

HIPPOCAMPAL DAMAGE AND NOVELTY PREFERENCE IN THE ISCHEMIC
GERBIL: DISSOCIATING OBJECT AND ARRANGEMENT MEMORY

by

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ABSTRACT

The most insidious consequences of transient ischemia are its effect on the hippocampus and the memory systems it serves. The novelty preference test is a direct measure of memory function and has been used in the rat and primate animal models. The gerbil animal model has been used extensively to study the mechanism of ischemic brain damage; however, the novelty preference paradigm has not been used to study memory impairment in this species. In addition, the novelty preference paradigm has not been tested with models of ischemia. In the present experiment, Mongolian gerbils were tested in two different types of novelty-preference tasks (Object and Arrangement) following either ischemic insult or a sham control surgery. There was no significant difference in preference for novel objects between ischemic and sham groups. However, ischemic gerbils showed a significant decrease in preference for novel arrangements relative to shams. These findings are consistent with previous studies that have demonstrated the dissociation between object and arrangement memory using rats and primates. The results of the present study indicate that the novelty preference paradigm can be successfully used with the gerbil model for ischemic brain damage to study memory impairment and neuroprotective agents.

INTRODUCTION

Stroke

Stroke is the third leading cause of death in the United States (Mayo Clinic, 2005) and can result from any cardiovascular disease or event that affects the arteries within, or leading to, the brain. In general, a stroke occurs when the brain fails to receive blood from the vascular system. More specifically, a stroke occurs when the brain is deprived of the nutrients delivered by the blood (e.g., glucose and oxygen) that are necessary to carry out normal metabolic functioning. Without oxygenated blood, the cells in the brain will begin to die after only a few minutes. Mayo Clinic (2005) estimated that 700,000 Americans experience a stroke each year and that about 160,000 of these people die as a direct result of the stroke or its complications.

Stroke is a broad term that can be applied to several clinically distinct phenomena. Stroke has two general causes: Blood flow can be interrupted, or it can be diverted. An ischemic stroke occurs when blood flow to the brain is interrupted due to either a blockage in an artery or cardiac arrest. A hemorrhagic stroke occurs when blood flow to the brain is diverted because of a rupture in an artery. A hemorrhagic stroke is often devastating. The brain is extremely sensitive to bleeding, both the blood itself and the pressure it exerts on the brain as it accumulates in the skull can be severely damaging. Although the hemorrhagic stroke can be further differentiated and has its own etiology, it is beyond the scope of the present study to go much further. However, it is important to note that treatment options are more limited and the prognosis generally much poorer for

someone suffering a hemorrhagic stroke. Unfortunately, hemorrhagic strokes often result in death (the American Heart Association).

Ischemic strokes accounts for about 80% of all strokes (Mayo Clinic; the American Heart Association). There are many causes of an ischemic insult. A clot in an artery that prevents blood from reaching the brain is referred to as a thrombus. A clot that travels through the arteries to block blood flow in a distant location, like the brain, is referred to as an embolus. Aside from a direct clot, ischemia can also be caused by a narrowing of the arteries leading to the brain, referred to as carotid stenosis. Finally, ischemia can be traced to several cardiac diseases that include arrhythmia and inflammation.

Cerebral ischemia can be further differentiated as either focal or global (the American Stroke Association). Focal ischemia results from an interruption of blood flow to only a portion of the brain. This interruption can happen when a clot dislodges from an artery wall and travels to the brain where it lodges and causes very selective, or focused, cell death. This type of ischemia can also be caused by an interruption of blood flow outside the brain, such as when one side of the common carotid becomes blocked and restricts blood flow to an entire hemisphere of the brain. Global ischemia is characterized by a restriction of blood flow to the entire brain. Cardiac arrest and other conditions that completely disrupt blood flow to the brain can lead to global ischemia.

While an ischemic episode does not generally result in death, its consequences are in many ways equally devastating. All the cells of the brain require nutrients and oxygen to survive, but the cells of the hippocampus, specifically in the dorsal region of the

hippocampus commonly referred to as the Cornu Ammonis (CA) 1 region, are particularly sensitive to ischemic insult (Ito, Spatz, Walker, & Klatzo, 1975). Even a brief ischemic episode of only 5 min can lead to cell death in the CA1 region of the hippocampus in animal models (Kirino, 1982). Though the cell death caused by this type of stroke can be restricted to the hippocampus, the cognitive and behavioral effects can be far reaching.

Stroke research has gained considerable ground since the 1950s when scientists first identified the buildup of fatty tissue along the inner wall of an artery (atherosclerosis) as a possible cause for some types of stroke. Today, we are aware of numerous risk factors that either directly or indirectly increase a person's susceptibility to stroke: age, heredity, gender, cigarette smoking, diabetes mellitus, high blood cholesterol, previous stroke, and obesity are prominent among the list (American Heart Association). Knowledge of these risk factors has given rise to a host of preventative measures to lower a person's risk for stroke. Though one cannot change his or her age, convincing a person with diabetes to quit smoking is at least a step in the right direction.

