OBJECT AND SPATIAL RECOGNITION

IN THE ISCHEMIC GERBIL

by

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ABSTRACT

The Mongolian gerbil is extensively used to model transient cerebral ischemia, a type of stroke that can occur with anoxia and cardiac arrest. A global ischemic insult in the gerbil produces damage to hippocampal CA1 pyramidal cells comparable to that observed in humans. A limited number of models are available to evaluate the behavioral consequences of cerebral ischemia in the gerbil. The goal of the present experiments was to evaluate the impact of transient cerebral ischemia on object and spatial recognition memory as these tasks have not been previously utilized with the gerbil model. Following ischemic insult (5-min bilateral carotid occlusion) or sham procedure, gerbils were tested in a familiar environment with novel objects. A familiarization phase followed by separate test phases for presentations of a novel object or object location were conducted. Exploratory behavior for the novel object or object location was evaluated using an automated tracking system. Results indicated that both ischemic and sham subjects were able to recognize the novel object when placed in the environment. However, when confronted with a familiar object, placed in a novel location, neither group exhibited a significant increase in exploratory behavior. A second experiment was conducted to further investigate the spatial recognition task. Subjects were habituated to the apparatus in addition to the experimental objects. Under this experimental condition, both groups exhibited significant exploratory behavior for the object placed in the novel location. The ischemic and control groups differed from each other during habituation with ischemic subject showing significantly higher activity levels. It is possible that differences between the groups remain but that these recognition findings are a result of extended habituation to the experimental objects. Further investigation of this matter is needed to determine the effect of prior object exposure on exploratory behavior in the spatial recognition task.

INTRODUCTION

Each year 795,000 individuals in the United States will suffer a stroke. Over six hundred thousand of these are new cases while the remaining 185,000 represent recurrences of stroke. Worldwide 15 million individuals will suffer a stroke each year (Stroke Center, University of Washington in St. Louis, 2009). A vast majority of stroke victims (85%) will suffer an ischemic stroke; ischemia occurs when a blood vessel is constricted or occluded decreasing blood flow to the brain (American Stroke Association, 2009). This phenomenon has been deemed "blood starvation" as blood is no longer able to circulate and provide oxygen to the surrounding tissue (Martini, 2003). The remaining 15% of stroke victims will suffer a hemorrhagic stroke which occurs when a blood vessel ruptures and bleeds into the surrounding brain tissue. As blood accumulates, elevated pressure can damage the adjacent tissue (American Stroke Association, 2009).

In order to experimentally study the effects of cerebral ischemia, animal models must be utilized. An *in vivo* model of cerebral ischemia is essential because this type of insult involves a series of highly diverse processes that would be impossible to replicate *in vitro*. Rat and gerbil models have predominantly been used to study transient cerebral ischemia because these rodents have similar vasculature to that of higher species (i.e. cats, dogs and humans). These two species represent fairly homogenous groups of animals thus limiting variability between individual subjects and allowing researchers to maintain statistical power while utilizing relatively small groups of animals (Ginsberg & Busto, 1989).

Models of Transient Cerebral Ischemia

Transient cerebral ischemia causes damage to the hippocampus, a limbic structure that plays a crucial role in learning and memory (Martini, 2003). The hippocampus proper is comprised of three distinct sub regions: the CA1, CA2 and CA3 (van Strien et al., 2009) (see Figure 1).

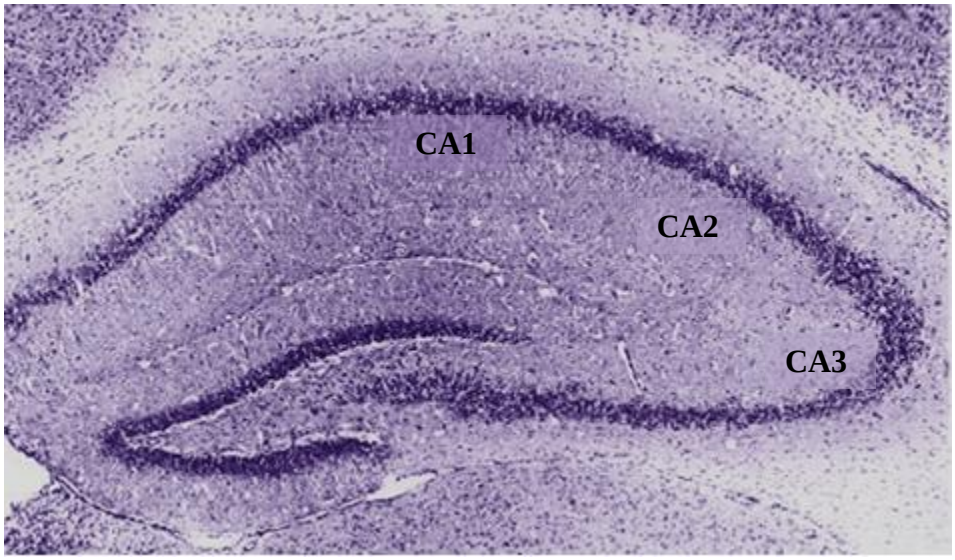

Figure 1. Cytoarchitecture of the Hippocampus. Coronal stained section delineating the sub regions of the hippocampus proper: CA1, CA2 and the CA3. The pyramidal cells in the CA1 region of the hippocampus are extremely sensitive to ischemic insult.

To produce global cerebral ischemia in rats, a 2- or 4-vessel occlusion model can be utilized. The 2-vessel occlusion model requires hypotension during bilateral carotid artery occlusion. Hypotension is produced by a controlled arterial bleed that decreases blood pressure. When blood pressure is reduced to 50 mm Hg, the common carotid arteries are occluded and this combination produces a cerebral ischemic insult. After the ischemic insult is produced, the removed blood is replaced. The ensuing insult can be

verified histologically as it produces delayed cell death in the CA1 pyramidal cells of the hippocampus. (Ginsberg & Busto, 1989) (see Figure 2).

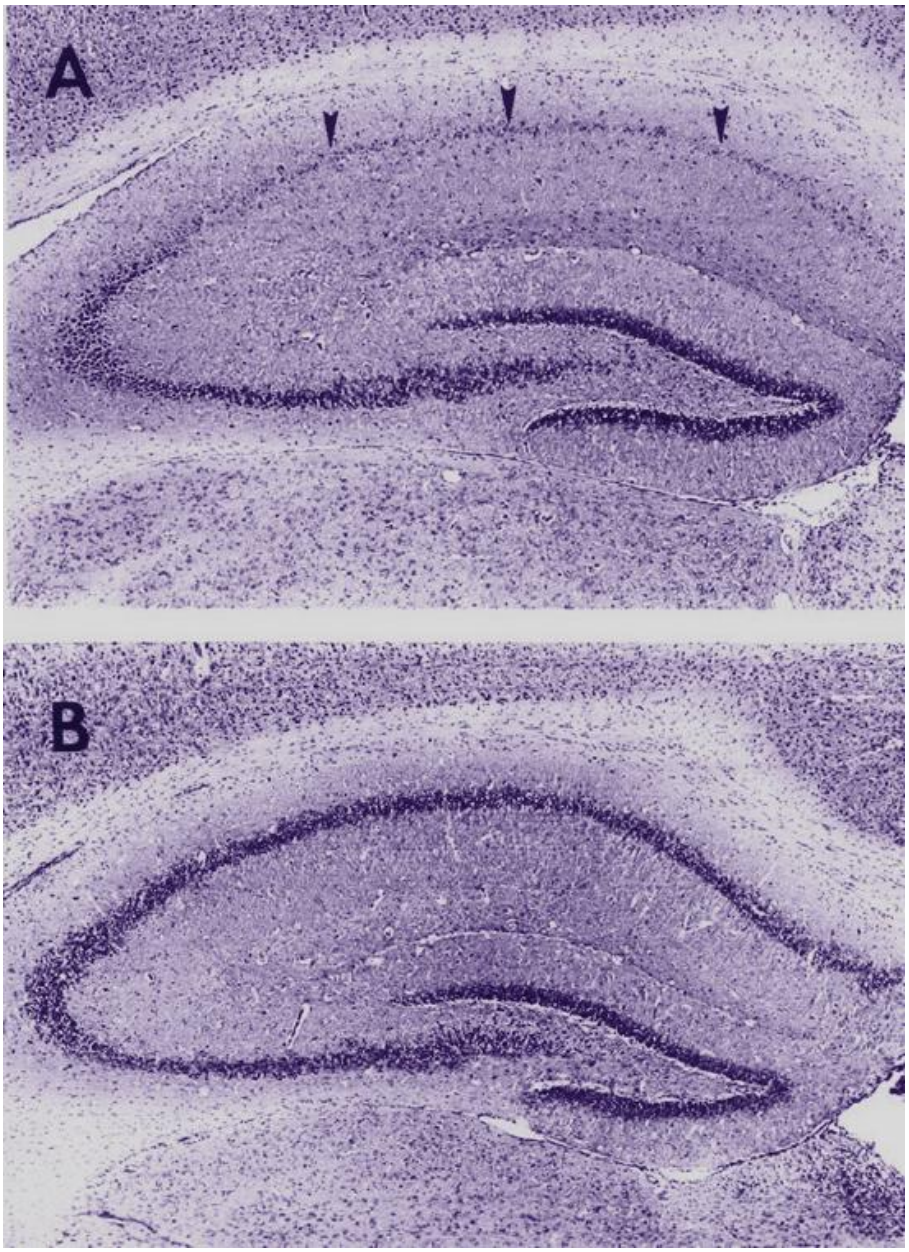

Figure 2. Ischemic Damage to the Hippocampal CA1 Region and Control Comparison. Coronal stained section of the CA1 region of the hippocampus in an ischemic and a control subject. A. Ischemic damage produces delayed cell death in the CA1 region of the hippocampus (indicated by arrows). B. A stained hippocampal section from a control subject. In comparison to the ischemic subject the pyramidal cells in the CA1 region of the control subject are healthy and intact.

