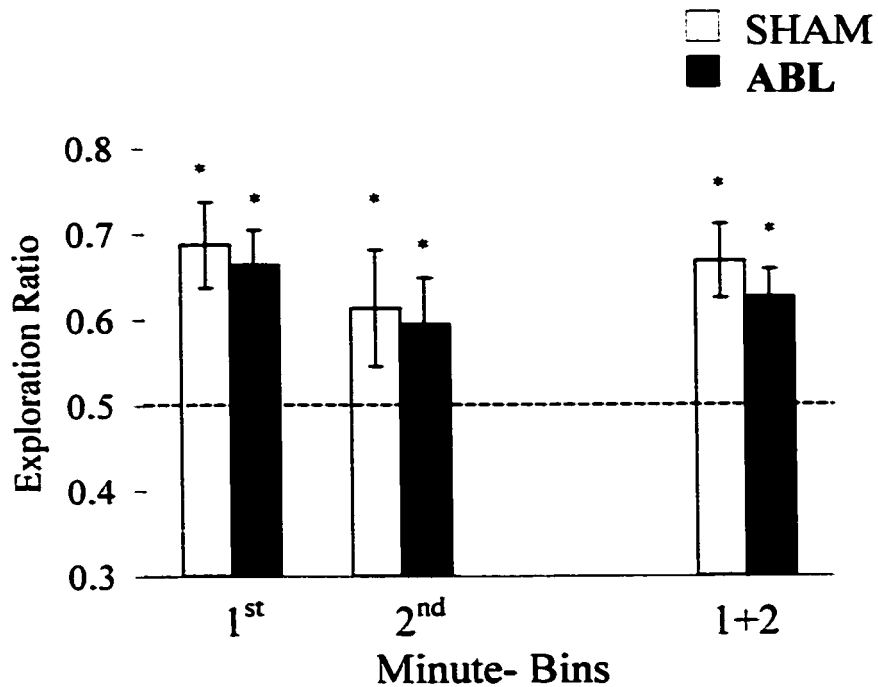
Figure 16. Mean exploration ratios for SHAM and ABL rats

in each of the first 2 minutes and the first 2 minutes combined

onthe Object version of the novelty-preference task with the

15-minute retention delay (Error bars represent the standard

error of the mean).

* Significant difference from chance (t-tests, p < .05)

OBJECT (24-hour retention delay)
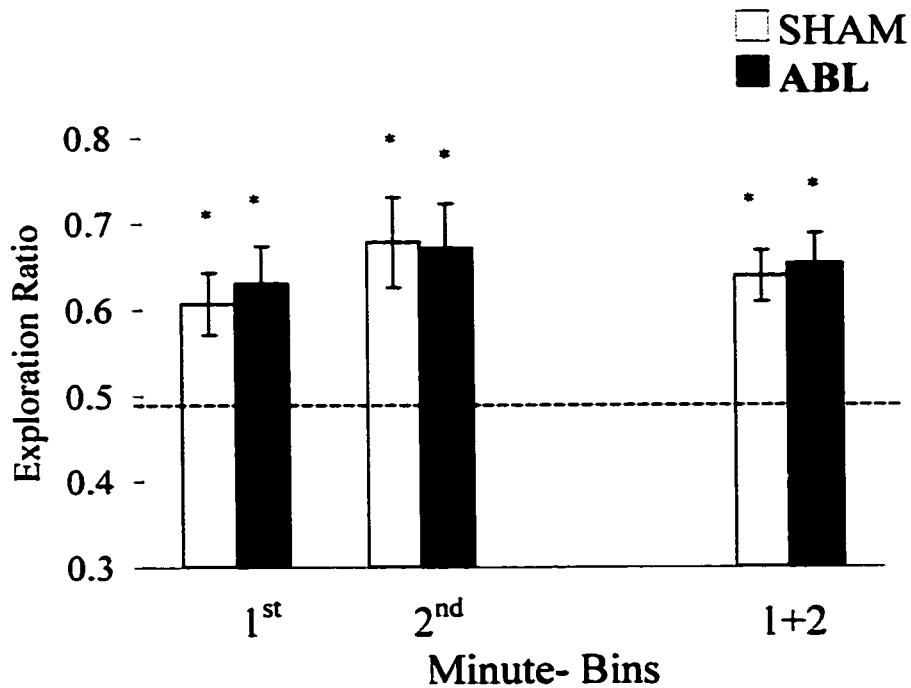
Figure 17. Mean exploration ratios for SHAM and ABL rats in each of the first 2 minutes and the first 2 minutes combined on the Object version of the novelty-preference task with the 24-hour retention delay (Error bars represent the standard error of the mean).