Research has provided similar insights for the treatment of stroke. In the 1970's, before the introduction of computerized tomography (CT), health care professionals had little to distinguish between a hemorrhagic and ischemic stroke. Though aspirin had been shown effective at lowering ischemic stroke risk because of its anti-coagulant properties, if given to someone suffering a hemorrhagic stroke its anti-coagulant properties would be lethal. Therefore, aspirin was only a viable option when a stroke could be successfully diagnosed as an ischemic episode (the American Heart Association). Today, CT

technologies have disseminated beyond major hospitals and are starting to reach into rural areas of the country making stroke screening more accessible and thus anti-coagulant agents, such as aspirin, a reliable option for the immediate treatment of an ischemic stroke.

One of the most exciting advances in stroke treatment is the ability to treat a patient before and/or after an ischemic episode. In the past, a victim of an ischemic stroke would have to find immediate treatment, that is *during* the ischemic episode, in order to receive anti-coagulants that could help blood flow through the occluded vessel and back to the brain before the brain suffered any damage. Today, neuroprotective agents are being researched that could either be administered under the presumption that one is likely to have a stroke and needs protection or administered sometime after the stroke episode, both of which afford health professionals more time for diagnostic and logistic concerns (e.g. does the patient need to be moved from a rural area to a nearby hospital with CT access).

For instance, Dong, Moody-Corbett, Colbourne, Pittman, and Corbett (2001) have convincingly demonstrated the neuroprotective effects of hypothermia after an ischemic insult. Ischemic gerbils were treated one full hour after the ischemic episode with 2 days of hypothermia. After 5 weeks, the animals were euthanized and electrophysiological recordings were taken of the hippocampal CA1 region; the hypothermic treatment afforded virtually complete histological protection, whereas the ischemic gerbils without the hypothermic treatment exhibited nearly complete loss of the CA1 neurons. The clinical applications of reducing temperature for post-ischemic neuronal protection are

reviewed elsewhere with regard for current drug therapies (Colbourne, Sutherland, & Corbett, 1997).

Hippocampus and Memory

The hippocampus is widely accepted as a key neurological structure for learning and memory; however, the exact role of the hippocampus in learning and memory remains unknown (Eichenbaum, Otto, & Cohen, 1992). Early case studies with patients such as H.M. (Scoville & Milner, 1957) revealed a complex role for the hippocampus and its associated structures in the temporal lobe. Although H.M. failed to recall or even recognize nearly every item presented to him (e.g. words, digits, paragraphs, faces, names, maze routes, spatial layouts, geometric shapes, nonsense patterns, nonsense syllables, clicks, tunes, tones, as well as public and personal events) (Cohen, 1984), he retained much of his perceptual, cognitive, linguistic, and motor capacities (Scoville & Milner, 1957). It became apparent that the hippocampus and its associated structures in the temporal lobe are only critical for certain aspects of memory.

There is accumulating evidence that many of the cells in the hippocampus are *place* cells, cells that spike consistently only when the animal is in one specific location and after repeated exposure that particular environment (O'Keefe & Dostrovsky, 1971; O'Keefe & Speakman, 1987). However, there has also been research showing non-spatial correlates to hippocampal cell spikes (Eichenbaum & Cohen, 1988; Wible, Findling, Shapiro, Lang, Crane, & Olton, 1986; Wood, Dudchenko, & Eichenbaum, 1999). The

configural association theory (Sutherland & Rudy, 1989) attempts to bring these findings together and explain the role of the hippocampus in learning and memory.

With the configural association theory, Sutherland and Rudy suggested that the hippocampus is responsible for the recognition of relational identities. A relational identity is derived from the characteristics of interaction that the object has with the environment, not its own internal characteristics. Intrinsic characteristics (e.g., weight, height, color, and shape) are the basis of object identity; extrinsic characteristics (e.g., orientation and proximity as well as abstractions like biggest and loudest) are the basis of relational identity.

Lesion studies have shed light on the role of the hippocampus by dissociating relational memory from object memory using the novelty preference paradigm with rats (Busey, Duck, Muir, & Aggleton, 2000; Busey, Muir, & Aggleton, 1999; Moses, Sutherland, & McDonald, 2001). Moses, Sutherland, and McDonald's (2001) lesion studies of the hippocampus and amygdala complete the differentiation of the two memory systems through double dissociation of object and arrangement memory. Rats with hippocampal lesions showed a decrease in preference for novel arrangements, while maintaining preference for novel objects; whereas, rats with lesions of the amygdala showed a decrease in preference for novel objects, while maintaining preference for novel arrangements. This research points to the amygdala as being a necessary component of object memory and to the hippocampus as being a necessary component of relational memory.

From the perspective of understanding the behavioral consequences of transient cerebral ischemia, the dissociation of these memory systems is very interesting. If a treatment is found to be neuroprotective through *in vitro* studies, the treatment can be tested using the novelty preference paradigm. This would allow researchers to determine if the treatment preserved the integrity of the hippocampus and preserved the relational identity memory system that the hippocampus serves.