The 4-vessel occlusion model involves two separate procedures. First, rats are anesthetized and the vertebral arteries are cauterized permanently through a dorsal incision. The ischemic insult is achieved by clamping the common carotid arteries after vertebral cauterization. The duration of blood flow interruption can range from 10 to 30 min. Damage to the CA1 region of the hippocampus is observed in 10 to 20 min, while additional damage to the striatum occurs following 20 to 30 min of ischemia. Although this model has been widely utilized, only about 50% of subjects survive vertebral cauterization (Ginsberg & Busto, 1989).

The 2-vessel occlusion rat model has become more popular as an alternative to 4-vessel occlusion because it does not require multiple surgical procedures to achieve an ischemic insult. This method has a higher success rate with fewer subjects lost compared to the 4- vessel occlusion model. However, one major disadvantage of the 2-vessel occlusion model is that it can be difficult to maintain hypotension during the ischemic insult (Ginsberg & Busto, 1989).

While the 2-vessel and 4-vessel occlusion models are widely utilized in rats there are two different methods for producing an ischemic insult in gerbils. Transient cerebral ischemia can be produced in the gerbil using either unilateral or bilateral common carotid artery occlusion. Production of ischemia following common carotid artery occlusion alone is possible because gerbils lack posterior communicating arteries, namely a Circle of Willis (Berry et al., 1975). The Circle of Willis, or cerebral arterial circle, connects the dorsal vertebral and basilar arteries to the ventral carotid arteries. This vascular arrangement decreases the likelihood that blood circulation will be interrupted completely

and thus represents a mechanism that protects an organism from interrupted blood flow in the brain (Martini, 2003).

Unilateral common carotid occlusion produces ipsilateral ischemic damage. This damage is restricted to the pyramidal cells of the CA1 region of the hippocampus and can be produced in as little as 5 min (Ginsberg & Busto, 1989).

Very similar to unilateral common carotid occlusion, bilateral common carotid occlusion in the gerbil involves a surgery where both common carotid arteries are isolated and clamped (Kahn, 1972). This method produces bilateral delayed cell death in the pyramidal cells in the CA1 region of the hippocampus (Levine & Sohn, 1969; Kirino, 1982; Kirino et al., 1984). The chief advantage of utilizing this model is that it is a more straightforward, replicable model compared to either the 2-vessel or 4-vessel occlusion model in rats. The current research utilizes the bilateral common carotid artery occlusion in gerbils to model a global ischemic insult.

Models of Neurotoxic and Mechanical Lesions to the Hippocampus

In addition to rodent models of ischemia, researchers have utilized neurotoxins to injure the hippocampus and study subsequent behavioral effects. Hippocampal lesions can be produced by injecting colchicine, a poisonous alkaloid that inhibits mitosis, N-methyl-D-aspartate (NMDA), a synthetic amino acid or ibotenic acid (Lee et al., 2005; Mumby et al., 2002; Gaskin et al., 2003; Ainge et al., 2005; Volpe et al., 1992). Studies utilizing neurotoxins have helped elucidate the influence of different hippocampal regions on learning and memory (Lee et al., 2005; Duva et al., 1997; Gaskin et al., 2003;

O'Brien et al., 2006). Employing neurotoxins is an attractive method because it affords a high degree of control when these agents are injected into specific sub regions of the hippocampus.

Another method by which damage to the hippocampus can be procured is through aspiration; a mechanical lesion. Aspiration lesions to the hippocampus are made using suction to remove cerebral tissue (Aggleton et al.,1986).

Behavioral Testing

A variety of experimental tasks have been used to study the behavioral impact of cerebral ischemia and neurotoxic/mechanical lesions to the hippocampus. Demonstrating behavioral impairment is important for validating these models as well as testing potential neuroprotective agents that might be subsequently used for human treatment.

Locomotor Activity

Several researchers have included measures of locomotor activity when investigating the effects of ischemic or neurotoxic damage to the hippocampus in rats. These measures include observation of subjects in an open field apparatus to determine overall activity levels.

Plamondon and Kahn (2005) conducted a study to investigate the effects of global ischemia on subsequent locomotor activity. These investigators utilized the 4-vessel occlusion rat model to induce ischemia and then observed subjects in an open field apparatus for a single 30-min session. Total distance traveled, amount of time spent grooming and amount of time immobile were measured. Analysis revealed that ischemic

subjects, compared to controls, traveled farther in the open field and spent less time resting. All subjects showed higher activity at the beginning of the 30 min testing interval that gradually declined; however, ischemic subjects exhibited significantly higher activity levels.

In a related study, Plamondon et al. (2008) investigated the effects of global ischemia on locomotor activity in the open field when subjects are preconditioned with a short 3-min ischemic insult that was followed by a 6 min insult. After surgery subjects were observed in the open field for 30 min and total distance traveled, rearing and grooming data were collected. All ischemic subjects exhibited heightened activity levels compared to controls. While ischemic subjects traveled significantly farther than controls in the open field both groups' activity levels decreased as time progressed.

Milot and Plamondon (2008) also investigated locomotor activity following global ischemia in rats by manipulating the lighting conditions where subjects were tested. Ischemic insult was achieved with the 4-vessel occlusion model and subjects were tested in the open field for 30 min under either a bright or dim lighting condition. Analysis revealed that when ischemic subjects were tested under bright lighting conditions they exhibited heightened activity levels compared to ischemic subjects that were tested in the dim lighting condition. Control subjects showed the opposite effect; they explored more under a dim versus bright lighting condition.

The studies demonstrate that heightened activity is a common, replicable effect that follows global ischemia in rats. This hyperactivity can be used to predict successful ischemic insult prior to histological evaluation.

In addition to locomotor testing in the open field the majority of research investigating the behavioral effects of hippocampal damage has been conducted in rats. Object recognition following damage to the hippocampus has been studied over many years and constitutes a large body of research literature. Spatial recognition has not been studied as extensively as object recognition and it represents an area of behavioral research that has not yet been fully investigated. The following sections will provide an overview of the current object and spatial recognition literature. Rats have been used extensively in these tasks and these data comprise the foundation for what is known about object and spatial recognition.

Object Recognition

To investigate the effects of ischemic or neurotoxic hippocampal damage, rodents can be tested using object recognition tasks. Rats tend to show a propensity to explore novelty in their environment. In a study conducted by Ennaceur and Delacour (1988), rats were exposed to two identical objects in the environment and were allowed to explore them for several minutes. Then subjects were removed from the apparatus for a retention interval after which they were placed in the apparatus. In this second phase, subjects were confronted with one of the original objects as well as a novel object. Normal rats spend more time exploring the novel object than the object that had been encountered previously. Observing this exploratory preference, researchers have inferred that if the subject spends more time with the novel object in the environment then it remembers the previously encountered object that was included in both phases of testing. Quantifying

changes in object exploratory behavior can be used to assess recognition of a novel object placed in a familiar testing environment.

The investigation of novel object recognition began in the 1980s when investigators started studying how damage to the hippocampus could induce behavioral impairment. Aggleton et al. (1986) tested rats in an object recognition task following aspiration lesions to the hippocampus. After surgery subjects underwent a delayed matching to sample (DNMS) object recognition task. In the first phase of this task rats are confronted with two identical objects. Regardless of which object is explored in this phase the rat is rewarded with a food pellet. In the next phase the rat is confronted with one of the original objects and a novel object. If the rat explores the novel object it is rewarded with another food pellet.

Results from this study demonstrated that even with varying retention intervals of 0, 20 or 60 s, hippocampal damaged subjects were able to learn the DNMS task without impairment. This was the first study to posit the idea that damage to the hippocampus does not result in object recognition deficits.

When the Aggleton et al. (1986) findings were reported others had suggested that object recognition was mediated by a connection between the hippocampus and amygdala with other diencephalic structures. This hypothesis was derived from lesion studies conducted in monkeys. It was presumed that hippocampal lesion studies in rats were not yielding object recognition deficits because an intact amygdalo-thalamic circuit could compensate for disruption in the hippocampal-thalamic circuit that was caused by hippocampal lesions (Mishkin, 1978).