* Significant difference from chance (t-tests, $p < .05$)

Summary

The main findings of Experiment 2 were that both SHAM and HPL rats displayed a preference for the novel object with retention delays of either 5-minutes or 24-hours. These results suggest that discrete lesions of the HPC do not impair object recognition-memory in the novelty-preference task at retention delays of up to 24 hours. These results support the hypothesis that HPC ablation would not result in deficits on the object version of the novelty-preference task, and are consistent with most studies in which object-recognition memory was spared in rats with HPC lesions (Aggleton, Hunt, & Rawlins, 1986; Cassaday & Rawlins, 1997; Kesner et al., 1993; Mumby, Gaskin et al., 2002). Thus, these results support the notion that the HPC does not play an essential role in object-recognition memory. An alternative explanation for these results may have been the large variability of the extent of the lesions within the animals. However, the extent of HPC-damage was too large for HPC function not to have been severely compromised. In addition, the same animals were significantly impaired on a test of retrograde object-recognition memory in a previous study (Gaskin et al., 2001, Society for Neuroscience Abstracts)

There are two studies in which rats with lesions of the HPC showed evidence of object-recognition impairments, one using the DNMS task (Clark, West, Zola, & Squire, 2001) and the other using the novelty preference paradigm (Clark et al., 2000). However, the results of both of these studies may lead to misinterpretation. In the first, some of the rats with HPC-ablation sustained damage to the perirhinal cortex. This fact constitutes a confound in that damage to this area alone is well known to cause object-recognition

memory deficits on the DNMS task (Mumby & Pinel, 1994). In the second, rats with ablations of the HPC were impaired at retention delays of 10 minutes and 1 hour but were not impaired with a 24-hour delay.

GENERAL DISCUSSION

The findings of Experiment 2 provide evidence that the object-recognition memory deficits found in the ischemic rats in Experiment 1 were not due to the damage observed in the HPC. If these deficits were due to HPC damage then complete ablation should have resulted in object-recognition memory deficits in Experiment 2. Taken together, the findings from both experiments suggest that alternative explanations must be sought in order to explain the object-recognition observed after ischemia, and perhaps some of the spatial-memory deficits as well.

Combined with the findings of Mumby et al. (2002) in which rats were impaired on the Place and Context version of the novelty-preference task, the present findings support the notion that the HPC is necessary to process information about the relationship among stimuli that constitute an event (Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila, 1999). For example, in both the Place and Context versions, the rat must encode the relationship between the objects and environmental cues in order to detect a change in the position of one of the objects in the Place version and of the context in the Context version. However, the HPC may not be necessary for processing elemental stimuli that are part of an event. For example, in the Object version of this study rats may have been

said to be familiar with an object but showed no evidence of "knowing" where or in what context it was seen as displayed by the deficits on the place and context versions.

Although studies that demonstrate object-recognition memory deficits following ischemia report damage restricted to the HPC, closer examination of the histological results often show damage to the thalamus, perirhinal and entorhinal cortices (Clark et al., 2000; Zola-Morgan et al., 1992). However, relative to damage to the HPC, damage to these areas is often discarded as being irrelevant to the observed object-recognition memory deficits. This hippocampocentric view of memory impairments following ischemia is erroneous in the light that damage to the three structures mention above can lead to such deficits.

Because HPC-ablation did not result in any deficits on the Object version of the novelty–preference task in Experiment 2, the notion that HPC damage alone can cause object-recognition memory deficits following ischemia is untenable. A more plausible explanation is that the observed deficits, on the Object version of the novelty-preference task following ischemia are due to extra-hippocampal damage.

Experiments that investigate the mnemonic effects of ischemia typically resort to cell counts in order to determine the extent of brain damage produced by the ischemia. However, this method can only assess structural aspects of a neuron. This poses an analytical problem, as neurons may seem to be intact yet actually be functionally abnormal. Evidence for this phenomenon is reported by Jasper (1990). In that study, rats that sustained 20 minutes of ischemia were impaired on a spatial-memory task while displaying no observable neuropathology. Thus, assessing whether cells are functional may be more useful in attempting to explain the memory deficits following ischemia,

than merely the assessment of their structural integrity. These considerations may also suggest that positive correlations may sometimes be found between the extent of damage to the HPC and object-recognition memory deficits following ischemia, because both variables are correlated with undetected brain damage in other brain areas.