Novelty Preference Paradigm

Novelty preference can be tested in incidental learning paradigms where the animals are not explicitly taught any relationship between their behavior and the cues in the environment. In these tasks, animals are allowed to freely explore an environment and natural responses are observed and recorded. Moses, Sutherland, and McDonald (2002) argued that two types of behaviors can be recorded in an incidental learning paradigm. The first type of behavior is characterized by being disproportionately targeted toward novel stimuli, such as approach behavior. The second type of behavior that can be recorded is characterized by its lack of selectivity towards cues in the environment. This latter type of behavior is said to be indirect. Indirect behaviors, such as locomotor activity, are by definition mediated by a second component. In the case of the hyperactive locomotor behavior observed in ischemic rodents with damage to the hippocampus, the interpretation is that the increased locomotion is indicative of the animal's attempt to familiarize with its surroundings (Babcock, Baker, & Lovec, 1993). In other words, the

rodents are moving more because they are stuck in search mode, continually investigating old and new objects indiscriminately.

The novelty preference paradigm was originally developed by Ennaceur and Delacour (1988) to investigate the observation that rhesus monkeys exhibit altered responses to objects and contexts following bilateral temporal lobectomy (Kluver & Bucy, 1939). When Kluver and Bucy removed the temporal lobes of their subjects, they removed both the hippocampus and amygdala. Without the hippocampus and amygdala, Kluver and Bucy's monkeys could no longer recognize objects in the world, neither by intrinsic nor extrinsic characteristics. Their lack of object and relational memory was termed *psychic blindness* (Kluver & Bucy, 1939). However, this technique represented a crude method for establishing a neural map for cognitive function. A refined technique with more precision for targeting neural structures was required to understand the behaviors associated with the structures of the temporal lobes.

Ablating or lesioning the brain with a neurotoxic agent causes isolated and controlled cell death. In fact, the majority of research using the novelty preference paradigm has involved studies in which the brain region is lesioned with a neurotoxin. These studies remain extremely beneficial for anatomically mapping cognitive function. However, there are important differences between lesion studies of the hippocampus, where cell death is caused by introducing a neurotoxin, and ischemic studies of the hippocampus, where cell death is caused by cellular process resulting from anoxia and reperfusion. Using an electrolytic sensorimotor cortex lesion in rats, Kozłowski, James, and Schallert (1996) found that increased use of the limb associated with the lesion

resulted in a significantly greater cell death; whereas Colbourne, Auer, & Sutherland, (1998) found that repeated behavioral testing did not exacerbate cell death in ischemic gerbils. The potential difference in the mechanisms of cell death increases the need to replicate the findings of lesion studies with the models of ischemia. As of yet, the novelty preference paradigm has not been tested in the gerbil model by lesion or ischemic damage to the hippocampus

Ischemia and the Gerbil Model

The rodent models of ischemic insult include the rat and gerbil. These animals are used extensively in stroke research both in the search for treatment options and in uncovering the mechanism(s) behind neuronal cell death caused by stroke. In particular, global cerebral ischemia in rodents is an excellent research model for cardiac arrest encephalopathy, a type of transient cerebral ischemia in humans (Colbourne et al., 1998).

There are several models of global cerebral ischemia in the rat species (Ginsberg & Busto, 1989). There are two that closely resemble global cerebral ischemia in the gerbil species. The two-vessel occlusion model of ischemia isolates and occludes the common carotid artery (CCA) while inducing hypotension. The four-vessel occlusion model of ischemia occludes the CCA and permanently occludes the vertebral arteries through cauterization.

The primary advantage of using the gerbil model is that the surgical procedure for inducing global cerebral ischemia, the bilateral CCA occlusion, is much simpler and permits the study of many animals (Ginsberg & Busto, 1989). The lack of posterior

communicating arteries in the gerbil species (Levine & Sohn, 1969) makes them highly susceptible to these types of ischemia (Berry, Wisniewski, Svarzbein, & Baez, 1975).

The gerbil's susceptibility to ischemia and the simplicity of the CCA occlusion procedures renders the gerbil model an excellent candidate for screening neuroprotective agents (Ginsberg & Busto, 1989).

Although there are also notable disadvantages of the gerbil model, such as their relatively small size, many of those disadvantages are ameliorated by the intent of the novelty preference paradigm. Since the direct and indirect measurements of behavior afforded by the novelty preference paradigm are meant to test cognitive function and are not concerned with investigating pathomechanisms of ischemia, monitoring procedures (e.g. arterial venous catheterization and endotracheal intubation), which are made extremely difficult because of the gerbils small size, may be excluded from the research design. The gerbils' susceptibility to seizures that has been established is often viewed as a disadvantage of the model (Kaplan & Mizejeski, 1972; Loskota, Lomax, & Rich, 1974). However, in the case of the novelty preference paradigm, the gerbils' behaviors are monitored under several conditions. Behavioral monitoring allows researchers to visually identify seizure activity (e.g., hemiparesis, circling, and clonic convulsions) during testing and confidently exclude them from normal analysis.