Taking these hippocampal connections into account Aggleton et al. (1989) followed up their original study by investigating the relationship between object recognition and damage to the hippocampal-thalamic circuitry. Subjects underwent neurotoxic lesions to the amygdala (ibotenic acid), neurotoxic lesions to the amygdala (ibotenic acid) plus surgical aspiration of the hippocampus or sham procedure. After surgery subjects underwent a DNMS task. Results revealed that all groups were able to acquire a DNMS object recognition task with a retention delay of 0 s. When the delay was increased to 20 or 60 s, subjects with amygdala plus hippocampal damage were impaired suggesting that to other structures like amygdala, in addition to the hippocampus, is important in object recognition (Aggleton et al., 1989).

Mumby et al. (1992) conducted a study that evaluated hippocampal interconnections with adjacent structures in the brain like the amygdala. Four groups were included in this study were: hippocampal lesions only, amygdala lesions only, hippocampal plus amygdala lesions, and a control group. This study utilized a DNMS task with a variety of retention intervals (4, 15, 60, 120 and 600 s). All lesion groups were able to acquire the task at control levels with intervals of 4, 15, 60 or 120 s. However, after the 10 min interval all lesion groups showed a significant performance deficit compared to control subjects. These results suggest that other structures, like the amygdala, may play an important role in object recognition as delay between trials increases.

Duva et al. (1997) conducted a study to investigate the effects of neurotoxic lesions on DNMS object recognition task performance. Rats were given NMDA

injections into the dorsal hippocampus. One group of rats had preoperative training and the other did not. After surgery both groups underwent the DNMS task but neither showed impairment in performance. Post mortem histological analysis revealed that the lesions covered a substantial area of the CA1 region of the hippocampus. These results indicate that neurotoxic damage to the CA1 region of the hippocampus is not sufficient to impair object recognition.

Mumby (2002) also investigated how NMDA lesions to the hippocampus affected performance in a standard novelty preference object recognition task. Rats were placed in the apparatus with two identical objects for 5 min. After a retention interval spent outside the apparatus, subjects were placed in the apparatus with one previously encountered object and one novel object. Results indicated that hippocampal damaged subjects were able to perform at control levels.

Gaskin et al. (2003) created substantial lesions to the hippocampus using NMDA injections. After surgery, rats underwent an object recognition task and again both hippocampal damaged and control subjects showed a preference for the novel object. These results indicate that hippocampal damage does not impair performance in object recognition tasks. It appears that the hippocampal formation is not crucial for recognizing differences between objects. Object recognition has also been evaluated in rats following ischemic damage to the hippocampus. Although ischemia can produce damage to other areas of the brain like the striatum, thalamus and cerebellum (Mumby et al., 1996), it was widely accepted that behavioral impairments are due to damage in the CA1 region of the hippocampus. Several studies have shown that hippocampal damage caused by ischemic

insult results in memory deficits (Bachevalier & Mishkin, 1989; Wood et al., 1993; Zola-Morgan et al., 1992).

Bachevalier and Mishkin (1989) conducted a study in monkeys investigating the effects of ischemic damage to the hippocampus on DNMS task performance. Before surgery subjects were exposed to a single object on the center food well that concealed a reward. Next, subjects were presented with the original object and a novel object each placed on a lateral food well. Subjects were rewarded when they displaced the novel object. After training, subjects underwent ischemic insult via permanent posterior cerebral artery occlusion or sham procedure. Ischemic subjects made more errors relative to controls. Histological evaluation revealed that the extent of hippocampal damage varied between subjects. Control subjects had no damage and performed without error on the DNMS task. One ischemic subject had little to no brain damage and maintained DNMS scores that were similar to controls. The other ischemic subjects sustained quantitatively more hippocampal damage and had lower DNMS scores.

Zola-Morgan et al. (1992) conducted a similar study in monkeys to investigate ischemic damage to the hippocampus and its effect on a subsequent DNMS task. Ischemic insult was achieved by bilateral carotid occlusion in addition to hypotension or sham procedure. After surgery subjects underwent a DNMS task; they were first confronted with a single object placed on a food well that concealed a reward. Following a delay subjects were exposed to the previously encountered object and a novel object. When they displaced the novel object they were given a reward. Results indicated that ischemic subjects were impaired on this task with a delay of 15 s, 60 s or 10 min

compared to control subjects. The greatest discrepancy between the groups was revealed during the 10 min delay. Histological evaluation revealed bilateral damage restricted to the CA1 region of the hippocampus.

Wood et al. (1993) investigated the effect of ischemic damage to the hippocampus in rats and replicated the findings of Bachevalier and Mishkin (1989) and Zola-Morgan et al. (1992). First, rats were trained to run between the ends of a rectangular apparatus with both ends of the apparatus containing guillotine doors. Subjects were baited to one end of the apparatus where they encountered two different objects; one concealed a food pellet (S+) and the other did not (S-). The subjects were rewarded for selecting the S+ object and received the food pellet. After the subject ate the food pellet, it was required to return to the opposite end of the apparatus where it encountered the same objects, S+ and S-. While the object placement (left or right) was varied randomly throughout training the subjects was always rewarded for displacing S+ and never for displacing S-.

Following an ischemic insult, subjects were tested on this discrimination task and underwent a DNMS task. On each trial subjects were positioned in the middle of the apparatus with both guillotine doors down. One of the doors was opened and the subject encountered the sample object that concealed a food pellet. After the subject ate the reward the door was lowered and the door at the opposite end of the apparatus was opened. Now the subject encountered the sample object and a novel object that concealed a food pellet. Reward was only given for displacement of the novel object. Subjects were tested with multiple interval delays: 4, 15, 30, 60, 120 and 300 s.

Results demonstrated that before surgery all subjects learned the discrimination task within five sessions. After surgery both ischemic and control subjects required only two sessions to reacquire the task; there were no significant differences between the groups. Results for the DNMS task were markedly different from the discrimination task. Ischemic subjects took significantly longer to acquire the DNMS task compared to controls. Further analysis revealed significant differences between the groups for delay intervals of 15, 30 and 60 s. Histological evaluation revealed a significant negative correlation between the amount of hippocampal CA1 region cell death and time required to acquire the DNMS task.

Collectively, these studies demonstrate object recognition impairment in rats following ischemic damage to the CA1 region of the hippocampus. In summary, there are several different ways to experimentally damage the hippocampus: neurotoxic lesions, aspiration and ischemic insult. Previous research demonstrates that damage to the hippocampus via neurotoxic or mechanical lesions is not sufficient to impair rats on object recognition tasks. In contrast, ischemic damage to the hippocampus produces deficits in object recognition in rats and monkeys. These divergent results suggest that ischemic damage to the hippocampus differs from aspiration and neurotoxic lesions produced in the same area.

Spatial Recognition Task

A second type of task, spatial recognition, investigates the ability of the subject to recognize that a familiar object has been moved to a novel location. Rodents will show a

propensity to explore familiar objects that have been moved to a novel location within an environment (Mumby et al., 2002).

Compared to the object recognition literature, there has not been extensive research investigating object spatial recognition in rodents. Volpe et al. (1992) investigated how neurotoxic lesions or ischemia would affect behavior in a tactile and spatial discrimination task. Rats were either subjected to a neurotoxic injection of ibotenic acid into the hippocampus or to forebrain ischemia. All subjects were tested in a modified T-maze. The apparatus consisted of a standard T maze with a division of the main stem into two alleyways that were lined with rough or smooth texture. Each alleyway could be closed off near the far end of the maze where the goal boxes resided. Subjects were trained to choose the specific alleyway based on tactile cue (smooth or rough floor covering). After subjects chose an alleyway they then had to choose one of the arms (with its respective goal box) at the end of the maze. During the first trial, one of the goal boxes was blocked and therefore the choice was forced. On the next trial, when both arms were open, subjects were reinforced with food for the opposite goal box. In this way they completed a tactile discrimination task followed by a spatial recognition task. Investigators found that ischemic subjects and those that received ibotenic acid performed more poorly on both tasks relative to controls (Volpe et al., 1992).

Duva et al. (1997) conducted a study that investigated spatial recognition after subjects had received neurotoxic lesions (NMDA) to the hippocampus. After surgery subjects were trained on the Morris water maze. In this task, subjects were released into a round pool of water and required to locate a hidden platform. Subjects with hippocampal

lesions that included the CA1 region took significantly longer and swam significantly farther to find the hidden platform that subjects that underwent a sham procedure.