Mumby et al. (1996) proposed that post-ischemic seizures originating in the HPC might cause damage in areas in which the HPC has efferent connections, and which themselves are critical for normal object-recognition memory. Support for this notion comes from a study in which ablation of the HPC shortly after ischemia attenuated ischemia-induced DNMS deficits. Although, no observable seizures occurred in the rats in the present study, it is plausible that covert seizures in the HPC resulted in this scenario. Furthermore, preliminary evidence from this laboratory suggests that 10 minutes of ischemia can disrupt electrical activity in the perirhinal cortex and thalamus.

Another interesting finding conveyed by the results of Experiment 1 is that ischemia resulted in object-recognition impairments with the 24-hour but not with the 5-minute delay. This result may signify that the brain areas damaged in ischemia are important for long-term memory but play a less important role for short-term memory. The question of whether short-term and long-term memories are subserved by two distinct systems was raised by McGaugh (1966). Support for this idea may be shown by an experiment in which long-term and short-term memory are differentially affected in the same animal. The findings of Experiment 1 provide such a demonstration. A more parsimonious explanation would be that the brain damage caused by the ischemia either prevented the storage of information for an extended time-period or prevented the recognition or recall of more remote information.

Although HPC-ablation is known to cause spatial (Nadel, 1991) and contextual-memory (Anagnostaras et al., 2001; Fanselow, 2000; Maren et al., 1997) impairments, the deficits displayed by ischemic rats in the place and context versions of the novelty-preference task may not have been entirely due to HPC-damage. Histological analyses revealed relative sparing of the CA3 and dentate-gyrus cell fields of most animals. Additionally, brain regions in the mid-diencephalon, which are affected ischemia (Kato, Araki, Itoyama, & Kogure, 1995; Northington, Ferriero, & Martin, 2001), have also been shown to impair spatial-memory (Sziklas & Petrides, 2000). .0

The results of this study further support the view that ischemia induced object-recognition memory deficits are not due to HPC-damage, and cast further doubt on the notion that object-recognition memory is dependent on an intact HPC. Several researchers have provided support for this idea (Aggleton et al., 1986; Cassaday & Rawlins, 1997; Kesner et al., 1993; Mumby, Gaskin et al., 2002; Mumby et al., 1996; Rothblat & Kromer, 1991) . However, none of them have showed both the effects of ischemia and HPC-ablation in the same testing conditions and within the same study, or with retention delays of more than a few minutes. For the first time, it was demonstrated that ischemia can cause object-recognition memory deficits whereas HPC-ablation does not, within the same study, using the same apparatus and the same procedure. A major shortcoming to this study is that the histological analysis was limited to cell counts within the HPC. Therefore, the only conclusion that could be drawn from the results of the present study is that it is unlikely that HPC damage is responsible for the object-recognition memory deficits observed in ischemic rats. Further studies investigating the effects of ischemia on object-recognition memory should therefore focus on using

histological techniques that permit the assessment of the functionality rather than the

viability of neurons. The results of the present study suggest that detection of ischemia-

induced neuropathology in extrahippocampal brain areas may ultimately explain some of

the memory impairments following ischemia.

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APPENDIX A

Stereotaxic coordinates used to make NMDA lesions of the hippocampal formation

Table 1

Stereotaxic CoordinatesUsed to make NMDA Lesions of the Hippocampal Formation. All Values are Listed as the Number of Millimetres relative to Bregma.

Injection Site	Anteroposterior	Mediolateral	Dorsoventricular
1	-3.1	+/-1.0	-3.6
2	-3.1	+/-2.0	-3.6
3	-4.1	+/-2.0	-4.0
4	-4.1	+/-3.5	-4.0
5	-5.0	+/-3.0	-4.1
6	-5.0	+/-5.2	-5.0
7	-5.0	+/-5.2	-7.3
8	-5.8	+/-4.4	-4.4
9	-5.8	+/-5.1	-6.2
10	-5.8	+/-5.1	-7.5

APPENDIX B

Analysis of Variance Source Tables

Table 1

Analysis Of Variance For The Total Time Spent Exploring Objects During The Sample Phases Of The Object, Place And Context Versions Of The Novelty-Preference Task With The 5-Minute Retention Delay .