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Rationale

The purpose of the present study was to demonstrate that the gerbil animal model can show dissociation of object and location memory in the novelty preference paradigm. The simplicity of the CCA procedure in the gerbil model of cerebral ischemia combined with the direct and indirect measures of behavior offered by the novelty preference paradigm would make an excellent platform for researching neuroprotective agents.

In the present study, ischemic gerbils are expected to show the same preference for novel objects as gerbils in the sham group. Consistent with the literature of object and arrangement memory using the rat model, gerbils will show the typical preference for novel objects by spending more time approaching those objects (Moses, Sutherland, & McDonald, 2002; Mumby et al., 2002). More importantly, ischemic gerbils will show a significant decrease in preference for novel arrangements by spending less time approaching the novel arrangement as compared with gerbils in the sham group.

METHOD

Subjects

The subjects of the present study were 14 male Mongolian gerbils (*Meriones unguiculatus*). Each gerbil was housed individually with continuous access to food and water while under a 12:12 light-dark cycle (light onset at 7:00 am) and a controlled temperature environment (23° C). The subjects weighed approximately 80 g at the time of surgery. The University Institution Animal Care and Use Committee approved all experimental animal procedures.

Surgery

The gerbils were anaesthetized using isoflurane. Core-body temperature was maintained between 37-38° C during surgery using a homeothermic blanket (Harvard Apparatus, South Natick, USA). A midline incision was made in the neck and the common carotid arteries were isolated, then occluded for 5 min using 85-gm pressure aneurysm clips (ISCH; n = 8). A second group of gerbils (SHAM; n = 6) underwent the identical surgery except that the carotid arteries were not clamped. The incision was sutured and each gerbil was placed in a warmed cage and observed for 30 min. Tylenol (8 mg/ml) was added to the drinking water to provide postoperative analgesia.

Apparatus

A digital video camera was used in conjunction with a commercial tracking program (ANY Maze, Stoelting Co.) to collect behavioral data during both phases of the experiment. Some data were coded by a research assistant blinded to the conditions using a standard coding program, E-prime (Schneider, Eschman, & Zuccolotto, 2002).

Both location and object tasks were performed in an open field arena (77 cm x 77 cm). The open field arena walls were constructed of particleboard and heavy white mat paper to enhance illumination and video tracking. The arena floor was constructed of a mesh wire to allow waste to fall out and away from the apparatus. The arena itself sat approximately 3 ft off the floor to allow researchers to sit out of view of the subjects and monitor each experiment without confounding distal cues in the environment.

The five stimuli objects were composed of metal, plastic, or wood (see Appendix) and were pseudo-randomized in their use throughout the experiment. No single object appeared in the same “position” of the experiment more than 50% of the time. In addition, there were two copies of each stimulus so that the objects could wash and dried between experimental phases in a 10% ETOH solution. This prevented biological markers (urine, mucus, hormones, etc) from confounding the proximal cues in the environment. The height of the objects ranged from 5-15 cm, and the width of the objects ranged from 5-10 cm.

Procedure

In order to maximize any novelty the gerbils would perceive of the stimuli, the subjects were introduced to the open field apparatus three days prior to the novelty preference tests and approximately seven days after surgery. Subjects were placed in the center of the open field apparatus and allowed to explore for 5 min and were then returned to their home cages.

Two different types of novelty-preference tasks were employed to evaluate memory: novel object preference and novel arrangement preference. For both the object and location preference tests, the objects were spaced greater than 10 cm from any wall to prevent escape behavior from being misinterpreted as approach behavior; furthermore, the objects were kept at least 30 cm from each other in the arena.

Both the object and arrangement novelty preference test consisted of a 5min familiarization phase followed by a 3 min ITI and ended with a 5 min preference phase in which a novel object or arrangement was introduced. The gerbils were placed in the arena from the same side and near the center with their head pointed away from the experimenter. During the ITI, each gerbil was removed from the arena and returned to its home cage. For the preference phase, gerbils were placed back into the arena with the same regard for placement and orientation and allowed to explore the apparatus for 5 min.

In the familiarization phase of the object novelty preference test, the gerbil was placed into the arena with two identical objects (Figure 1A). During the ITI,

experimenters replaced one of the objects with a novel object. In the preference phase of the object recognition task, the gerbil was placed back into the arena with a familiar object and a novel object (Figure 1B).

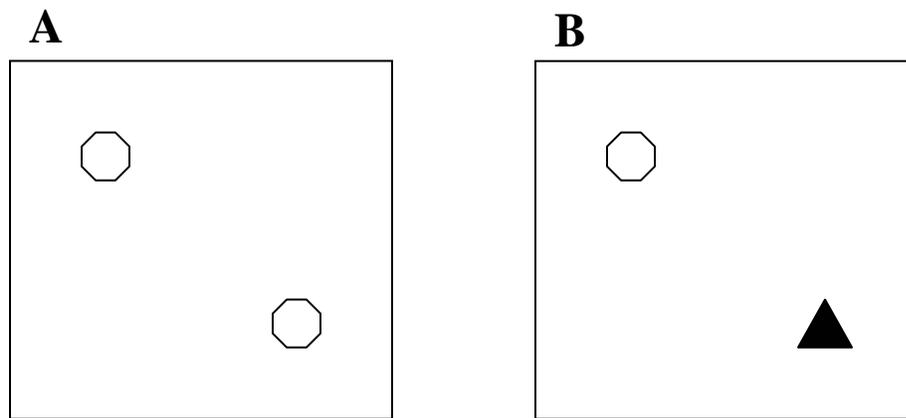


Figure 1. Schematic of the Novel Object Preference Test at the Familiarization (A) and Preference (B) phases. In the Familiarization phase (A), two identical objects are presented concurrently within the open field apparatus. In the Preference phase (B), one of the objects is replaced with a different object to introduce novelty.