Another study investigated the effect of hippocampal lesions produced via NMDA injections, on two variations of spatial recognition (Mumby et al., 2002). Animals were tested in spatial and contextual recognition tasks. In the spatial task, subjects were exposed to two identical objects and allowed to explore them for 5 min. After a retention interval they were allowed to explore the same objects except one was now placed in a novel location in the apparatus. In the contextual task subjects were exposed to two identical objects (object set A) for 5-min and then moved to a different room (i.e. different context) and allowed to explore a different pair of identical objects (object set B). After a retention interval subjects were confronted in one of the two previously encountered contexts with one object from set A and one object from set B. They found that subjects that underwent the sham procedure showed novelty preference in both spatial and contextual tasks; they were able to recognize previously encountered objects and subsequently showed a preference for an object in a novel location. In the contextual task, subjects that had undergone the sham procedure were able to recognize the object they had originally encountered in that context and subsequently spent time exploring the other object that was not familiar to them. Subjects with hippocampal lesions were unable to identify the objects that had been moved to novel locations or new objects that were encountered in a familiar context. These results suggest that hippocampal damage is sufficient to disrupt the processing required to recognize spatial relationships between objects encountered in familiar environments.

Lee et al. (2005) investigated the role of various hippocampal subregions in object spatial recognition. Investigators utilized colchicine injections to damage different regions of the hippocampus: CA1, the dentate gyrus or CA3 regions. After surgery subjects underwent an object recognition task where they were familiarized with several objects in set locations in the apparatus. Hippocampal lesioned and control subjects were able to recognize previously encountered objects and subsequently spent time exploring the novel object in the environment.

Next, subjects underwent a spatial task where objects encountered in the object recognition testing phase were moved to novel locations in the apparatus. Subjects with lesions in the dentate gyrus or CA3 region were severely impaired on the spatial recognition task. Interestingly, the group that received lesions to the CA1 region of the hippocampus were only mildly impaired on the spatial recognition task. These results suggest that the CA1 region of the hippocampus is not crucial for recognizing spatial relationships between objects in a familiar environment. This study also suggests that other hippocampal subregions like the dentate gyrus and the CA3 may play a more important role in recognizing spatial orientation relationships.

Investigators also measured to locomotor activity of rats throughout the various phases of testing. They found that all groups' (control, CA1 damaged, CA3 damaged and dentate gyrus damaged) activity levels declined significantly over several days. The decrease in overall activity was interpreted as the subjects becoming habituated to an environment they were exposed to repeatedly. The dentate gyrus damaged group showed the highest level of initial activity, followed by the CA1 damaged, CA3 damaged and

control groups, respectively. These results suggest that damage to specific sub regions of the hippocampus produces heightened locomotor activity (Lee et al., 2005).

In summary there is converging evidence suggesting that neurotoxic lesions to the hippocampus do not impair rats in object recognition tasks. In contrast, some studies suggest that ischemic damage to the hippocampus can impair performance on this task. Similarly, object spatial recognition can be impaired when the hippocampus is damaged via ischemic or neurotoxic lesions. Although the object spatial recognition has not been extensively investigated, the current literature suggests that the hippocampus is crucial for recognizing spatial relationships.

Gerbil Behavioral Models

There have been relatively few behavioral models used to investigate the effects of cerebral ischemia in the gerbil. The majority have used open field apparatuses to evaluate locomotor activity (Babcock, 1993; Janać et al., 2006). Babcock et al. (1993) reported that ischemic subjects exhibit hyperactivity within 24 hrs of insult. This finding has been reported by others (Chandler et al., 1985; Mileson & Schwartz, 1991; Wang & Corbett, 1990). The observed hyperactivity diminishes over time and has been considered a transient effect of cerebral ischemia (Katoh et al., 1992). Babcock et al. (1993) evaluated the effects of delayed testing and changes in testing environment on ischemia-induced hyperactivity. A group of subjects was tested 14 days after the ischemic insult and they showed hyperactivity levels comparable to those tested immediately after ischemic insult. After hyperactivity levels had decreased over several daily exposures to the apparatus, subjects were retested in a novel location. Testing in this novel

environment resulted in a reappearance of hyperactivity in the ischemic gerbils. These findings suggest that hyperactivity observed following ischemia may in part represent an impairment in habituation.

Anderson et al. (1997) utilized an open field apparatus to investigate behavioral changes and their correlation to damage in the CA1 region of the hippocampus. Subjects underwent 2, 4, 6, or 8 min of bilateral carotid occlusion or a sham procedure. After surgery subjects were tested in the open field. Results revealed that damage to the CA1 region of the hippocampus and heightened activity were positively correlated. In addition, results indicated that duration of ischemic insult had an impact on overall activity levels; subjects that underwent an 8 min ischemic insult traveled significantly farther relative to other ischemic groups.

Janać et al. (2006) utilized a similar open field model to investigate behavioral changes that accompany varying durations of cerebral ischemia (5, 10 or 15 min bilateral carotid occlusion). Motor activity was evaluated based on measures such as total distance traveled, stereotypic behavior (grooming) and resting. Investigators found that cerebral ischemia increases locomotor and stereotypic behavior and the magnitude of this effect was dependent on the duration of cerebral ischemia. The group that underwent 15 min bilateral carotid occlusion traveled the greatest distance and exhibited increased stereotypic behavior compared to the other groups. After testing lesions were evaluated histologically; increased duration of ischemia produced greater damage to cells in the striatum.

Other investigators have studied working memory using a T-maze design consisting of a center stem with two side arms at one end (Imamura et al., 1991). In this task the subject were forced and rewarded for entering one of the side arms on the first trial. On the following trial both arms are open and the subject was rewarded when it chose the opposite arm. Ischemic gerbils are impaired on this task (Imamura et al., 1991).

Thus far, researchers have only utilized open field and T-maze testing to investigate the behavioral effects of ischemic insult in gerbils. The goal of the present study was to develop and evaluate a novel behavioral model for use with ischemic gerbils. These experiments evaluated normal and ischemic gerbils in both object and spatial recognition tasks.

EXPERIMENT 1

The purpose of this experiment was to evaluate the utility of standard object and spatial recognition tasks in normal and ischemic Mongolian gerbils. Prior to evaluation on these tasks, gerbils were habituated to the apparatus in the absence of objects. Previous studies have shown that ischemic gerbils will exhibit increased activity when evaluated in an open field apparatus (Babcock et al., 1993; Janać et al., 2006).

Methods

Behavioral Apparatus and Experimental Objects

The apparatus was a square open field constructed of white PVC plastic. The base of the apparatus measured 76.2 cm square with walls 38.1 cm high. One wall was labeled with black electrical tape (diagonal stripes) to provide subjects with a spatial orientation cue. The bottom of the apparatus was designed to accommodate different drop-in floors depending on the experimental task. For open field habitation a white PVC plastic floor with no holes was used. For object and spatial tasks a white PVC plastic floor with two holes was used. This floor provided two orientations for objects during the spatial recognition task (see Figure 3).

Experimental stimuli for the object recognition task were a Matchbox car and two identical cylindrical kaleidoscopes. Experimental stimuli for the spatial recognition task were two identical golf balls. All stimuli were modified to fit into the holes in the floor of the apparatus. Nuts were soldered onto washers that were secured with epoxy to the

bottom of the Matchbox car and the kaleidoscopes. Each nut fit a butterfly bolt that could secure the object through the drop in floor and the base of the apparatus. The golf balls were fitted internally with long nuts that could be attached through the drop in floor and the base of the apparatus with a butterfly bolt (see Figure 3).

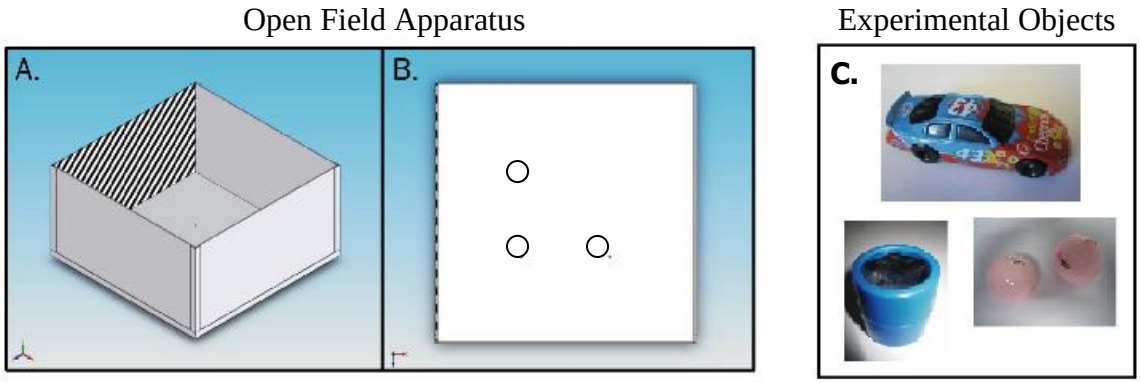

Figure 3. Behavioral Apparatus and Experimental Objects. A. The apparatus used for these experiments was 76.2 cm x 76.2 cm x 38.1 cm. B. Aerial view of open field apparatus showing the three positions where objects could be located. C. Photographs of experimental objects for object (blue kaleidoscope and Matchbox car) and spatial (golf balls) recognition tasks.

Data Collection

Video data were collected using a webcam interfaced to a computer and the ANY-maze tracking software (Stoelting, Wood Dale, Illinois). Each subject was kept outside the experimental room until the testing period began. After testing each subject was placed back in its home cage and removed from the testing room.