Source	SS	df	MS	F	P
Between Subjects					
Group	645.33	1	645.33	2.16	.162
Error	4473.17	15	298.21		
Within Subjects					
Task	2034.63	2	1017.31	4.33	.02
Group x Task	388.65		194.32	.82	.44
Error	7043.16	30	234.77		

Table 2

Analysis Of Variance For The Total Time Spent Exploring Objects During The Sample
Phases Of The Object, Place And Context Versions Of The Novelty-Preference Task
With The 24-Hour Retention Delay .

Source	SS	df	MS	F	P
Between Subjects					
Group	34.42	1	34.42	.14	.712
Error	3387.24	14	241.94		
Within Subjects					
Task	6992.62	2	3496.31	32.99	.00
Group x Task	81.82		40.91	.38	.683
Error	2967.32	28	105.97		

Table 3

Analysis Of Variance For The Change In Exploration Ratios From The 5-Minute
Retention Delay To The 24-Hour Retention Delay For The Object Version Of The
Novelty Preference Task (First 2 Minutes Combined).

Source	SS	df	MS	F	P
Between Subjects					
Group	.017	1	.017	1.571	.232
Error	.141	13	.010		
Within Subjects					
Delay	.082	1	.082	4.372	.057
Group x Delay	.001	1	.001	.060	.810
Error	.244	13	.018		

Table 4

Analysis Of Variance For The Change In Exploration Ratios From The 5-Minute

Retention Delay To The 24-Hour Retention Delay For The Place Version Of The Novelty

Preference Task. (First 2 Minutes Combined)

Source	SS	df	MS	F	P
Between Subjects					
Group	.009	1	.009	3.527	.081
Error	.036	14	.002		
Within Subjects					
Delay	.038	1	.038	3.201	.095
Group x Delay	.006	1	.006	.573	.462
Error	.148	14	.010		

Table 5

Analysis Of Variance For The Change In Exploration Ratios From The 5-Minute
Retention Delay To The 24-Hour Retention Delay For The Context Version Of The
Novelty Preference Task. (First 2 Minutes Combined)

Source	SS	df	MS	F	P
Between Subjects					
Group	.003	1	.003	.104	.752
Error	.432	14	.003		
Within Subjects					
Delay	.278	1	.278	12.426	.003
Group x Delay	.003	1	.003	.162	.694
Error	.313	14	.022		

Table 6

Analysis Of Variance For The Change In Exploration Ratios From The 5-Minute
Retention Delay To The 24-Hour Retention Delay For The Object Version Of The
Novelty Preference Task. (All 3 Minutes)

Source	SS	df	MS	F	P
Between Subjects					
Group	.007	1	645.33	.594	.454
Error	4473.17	13	1.305		
Within Subjects					
Delay	0.057	1	.057	4.74	.04
Group x Delay	.021	1	.021	1.79	.20
Error	.15	13	.12		

Table 7

Analysis Of Variance For The Change In Exploration Ratios From The 5-Minute
Retention Delay To The 24-Hour Retention Delay For The Place Version Of The Novelty
Preference Task. (All 3 Minutes)

Source	SS	df	MS	F	P
Between Subjects					
Group	.007	1	.007	1.709	.212
Error	.058	14	.004		
Within Subjects					
Delay	0.01	1	.01	1.679	.216
Group x Delay	.000	1	.000	.022	.884
Error	.128	14	.009		

Table 8

Analysis Of Variance For The Change In Exploration Ratios From The 5-Minute
Retention Delay To The 24-Hour Retention Delay For The Context Version Of The
Novelty Preference Task. (All 3 Minutes)

Source	SS	df	MS	F	P
Between Subjects					
Group	9.074	1	9.0744	12.061	.000
Error	.486	14	.034		
Within Subjects					
Delay	.165	1	.165	4.760	.047
Group x Delay	.004	1	.004	.117	.738
Error	.486	14	.034		

Table 9

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes of Exploration During The Test Phase of the Object Version of the Novelty-Preference Task For SHAM-Rats, Including Analysis Of The Linear Trend For the 5-Minute Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.271	2	.136	8.934	.003
Error	.213	14	.015		
Linear	.265	1	.265	19.264	.003
Error	.096	7	.013		