In the familiarization phase of the arrangement novelty preference test, the gerbil was placed into the arena, again with two identical objects (Figure 2A). During the ITI, experimenters move one of the familiar objects to a new location creating a novel arrangement for that object. In the preference phase of the arrangement novelty preference test, the gerbil was placed back into the arena, now with an object that had a relatively novel arrangement to proximal and distal cues and an object that had a relatively familiar arrangement to proximal and distal cues (Figure 2B).

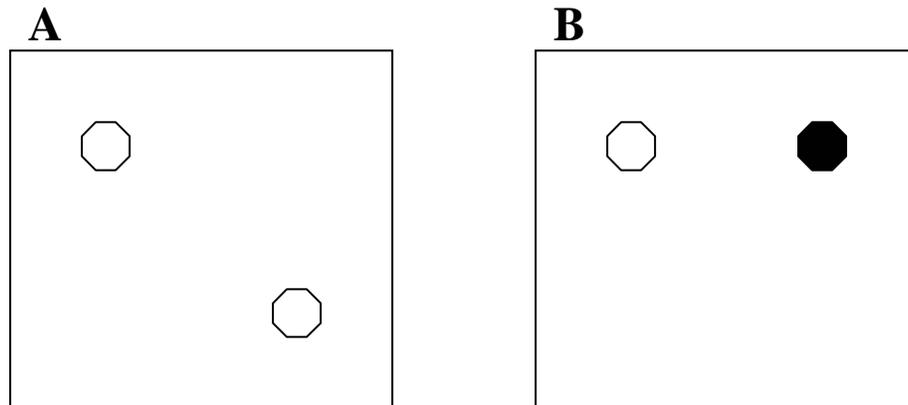


Figure 2. Schematic of the Novel Arrangement Preference Test at the Familiarization (A) and Preference (B) phases. In the Familiarization phase (A), two identical objects are presented concurrently within the open field apparatus. In the Preference phase (B), one of the objects is moved to a new location within the open field apparatus to introduce novelty.

Approach behavior was used as a measure of novelty preference (Moses, Sutherland, & McDonald, 2002; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002). Previous studies using approach behavior have defined it along several dimensions: proximity, head orientation, and activity. In the present study, gerbil activity was considered approach behavior if the following criteria were met: the gerbil was within 4 cm of the object, the gerbil's head was oriented 45° from the center of the object, and the gerbil was not physically engaging the object.

The data of the indirect measure (locomotor behavior), was collected with the aid of the ANYMaze tracking program. The direct measure, approach behavior, was analyzed from the video data by an undergraduate assistant unaware of the treatment conditions. Using E-prime, the undergraduate assistant coded the data using the criterion described above. The direct and indirect measures of behavior were evaluated using parametric statistics. The use of the one-tailed t test to determine the dissociation of object

from arrangement memory was used since previous research showing a decrease in preference for novel arrangements as a result of damage to the hippocampus (Aggleton, Hunt, & Rawlins, 1986; Cassady & Rawlins, 1997; Clark, Zola, & Squire, 2000; Dix & Aggleton, 1999; Sutherland & McDonald, 1990). Consequently, the prediction of diminished novelty preference of hippocampally damaged animals allowed for a directional hypothesis in this study, which satisfied the requirement for using the one tailed t-test (Hays, 1988; Keppel, 1991).

Following behavioral testing, subjects were euthanized with CO₂ and perfused with Phosphate Buffered Saline (PBS) followed by 4% paraformaldehyde. Brains were removed and post-fixed for at least 48 hr prior to collection of 40 µm vibratome sections through the hippocampal region. Sections were mounted on slides and stained with cresyl violet. Damage to the hippocampal CA1 region was evaluated without knowledge of the treatment condition by two independent observers using a 4 point rating scale (Babcock, Baker, & Lovec, 1993). A score of 0 (4-5 compact layers of normal neuronal bodies, 1 (4-5 compact layers with presence of some altered neurons), 2 (sparse neuronal bodies with “ghost spaces” and/or glial cells between them), 3 (complete absence or presence of only rare normal neuronal bodies with intense gliosis of the CA1 subfield) was assigned for each animal. Ratings were averaged and evaluated using nonparametric statistics (Kruskal-Wallis and Mann-Whitney U test; $p < 0.05$ considered significant).