Subjects and Surgery

Twenty male Mongolian gerbils (65-75 g) were housed individually in standard rodent cages. They were maintained on a 12-hr light-dark cycle. All behavioral testing

was conducted during the light phase of this cycle. Each gerbil was allowed access to food and water *ad libitum*.

Gerbils were randomly assigned to receive either an ischemic insult (n=12), or a sham procedure (n=8). For ischemia induction, subjects were anesthetized with isoflurane and core body temperature was maintained at 37-38º C during surgery using a homeothermic blanket (Harvard Apparatus, South Natick, USA). A midline incision in the neck was made and the carotid arteries were clamped for 5 min using 85 g pressure micro aneurysm clips. After ischemic insult, the clamps were removed and the muscle and skin were sutured closed. Subjects were placed back in their cages and monitored during recovery. Subjects in the control group underwent the same procedure without bilateral carotid occlusion. All subjects received Tylenol (300 mg/kg/day) mixed with their drinking water for two days post-surgery. All animal procedures were approved by the Montana State University IACUC.

Behavioral Procedure

After surgery subjects underwent a habituation period, 5 consecutive days, to acclimate to the apparatus. The apparatus floor that contained no holes was used for the habituation period. Each subject was placed in the center of the apparatus and was allowed to explore for 5 min each day. Object and spatial recognition testing commenced two days after the habituation period was complete.

Subjects in both the ischemic and control groups were tested in the Object and Spatial recognition tasks in a randomized, counterbalanced design. The Object recognition task consisted of two phases (see Figure 4). In Phase 1, subjects were placed

in the center of the apparatus with two identical objects (i.e. kaleidoscopes) and allowed to explore for 5 min. Next, subjects were returned to their home cages for a retention interval of 3 min. The apparatus and objects were cleaned with a 70% ethanol solution and one of the objects was replaced with the Matchbox car. Subjects were placed in the center of the apparatus and allowed to explore for 5 min (Phase 2).

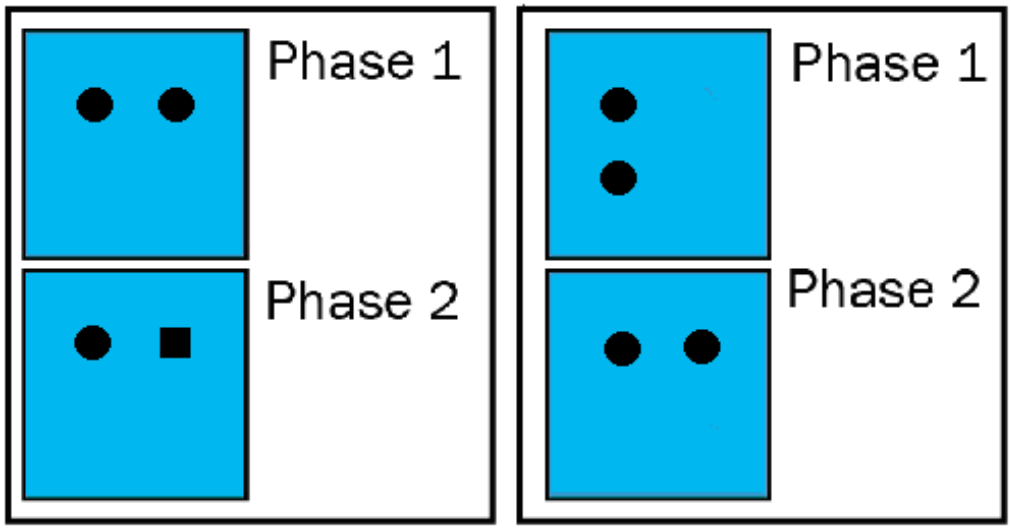

Figure 4. Object and Spatial Recognition Object Configurations. The blue shaded region represents the floor of the open field apparatus. The left panel depicts the configuration for the Object recognition task. In phase 1, two identical objects are placed in the apparatus. Next, one of the original objects is replaced with a novel object (square; phase 2). The right panel depicts the configuration for the Spatial recognition task. In phase 1, two identical objects are placed in the apparatus. Next, one of the original objects is moved to a novel location in the apparatus (phase 2).

The spatial task consisted of two phases (see Figure 4). In Phase 1 two identical objects (i.e. golf balls) were secured through the floor of the apparatus. Subjects were placed in the center of the apparatus and allowed to explore for 5 min. After Phase 1 subjects were removed from the apparatus and placed in their home cages for a retention interval of 3 min. The apparatus floor was repositioned such that one object was in a novel location for Phase 2. Both the apparatus and objects were cleaned with a 70%

ethanol solution. Subjects were placed in the center of the apparatus and allowed to explore for 5 min. After testing, subjects were placed in their home cages and removed from the testing room.

Histology

At 20 days after surgery subjects were euthanized with CO_2 and perfused intracardially with 0.9% saline and 10% buffered formalin. Brains were removed and stored in formalin for 96 hrs at 4º C. A tissue block was made by two coronal cuts to remove the anterior portion of the brain (anterior to the optic chiasm) and the cerebellum/brainstem. Tissue blocks were mounted with adhesive on a vibratome block and 50 μm sections were collected through the hippocampal region. Sections were mounted on slides and stained with cresyl violet. Four independent observers blinded to the conditions scored the damage using a rating scale described elsewhere (Coimbra & Cavalheiro, 1990). Experimenters evaluated damage to the CA1 region of the hippocampus and assigned a value ranging from 0 and 3 (0 = no damage, 3 = severe damage). Only subjects in the ischemic group with histological ratings of 3 were included in the final analysis.

Data Analysis

Activity was measured as the total distance traveled (m) in the apparatus and analyzed using a repeated measures ANOVA. Exploratory behavior for object and spatial recognition tasks was quantified using zones defined around each object with the ANY-maze program. Time spent in these zones (sec) was used as a measure of object

exploratory behavior. Exploratory behavior data were transformed into exploratory ratios (time spent with novel object/total time spent with both objects) as previously described (Mumby et al., 2002). For both object and spatial recognition tasks, exploratory ratios were analyzed using a mixed model repeated measures ANOVA.

Results and Discussion

Histology

Seven of 12 ischemic subjects were given a rating of 3 and were included in the final analysis. All control subjects (n=8) were assigned a rating of 0.

Locomotor Activity

Activity levels were measured as the total distance traveled (m) in the apparatus during the 5 min of daily testing. On Day 1 ischemic and control subjects traveled, on average, 64.3±4.4 m and 41.2±2.7 m, respectively. On the last day of habituation testing, Day 5, ischemic and control subjects traveled 38.8±5.0 m and 31. 9±3.9 m, respectively (see Figure 5).

Analysis revealed a main effect of testing Days, ($F(4, 52) = 14.715$, $p < .001$, $\eta_p^2 = .531$), and Treatment, ($F(1, 13) = 5.120$, $p = .041$, $\eta_p^2 = .283$). A significant interaction was also observed, ($F(4, 52) = 4.072$, $p = .006$, $\eta_p^2 = .239$). Additional *t*-test analysis revealed that ischemic subjects were significantly more active relative to controls on Day 1 ($p < .001$) and Day 2 ($p = .01$), but the groups no longer differed on Day 5, ($p = .29$). The Bonferroni correction was used to adjust the critical *p* value for these multiple comparisons. These findings replicate previous findings that demonstrate hyperactivity in

ischemic subjects that is attenuated with repeated exposure to the testing environment (Babcock et al., 1993; Janać et al., 2006).

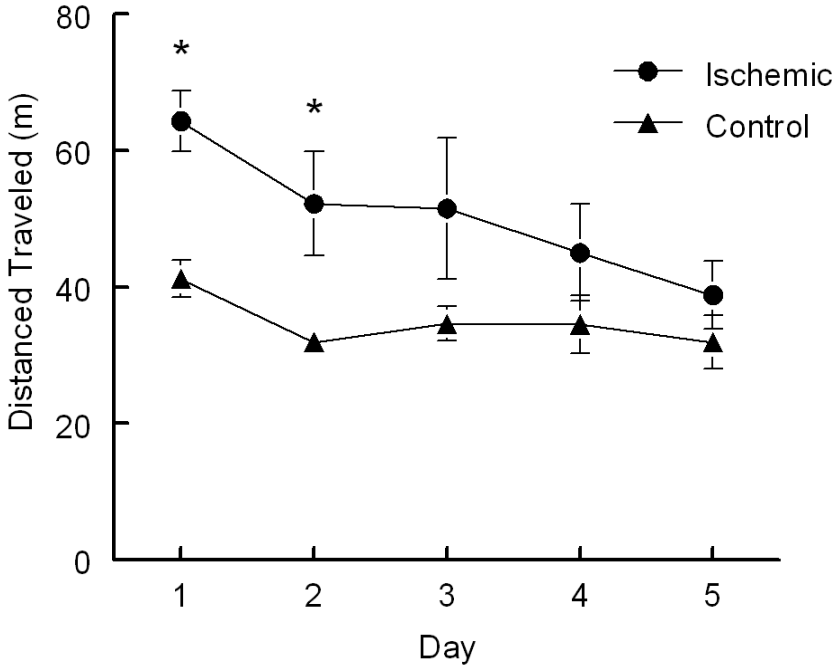

Figure 5. Experiment 1: Locomotor Activity. Locomotor activity of ischemic and control subjects during a 5 day testing period. Subjects were tested 5 min each day. Ischemic subjects showed significantly higher levels of activity on Day 1 and 2 of testing compared to control subjects. On Days 3-5, activity levels of ischemic and control subjects were no longer significantly different.