Table 10

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes of

Exploration During The Test Phase of the Place Version of the Novelty-Preference Task

For SHAM-Rats, Including Analysis Of The Linear Trend For the 5-Minute Retention

Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.090	2	.045	1.962	.177
Error	.322	14	.023		
Linear	.087	1	.087	2.821	.137
Error	.216	7	.030		

Table 11

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes of Exploration During The Test Phase of the Context Version of the Novelty-Preference Task For SHAM-Rats, Including Analysis Of The Linear Trend For the 5-Minute Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.312	2	.156	8.488	.004
Error	.257	14	.018		
Linear	.253	1	.253	12.888	.009
Error	.137	7	.017		

Table 12

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes of

Exploration During The Test Phase of the Object Version of the Novelty-Preference

Task For ISC-Rats, Including Analysis Of The Linear Trend For the 5-Minute Retention

Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.056	2	.028	1.283	.304
Error	.354	16	.022		
Linear	.015	1	.015	.656	.442
Error	.190	8	.023		

Table 13

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes Of Exploration During The Test Phase of the Place Version of the Novelty-Preference Task For ISC-Rats, Including Analysis Of The Linear Trend For the 5-Minute Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.140	2	.069	4.736	.024
Error	.236	16	.014		
Linear	.133	1	.133	7.494	.026
Error	.142	8	.078		

Table 14

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes of Exploration During The Test Phase of the Context Version of the Novelty-Preference Task For ISC-Rats, Including Analysis Of The Linear Trend For the 5-Minute Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.057	2	.028	.912	.421
Error	.502	16	.031		
Linear	.005	1	.005	.195	.670
Error	.205	8	.025		

Table 15

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes of Exploration During The Test Phase of the Object Version of the Novelty-Preference Task For SHAM-Rats, Including Analysis Of The Linear Trend For the 24-Hour Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.009	2	.004	.461	.640
Error	.138	14	.009		
Linear	.003	1	.003	.247	.635
Error	.085	7	.012		

Table 16

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes Of Exploration During The Test Phase of the Place Version of the Novelty-Preference Task For SHAM-Rats, Including Analysis Of The Linear Trend For the 24-Hour Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.108	2	.053	6.269	.011
Error	.120	14	.008		
Linear	.094	1	.094	16.386	.005
Error	.040	7	.005		

Table 17

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes of Exploration During The Test Phase of the Context Version of the Novelty-Preference Task For SHAM-Rats, Including Analysis Of The Linear Trend For the 24-Hour Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.312	2	.156	8.488	.004
Error	.257	14	.018		
Linear	.253	1	.253	12.888	.009
Error	.137	7	.019		

Table 18

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes of
Exploration During The Test Phase of the Object Version of the Novelty-Preference Task
For ISC-Rats, Including Analysis Of The Linear Trend For the 24-Hour Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.061	2	.030	2.338	.139
Error	.159	12	.013		
Linear	.049	1	.049	2.453	.168
Error	.120	6	.020		

Table 19

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes Of Exploration During The Test Phase of the Place Version of the Novelty-Preference Task For ISC- Rats, Including Analysis Of The Linear Trend For the 24-Hour Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.184	2	.092	3.078	.078
Error	.419	14	.29		
Linear	.070	1	.070	3.336	.111
Error	.147	7	.021		

Table 20

Analysis Of Variance For The Change In Exploration Ratios Across The 3 Minutes of Exploration During The Test Phase of the Context Version of the Novelty-Preference Task For ISC-Rats, Including Analysis Of The Linear Trend For the 24-Hour Retention Delay.

Source	SS	df	MS	F	P
Within Subjects					
Minute-Bins	.057	2	.028	.809	.465
Error	.494	14	.035		
Linear	.001	1	.001	.063	.809
Error	.200	7	.028		

Table 21

Analysis Of Variance For The Change In Exploration Ratios, For The SHAM and HPL

rats, From The 15-Minute Retention Delay To The 24-Hour Retention Delay For The

Object Version Of The Novelty Preference Task (First 2 Minutes Combined).

Source	SS	df	MS	F	P
Between Subjects					
Group	.025	1	.025	.889	.176
Error	1.109	38	.029		
Within Subjects					
Delay	.002	1	.002	.121	.729
Group x Delay	.000	1	.000	.027	.871
Error	.802	38	.021		