RESULTS

Histological

At 21 days post surgery, animals were euthanized for histological evaluation (see Figure 3). All gerbils in the sham group had no detectable damage to the hippocampus and received scores of 0. The hippocampal neurons showed compact layering (see Figure 3A). Magnification of the CA1 region of the hippocampus revealed the expected tight band of compact neuronal bodies (see Figure 3B). In contrast, gerbils in the ischemic group exhibited extensive loss of pyramidal neurons within the hippocampal CA1 subfield. Four of the six gerbils in this group showed complete absence of normal neuronal bodies with intense gliosis of the CA1 subfield and received a score of 3 (see Figure 3C). Magnification of the CA1 sub-region revealed a remarkable absence of the compact band of neuronal bodies typical of the CA1 pyramidal cells (see Figure 3D). Evaluation of the cresyl violet staining ratings revealed a significant difference between sham and ischemic gerbils ($p < 0.05$).

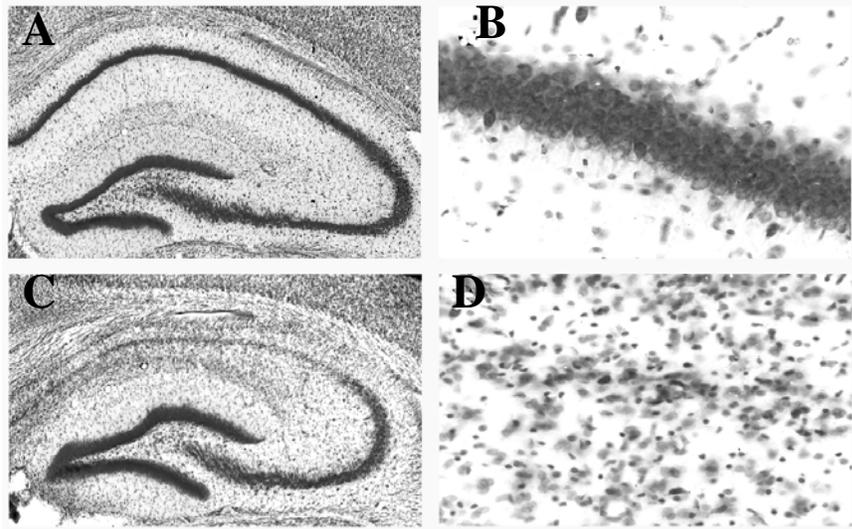


Figure 3. Histological Assessment of Hippocampus following Surgery. Sham gerbils showed normal neuronal bodies in the hippocampus (A) and tight clustering in the CA1 region (B). Ischemic gerbils showed intense gliosis in the CA1 region of the hippocampus (C), magnification revealed the complete absence of neuronal clustering in the CA1 region (D).

Behavioral

Locomotor behavior data were collected during the habituation phase of the experiment (see Figure 4). Ischemic gerbils were generally more active in the open-field apparatus 3 days after surgery. In the present study, locomotor behavior was evaluated as total distance traveled. Gerbils in the ischemic group were more active than gerbils in the sham group in terms of total distance traveled. The mean (\pm SEM) total distance traveled of the control gerbils was 72.7 (\pm 6) m, and the mean total distance traveled for ischemic gerbils was 129.4, (\pm 20) m. An independent samples *t* test revealed a significant difference in distance traveled between the sham and ischemic group, $t(10) = 2.63$, $p = 0.023$, $d = 1.52$.

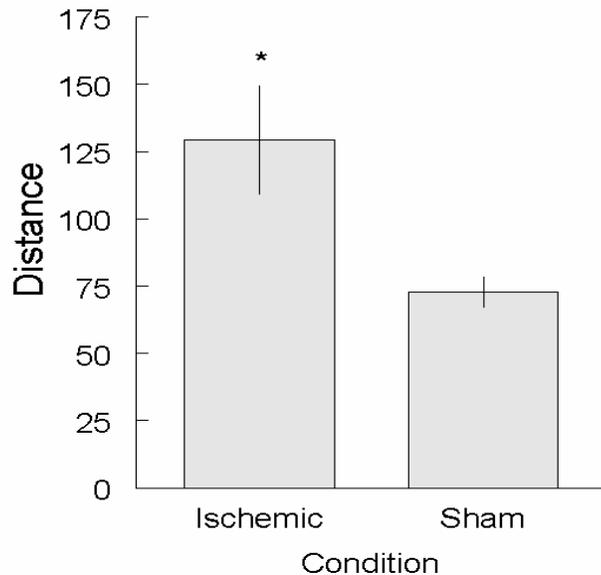


Figure 4. Mean Travel Distance of Sham and Ischemic Gerbils during Open-field Habituation Phase. Distance was measured in meters. Bar graphs include standard error values. Ischemic gerbils traveled a significantly greater distance than control gerbils.

Approach behavior collected during the preference phase of the novel object and arrangement preference tests was analyzed. Approach behavior time from trial 1 and trial 2 were not statically different and were averaged. The Exploratory Index was expressed as a percentage and calculated from the ratio of time spent approaching the novel stimuli over the total time the subject spent approaching all stimuli (both novel and familiar). In the novel object preference test, ischemic gerbils showed the same preference for novel objects that gerbils in the sham group exhibited (see Figure 5). The mean Exploratory

Index for ischemic gerbils was 53.04, (± 1.46), and the mean approach behavior percentage for gerbils in the sham group was 51.64, (± 4.24). An independent-samples t test revealed no significant difference in approach behavior between sham and ischemic gerbils, $t(10) = 0.311$, $p = 0.19$, $d = .16$.