Object Recognition

In Phase 1 (familiarization), ischemic and control subjects spent equal amounts of time exploring the target object; exploratory ratio means were .46±.06 and .48±.04, respectively. In Phase 2 (test), both ischemic and control subjects spent significantly

more time exploring the novel object; exploratory ratio means were .76±.06 and .64±.06, respectively (see Figure 6).

Comparing groups across the two experimental phases revealed a main effect of Phase, ($F(1, 13) = 13.491$, $p = .003$, $\eta_p^2 = .509$), but not for Treatment, ($F(1, 13) = 1.177$, $p = .298$, $\eta_p^2 = .083$). The interaction was not significant, ($F(1, 13) = 1.076$, $p = .319$, $\eta_p^2 = .076$).

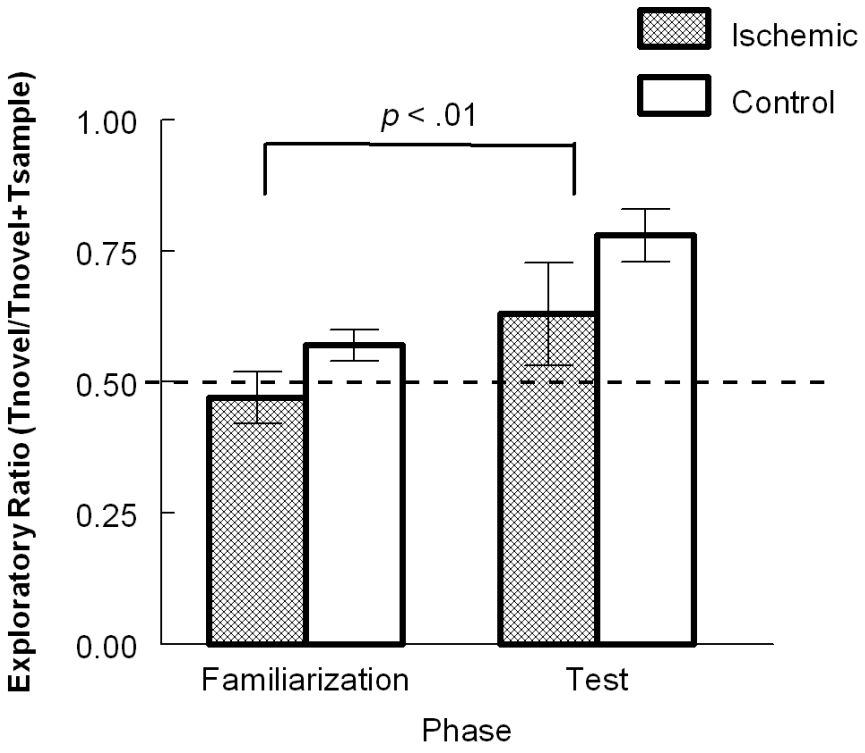

Figure 6. Experiment 1: Object Recognition. Exploratory ratios were compared between the familiarization and test phases. In the familiarization phase (left bars), subjects were exposed to two identical objects that they explored equally. In the test phase (right bars), subjects were exposed to a previously encountered object and a novel object. Both groups showed a significant preference for the novel object.

Spatial Recognition

In Phase 1 (familiarization), ischemic and control subjects spent more than 50% of the time exploring the target object; exploratory ratio means were .68±.04 and .59±.04, respectively. In Phase 2 (test), neither ischemic nor control subjects spent significantly more time exploring the object in the novel location; exploratory ratio means were .64±.05 and .60±.07, respectively (see Figure 7).

Comparing the groups across the two experimental phases did not reveal a main effect of Phase, ($F(1, 13) = .109$, $p = .746$, $\eta_p^2 = .008$), or Treatment, ($F(1, 13) = 1.863$, $p = .195$, $\eta_p^2 = .125$). The interaction was not significant, ($F(1, 13) = .256$, $p = .621$, $\eta_p^2 = .019$).

The results from the present experiment revealed that ischemic subjects exhibited hyperactivity during the habituation phase compared to control subjects. This difference decreased with repeated exposure to the apparatus.

The results from the object recognition task indicate that both ischemic and control subjects were able to recognize a novel object in the environment. Ischemic subjects tended to explore the novel object relatively more than controls; although not statistically significant, this trend is noteworthy. This point will be discussed further in General Discussion.

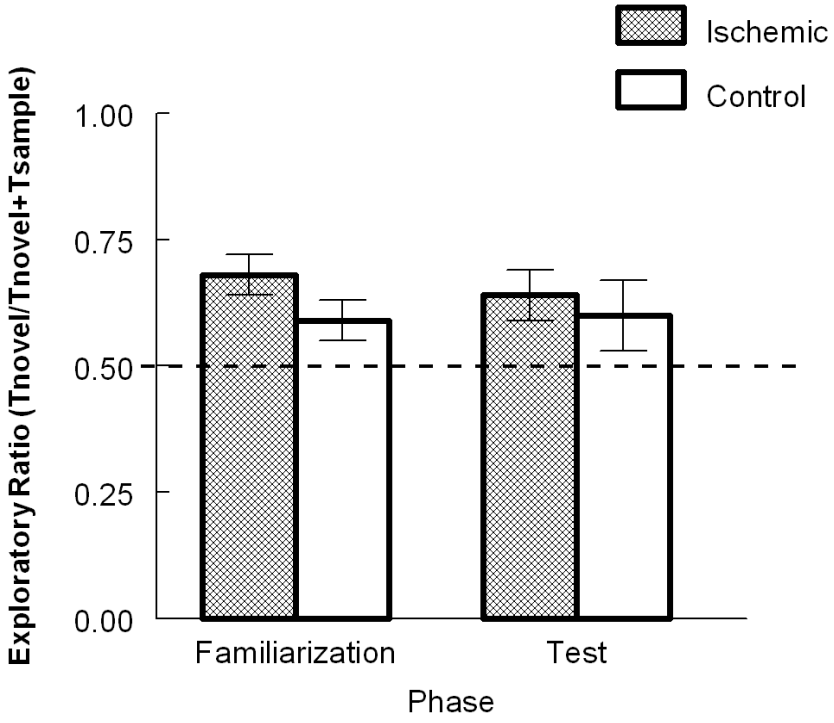

Figure 7. Experiment 1: Spatial Recognition. Exploratory ratios were compared between the familiarization and test phases. In the familiarization phase (left bars), subjects were exposed to two identical objects. Both ischemic and control subjects explored the target object more than 50%. In the test phase (right bars), subjects were exposed to the same objects but one of those objects was placed in a novel location. Neither group showed a preference for the object in the novel location.

The findings from the spatial recognition task suggest that neither group showed a preference for the novel location. Although determining the impact of ischemic damage on task performance was the goal of this study, it was expected that control subjects would exhibit a preference for the object in the novel location. This unanticipated result suggested the need for further research aimed at evaluating spatial recognition performance in gerbils.

EXPERIMENT 2

In Experiment 1 it was observed that neither group showed a preference for the object in the novel location. This finding is inconsistent with previous research (Mumby et al., 2002; Duva et al., 1997; Lee et al., 2005). The goal of Experiment 2 was to evaluate the effects of additional exposure to the experimental objects on subsequent performance in a spatial recognition task. It was hypothesized that extended exposure to the experimental objects should enable control subjects to exhibit the expected preference for the object in the novel location. This experiment also evaluated the impact of ischemic damage to the hippocampus on object exploration under this condition. Based on previous research, damage to the hippocampus should impair ischemic subjects on a spatial recognition task. (Volpe et al., 1992; Duva et al., 1997; Mumby et al., 2002; Lee et al., 2005).

Methods

Behavioral Apparatus and Experimental Objects

The apparatus and the experimental objects were identical to those used in Experiment 1.

Data Collection

Video data were collected using a webcam interfaced to a computer and the ANY-maze tracking software (Stoelting, Wood Dale, Illinois). Each subject was kept

outside the experimental room until the testing period began. After testing each subject was placed back in its home cage and removed from the testing room.

Subjects and Surgery

The subjects were twenty male Mongolian gerbils naïve to testing. The surgical procedure was identical to Experiment 1.

Behavioral Procedure

After surgery subjects were habituated to the apparatus and experimental objects for 5 min each day for 5 consecutive days. The habituation period was followed by the spatial recognition task described in Experiment 1. Subjects were placed in the apparatus with the objects in the same configuration as the habituation period and were allowed to explore for 5 min (Phase 1). Subjects were then removed from the apparatus and placed in their home cages for a retention interval of 3 min while the apparatus was reconfigured and wiped down with a 70% ethanol solution. Next, subjects were placed in the center of the apparatus and were allowed to explore for 5 min (Phase 2).

Histology and Data Analysis

Tissue harvest, histology and data analysis were identical to those in Experiment 1.

Results and Discussion

Histology

Five of 12 ischemic subjects were given a rating of 3 and included in the final analysis. All control subjects (n=8) were assigned a rating of 0.

Locomotor Activity

Activity levels were measured as the total distance traveled (m) in the apparatus. On Day 1 ischemic and control subjects traveled, on average, 68.2±6.3 m and 34.8±1.4 m, respectively. On the last day of habituation (Day 5), ischemic and control subjects traveled 45.8±6.4 m and 31. 6±3.7 m, respectively (see Figure 8). Analysis revealed a main effect of testing Days, ($F(4, 44) = 10.518$, $p < .001$, $\eta_p^2 = .489$), and Treatment, ($F(1, 11) = 11.668$, $p = .006$, $\eta_p^2 = .515$). A significant interaction was also observed, ($F(4, 44) = 9.078$, $p < .001$, $\eta_p^2 = .452$).

Subsequent *t*-test analysis revealed that ischemic subjects were significantly more active relative to controls on Day 1 ($p < .001$) and Day 2 ($p = .002$), but groups no longer differed on Day 5, ($p = .06$). The Bonferroni correction was used to adjust the critical *p* value for these multiple comparisons. These findings replicate previous findings that demonstrate hyperactivity in ischemic subjects that is attenuated with repeated exposure to the testing environment. (Babcock et al., 1993; Janać et al., 2006).

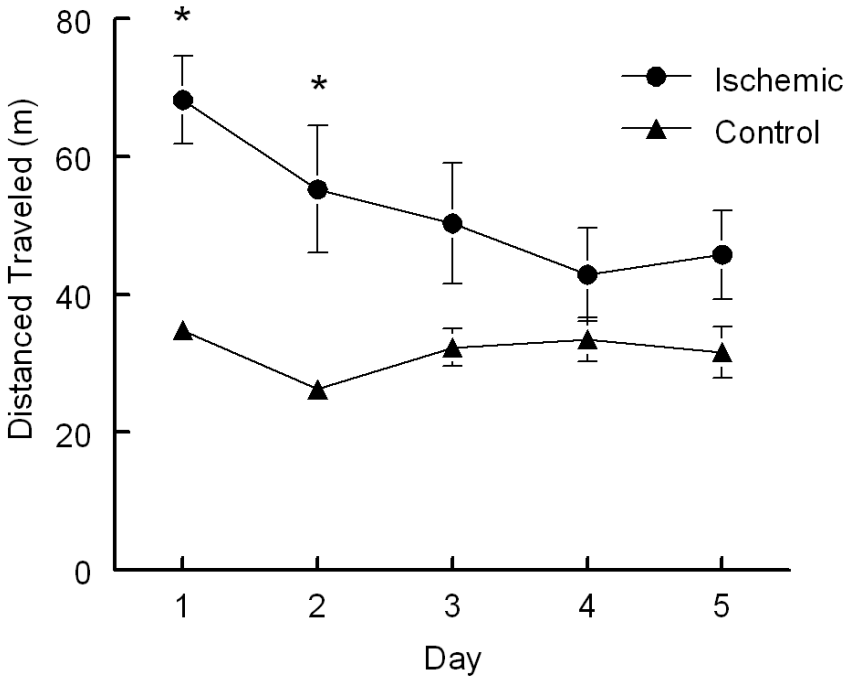

Figure 8. Experiment 2: Locomotor Activity. Locomotor activity of ischemic and control subjects during a 5 day testing period. Subjects were tested 5 min each day. Ischemic subjects showed significantly higher levels of activity on Day 1 and 2 of testing compared to control subjects. On Days 3-5, activity levels of ischemic and control subjects were no longer significantly different.

Spatial Recognition

In Phase 1 (familiarization), ischemic and control subjects spent about 50% of the time exploring the target object; exploratory ratio means were .47±.05 and .57±.03, respectively. In Phase 2 (test), both ischemic and control subjects spent more time exploring the object in the novel location; exploratory ratio means were .63±.09 and .78±.05, respectively (see Figure 9).

Comparing the groups across the two experimental phases revealed a main effect of Phase, ($F(1, 11) = .9.747$, $p = .01$, $\eta_p^2 = .470$) and Treatment, ($F(1, 11) = 5.899$, $p = .033$, $\eta_p^2 = .349$). The interaction was not significant, ($F(1, 11) = .247$, $p = .629$, $\eta_p^2 = .022$).

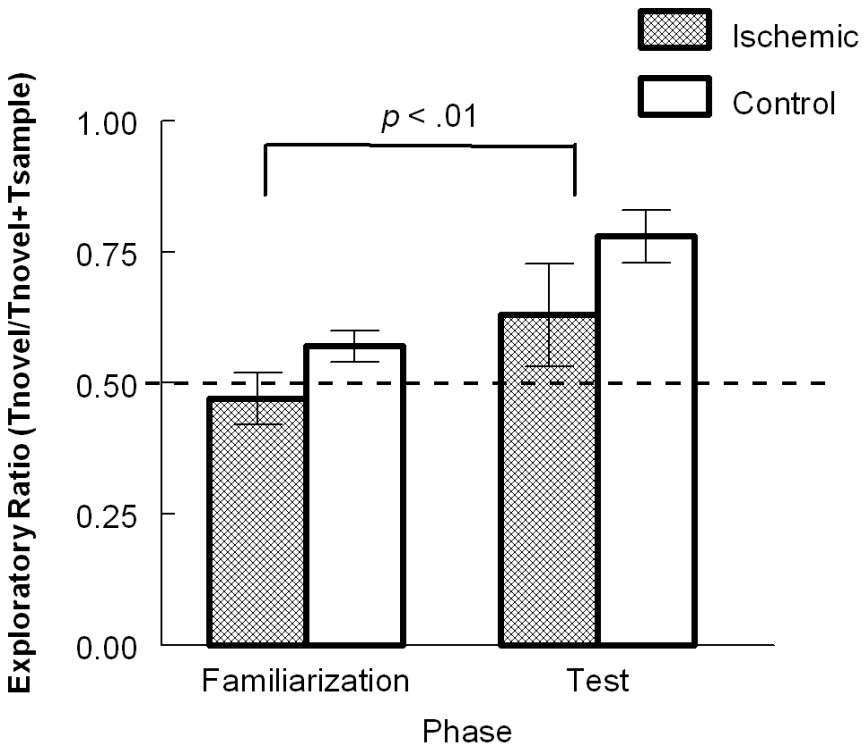

Figure 9. Experiment 2: Spatial Recognition. Exploratory ratios were compared across the familiarization and test phases. In the familiarization phase (left bars), subjects were exposed to two identical objects. Ischemic and control subjects explored the target object about 50% of the time. In the test phase (right bars), subjects were exposed to the same objects in the test phase but one of those objects was placed in a novel location. Both groups showed a significant preference for the object in the novel location.

The activity results from Experiment 2 revealed a pattern similar to that of Experiment 1; ischemic subjects were significantly more active compared to controls at

the beginning of the habituation phase, and this difference dissipated with repeated exposure to the testing environment.

Exploratory behavior during the spatial recognition task revealed that both groups were able to recognize that a familiar object had been moved to a novel location. This finding for control subjects was expected based on previous research (Mumby et al., 2002; Volpe et al., 1992; Duva et al., 1997). Damage to the hippocampus has been previously shown to impair spatial recognition in the rat (Mumby et al., 2002; Volpe et al., 1992; Duva et al., 1997). The results of the present study are inconsistent with these previous findings.

GENERAL DISCUSSION

Both experiments replicated the finding that damage to the hippocampus via ischemic insult causes hyperactivity in ischemic subjects (Plamondon & Kahn, 2005; Plamondon et al., 2008; Milot and Plamondon, 2008; Babcock et al., 1993; Janać et al., 2006; Chandler et al., 1985; Mileson & Schwartz, 1991; Wang & Corbett, 1990). Control subjects showed relatively stable activity levels throughout habituation; whereas, ischemic subjects showed significantly more locomotor activity. However, by the end of the habituation period activity levels between the groups were no longer significantly different from each other. Hyperactivity is a robust effect that can be replicated and is an excellent indicator of successful ischemic insult before ischemia can be verified histologically.

Plamondon and Khan (2005) argued that ischemia induced hyperactivity is an indicator of decreased anxiety. In this study rats underwent an ischemic insult and then were tested in an open field apparatus where they demonstrated heightened locomotor activity compared to control subjects. Next, ischemic and control subjects were tested in an elevated plus maze. Results revealed that ischemic subjects spent significantly more time in the open arms of the maze compared to control subjects. Time spent in the open arms is indicative of decreased levels of anxiety. While the exact process has yet to be elucidated, ischemia induced hyperactivity may be considered a form of disinhibition where ischemic subjects demonstrate less anxiety than control subjects.