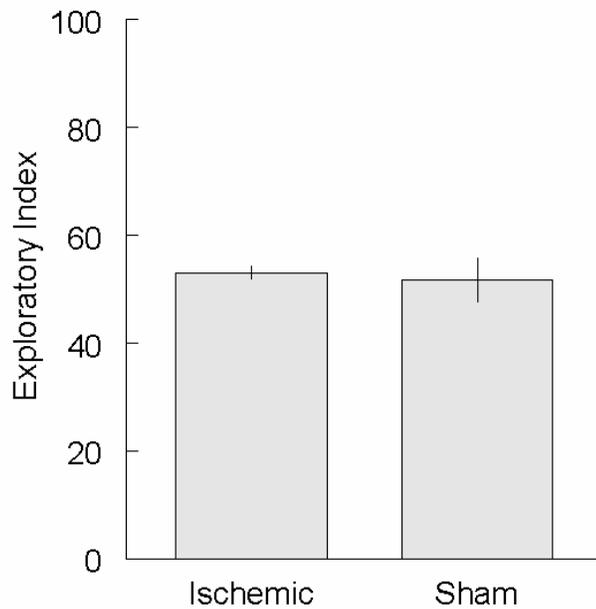


Figure 5. Mean Exploratory Index for Novel Objects of Sham and Ischemic Gerbils. The exploratory index was calculated by contrasting the amount of time spent approaching the novel object with the total amount of time spent engaging both novel and familiar objects as a percentage. Ischemic and sham gerbils showed no significant difference in preference for novel objects.

In the novel arrangement preference test, ischemic gerbils showed no preference for novelty as compared to gerbils in the sham. Sham gerbils exhibited a preference for novel arrangements by spending a greater percentage of their time approaching novel stimuli (see Fig. 6). The Exploratory Index for ischemic gerbils was 51.68, SEM = 3.27, and 60.90, SEM = 3.63, for gerbils in the sham group. An independent-samples t test revealed a significant difference in approach behavior between gerbils in the ischemic group and gerbils in the sham group, $t(10) = 1.88$, $p = 0.04$, $d = 1.09$ (one tailed).

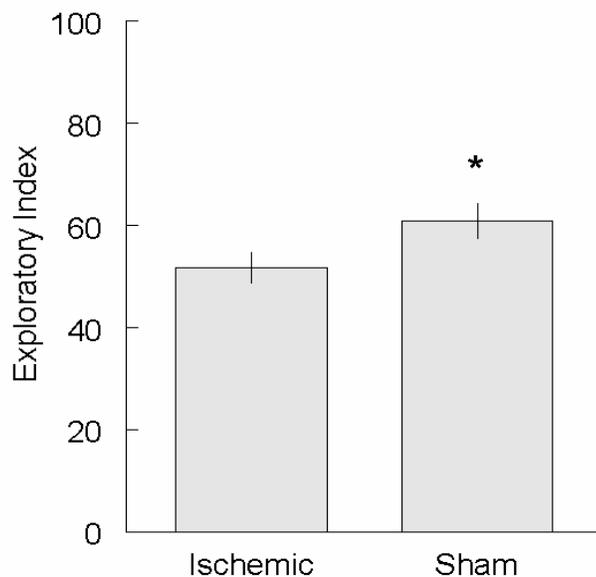


Figure 6. Mean Exploratory Index for Novel Arrangements of Sham and Ischemic Gerbils. The exploratory index was calculated by contrasting the amount of time spent approaching the novel arrangement with the total amount of time engaging both novel and familiar arrangements. As compared with control gerbils, ischemic gerbils spent significantly less time approaching novel arrangements.

DISCUSSION

The results of the present study indicate that the novelty preference paradigm can be used to evaluate gerbils following ischemic insult. Ischemic gerbils with damage to the hippocampus exhibited the same preference for novel objects that gerbils in the sham group exhibited but showed a significant decrease in preference for the novel arrangements as compared with gerbils in the sham group., these finding lend support for the use of the gerbil model with the novelty preference paradigm as a direct measure of cognitive function. This platform is of particular interest for the study neuroprotective agents that seek to protect the brain from ischemic damage and the memory systems affected by that damage.

Since the direct and indirect measurements of behavior afforded by the novelty preference paradigm are meant to test cognitive function and are not concerned with investigating pathomechanisms of ischemia, monitoring procedures, which are made extremely difficult because of the gerbils small size, may be excluded from the research design. Consequently, the relative simplicity of the bilateral CCA surgical procedure for inducing an ischemic episode in the gerbil model (Ginsberg & Busto, 1989; Levine & Sohn, 1969) could facilitate more efficient research. In short, the gerbil model provides reliable cerebral ischemia and requires the least invasive surgical procedure.