In the Object recognition task, both ischemic and control subjects exhibited a significant preference for the novel object in the environment. Interestingly, ischemic subjects spent more time exploring the novel object compared to control subjects. While this difference was not significantly different it may be due to decreased anxiety in ischemic subjects. In the wild, rodents are a prey species where approaching novelty with caution can be crucial for survival. It is possible that ischemic damage affects the brain in such a way as to make subjects less wary of novelty in the environment and therefore they spent more time exploring the novel object compared to control subjects.

In Experiment 1, both ischemic and control subjects were able to recognize a novel object in a familiar environment. Moving from Phase 1 (familiarization) to Phase 2 (test with novel object) both groups showed a significant preference shift for the novel object. This result is consistent with research that demonstrates neurotoxic damage to the CA1 region of the hippocampus is insufficient to produce deficits in object recognition tasks (Duva et al., 1997; Gaskin et al., 2003; Mumby et al., 2002). However, this finding is inconsistent with research that demonstrated ischemic damage to the hippocampus is sufficient to cause impairment in object recognition tasks (Bachevalier & Mishkin, 1989; Wood et al., 1993; Zola-Morgan et al., 1992, Wappler et al., 2009).

To reconcile these conflicting results, researchers have posited many ideas to explain why ischemic insult to the hippocampus might produce behavioral impairment when hippocampal lesions fail to produce the same behavioral deficit. Importantly, it has been shown that the methods used to quantify hippocampal damage in ischemia studies have not taken into account damage that may have occurred outside the hippocampus.

Extrahippocampal damage has been documented in ischemia research and it is crucial that it is taken into account when interpreting behavioral deficits in object recognition tasks like DNMS (Nunn & Hodges, 1994; Corbett & Nurse, 1998).

In addition, researchers have determined that any number of abnormalities may present themselves after an ischemic insult that do not have any evident histological correlate. This phenomenon, termed "covert damage", applies when functional deficits are obtained without corresponding histological damage (Corbett & Nurse, 1998). Although investigating a functional deficit in the absence of discernible physical damage presented some difficulty Mumby et al. (1996) was able to investigate this issue. First, forebrain ischemia was induced in one group of rats while another group underwent neurotoxic hippocampal lesions. While the rats with hippocampal lesions rats exhibited DNMS performance equal to that before surgery, the ischemic subjects showed impairment. Next, some of the rats with hippocampal lesions then underwent cerebral ischemia while the remainder of the hippocampal-lesioned group underwent a sham procedure. After the surgeries, the neurotoxic lesion + ischemic insult subjects did not exhibit impaired performance on the DNMS task.

This study suggests that neurotoxic and ischemic lesions to the hippocampus are different. Based on this study, it is presumed that ischemic insult damage to the hippocampus wounded the brain in such a way as to bring about the aforementioned behavioral impairment. This differed from the damage incurred from lesions directly to the hippocampus because those lesions did not produce any behavioral impairment. When hippocampal-lesioned subjects were given an ischemic insult, deficits in object

recognition were expected. Researchers presumed that connections in the brain that were left untouched by lesion damage would suffer under the ischemic insult and deficits in the object recognition task would be seen. Amazingly, Mumby et al. (1996) did not observe deficits in object recognition. These results are somewhat surprising and they suggest that ischemia may not be sufficient to create behavioral deficits in object recognition tasks. Moreover, when considering results from ischemia studies it is important to consider the role of extrahippocampal structures that are often damaged when ischemia is induced. Therefore, the results presented here support the hypothesis that forebrain ischemia does not impair subsequent performance in object recognition tasks.

The spatial recognition task in Experiment 1 did not reveal any trends or patterns. There are at least two possible explanations for this result. The first is that because subjects were only exposed to the object configuration of Phase 1 (familiarization) for 5 min before the target object was moved, it is possible that subjects did not have sufficient time to encode the locations of the objects. The findings in Experiment 2 are consistent with this hypothesis since control animals with extended exposure to the objects exhibited a shift in exploratory behavior to the relocated object. The fact that ischemic subjects also showed a preference for the novel location is an interesting finding.

The second explanation for the results of the impaired spatial recognition performance in Experiment 1 is that the counterbalanced tasks across days was a confounding variable. The group that underwent the object task followed by the spatial task showed a tendency to explore one of two identical objects (in Phase 1) more than the other object in the spatial recognition task. A tendency to explore one of the objects more

during the habituation phase could indicate that the subjects were responding to what appeared to be a change in environmental configuration.

In Experiment 1, both the ischemic and control groups were split in half (Isch1, Isch2, Sham1, Sham2). Group 1 consisted of Isch1 and Sham1 whereas Group 2 consisted of Isch2 and Sham2. Both groups underwent one test (object or spatial recognition) on Days 1 and 2 of testing. Across testing Days 1 and 2 Group 1 underwent spatial then object testing while Group 2 underwent object then spatial testing. Because Group 2 underwent object then spatial testing (see Figure 10) subjects were confronted with one configuration of objects in both phases of object testing. When spatial testing followed then Phase 1 (familiarization) exposed subjects to a completely different *configuration of objects* from object testing. Subjects in Group 2 spent more time exploring object 2 because, compared to the objects' configuration in object testing, it was now in a novel location. Despite the fact that both objects in Phase 1 on spatial testing were identical and therefore should be explored equally, object 2 may have been explored more because it resided in a novel location compared to objects in the object task. Due to small subgroup sizes, it was not possible to statistically test these observed trends. Nevertheless, it seems likely that the counterbalance design used in Experiment 1 may have confounded the results.

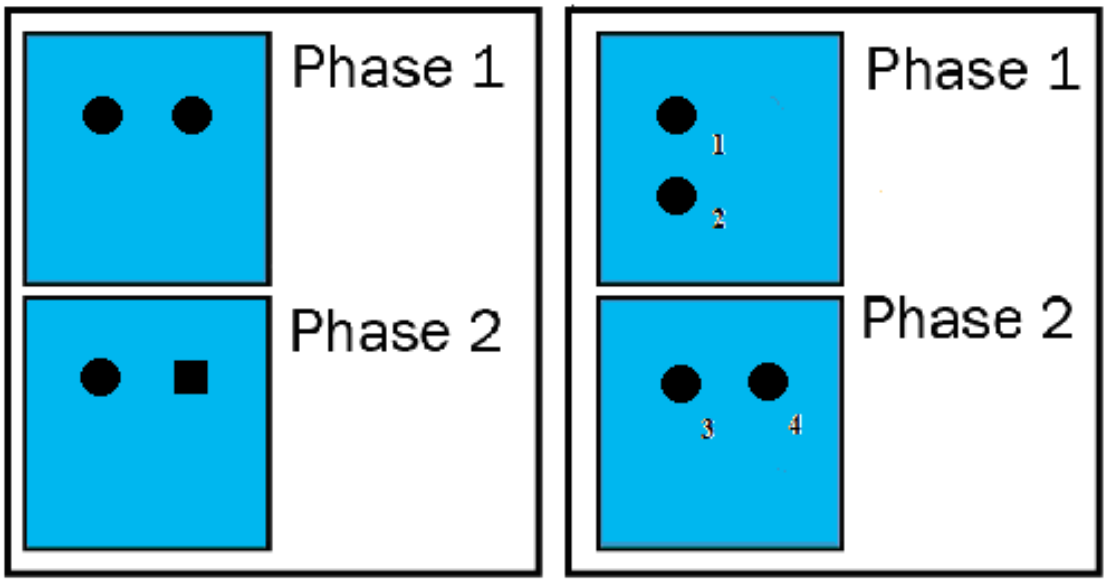

Figure 10. Object and Spatial Recognition Object Configurations. Left panel: Object recognition task. In both Phases 1 and 2 subjects encountered the same general configuration of objects (upper left and upper right locations remained constant throughout testing phases). Right panel: Spatial recognition task. Phase 1 presented a novel configuration (upper left, lower left) when the spatial task followed the object task. When subjects completed tasks in this order they spent more time exploring object 2, not because it was novel compared to object 1, but because the configuration of objects was novel in comparison to the general configuration of objects in the object recognition task.

Experiment 2 revealed that both ischemic and control subjects were able to recognize an object in an updated location. While performance on this task was not impaired by ischemic damage to the hippocampus, activity levels were different during initial exposure to the apparatus. One conclusion is that the spatial recognition task used in the present study is not sensitive to ischemic brain damage. An alternative possibility is that repetitive exposure to the test objects (Experiment 2) masked the effects of ischemia. In other words, allowing subjects additional time to explore the apparatus with the test objects may have given ischemic subjects enough time to establish the location of

the objects. Future research aimed at evaluating the importance of prior experience with the test objects is needed.

The findings of the present set of experiments extended the current literature regarding the effects of ischemia on object and spatial recognition tasks. This research also demonstrates the utility of these tasks in a different rodent model. Beyond the current studies presented here, establishing and refining the model will constitute some of the future research conducted in this laboratory.

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