More importantly, as a direct measure of cognition, novelty preference provides another means of testing some of the alternative hypotheses for treatment effects (e.g., motivation and hunger). Currently, much of the behavioral research using the gerbil

model of ischemia is focused on indirect measures of cognitive function, such as locomotion. The underlying assumption of this research seems to be that a treatment's success in reducing hyperactive locomotion, which is typically associated with ischemic rodents, is indicative of successful protection of the supporting cognitive structures. The direct measure of cognition afforded by the novelty preference paradigm effectively removes the need for this assumption.

The statistical analysis in the present study was intended to demonstrate the potential for dissociating object and arrangement memory by showing that preference for novel objects would not change as a result of ischemic damage to the hippocampus. Close examination of the Index preference for novel object reveals that neither sham nor ischemic gerbils showed a preference for novel object stimuli by spending a majority of the time approaching the novel object. This is inconsistent with previous studies that have shown a preference for novel objects. Although the reason that gerbils in the present study failed to show a preference for novel objects is unclear, there are several possible explanations.

The introduction of ischemic damage as the cause of cell death could have influenced the preference for novel objects. Since, to the best of our knowledge, the novelty preference paradigm has not been used in the study of ischemic brain damage, it is possible that the mechanisms of cell death in cerebral ischemia or the selectivity of cell death in the CA1 region of the hippocampus may have played a part in the gerbils' lack of preference for novel objects.

It might also be useful to analyze approach behavior minute by minute, since gerbils with intact memory systems would be expected to have the capacity to familiarize and eventually recognize an object. After familiarization, approach behavior becomes something else entirely (e.g., habituation behaviors like sitting immobile in the center of the open field apparatus, engagement behaviors like standing against the object, or escape behaviors like running past the object while skirting the perimeter of the open field apparatus looking for a way out). Therefore, a minute-by-minute analysis of approach behavior might reveal more of the expected preference for novel objects during the initial minutes of the test.

Another possible explanation for the gerbils' lack of preference toward the novel objects was that the intrinsic characteristics of the objects (see Appendix) were not distinct enough to elicit or sustain the perception of novelty. Future studies of the novelty preference paradigm using the gerbil species should consider more salient changes in shape and color between the object stimuli in order to maximize the perception of novelty.

Another consideration is that this experiment analyzed the histological data of the hippocampus in an attempt to confirm the dissociation of object from arrangement memory. However, there was no attempt to gather histological data on the amygdala to confirm the neurological integrity which, for the present study, was only a presumption based on behavioral measures of novel object preference. In light of the importance of the amygdala to the object/arrangement novelty preference paradigm (Moses, Sutherland, &

McDonald, 2002), the histology of the amygdala should be analyzed despite the fact that it is usually excluded from the damage done by transient cerebral ischemia.

In addition, research has suggested the importance of the perirhinal cortex for the cohesion of both object and arrangement memory (Buckley, Gaffan, & Murray, 1997; Bussey, Muir, & Aggleton, 1999; Murray & Richmond, 2001). A recurring inference from studies involving lesions to or removal of the perirhinal cortex is that the memory of an object's defining characteristics, both intrinsic and extrinsic, do not become associated with the object itself. This deficit has been observed to lead to difficulty in distinguishing objects which are defined by numerous and complex characteristics. This difficulty in distinguishing complex stimuli increased dramatically when researchers increased the feature ambiguity of the objects (Murray & Bussey, 1999). Perhaps the perirhinal cortex should also be included in the histological assessment despite that fact that it is typically not involved in the cell death caused by global cerebral ischemia.

In the present study, an object's relational identity was rendered novel by manipulating proximal cues in the environment (i.e., the position of the object itself). A third test in the novelty preference paradigm, aside from object and location, has been used to measure the relational memory system served by the hippocampus (Mumby et al., 2002). Manipulating distal cues in the environment to introduce novelty might be beneficial to include for future research.

It is important to note that the subjects' exposure to the open field apparatus only reduced the perceived novelty of the test environment during the tests for object novelty and arrangement novelty preference; previous studies of ablative lesions have suggested

that mental and physical activity can be harmful during the sensitive recovery period following damage to the CA1 region of the hippocampus. However, it has been shown that post-ischemic activity on both the behavioral and cognitive levels does not exacerbate the damage caused by cerebral ischemia (Colbourne et al., 1998).

Colbourne et al.'s finding raises important questions about the parallels drawn from lesion studies onto research of cerebral ischemia, and it highlights the importance of replicating lesion study findings that seem to shed light on the neurogenesis and prognosis of ischemic cell damage. Though there are reasons to reserve judgment regarding the success of the novelty preference paradigm in dissociating object and arrangement memory in the gerbil species, the results of the present study will hopefully encourage future research of global cerebral ischemia to consider the gerbil species in order to replicate the success this direct behavioral measure has demonstrated in the rat species. Ultimately, the introduction of more refined behavioral measurements to the gerbil models of stroke, like the novelty preference paradigm, will promote the use of the gerbil model and, as a result, potentially increase the efficiency of researching neuroprotective agents of cerebral ischemia.

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APPENDIX A

PRESENTATION OF STIMULI





