

NEUROPROTECTIVE POTENTIAL OF METHAMPHETAMINE:
BEHAVIORAL AND HISTOLOGICAL ANALYSIS

by

Christy Samantha Star Weeden

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

In

Psychology

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2007

© COPYRIGHT

by

Christy Samantha Star Weeden

2007

All Rights Reserved

APPROVAL

of a thesis submitted by

Christy Samantha Star Weeden

This thesis has been read by each member of the thesis committee and had been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the Division of Graduate Education.

Dr. A. Michael Babcock

Approved for the Department of Psychology

Dr. Richard A. Block

Approved for the Division of Graduate Education

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University-Bozeman, I agree that the Library shall make it available for borrowers under the rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed by the U. S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole in parts may be granted only by the copyright holder.

Christy Samantha Star Weeden

April 2007

ACKNOWLEDGEMENTS

I would like to thank my academic advisor, Dr. Mike Babcock, for his time and dedication to the completion of this research experiment and guidance throughout the thesis writing process. Also, I would like to thank Denean Standing, Katie Coombs, Damon McNeill, Rachel Rabenberg and Mike Cranston for their assistance with the experiments. Thank you to Dr. Dave Poulsen from University of Montana for working on this joint-university research project. I also wish to thank my graduate thesis committee for their editing expertise and advice in preparation for this submission.

TABLE OF CONTENTS

1. INTRODUCTION	1
Hippocampus.....	1
History of Discovery.....	1
Hippocampal Anatomy.....	1
Relationship to Memory	3
Stroke	6
Historical Background	6
Stroke Defined.....	6
Models of Stroke	11
Rodent Models of Ischemia	12
Gerbil Models.....	12
Rat Models	13
Behavior Paradigms	15
Radial Arm Maze.....	15
Barnes Maze	17
Morris Water Maze	17
Open Field Apparatus	18
Neuroprotection and Deficits	19
General Deficits	19
Hyperactivity	19
Treatments	20
Hypothermia	20
Thrombolytics and Vasodilators	21
Free Radical Regulators	22
Amphetamines	23
Methamphetamine	24
2. STATEMENT OF PURPOSE.....	26
3. METHODS	27
Subjects	27
Surgery	27

TABLE OF CONTENTS-CONTINUED

Drug Treatment	28
Behavioral Testing.....	28
Histology.....	28
Experimental Design.....	29
4. RESULTS	31
Open-field Task	31
Distance Traveled	31
Speed.....	35
Line Crossings	38
Histology.....	41
5. DISCUSSION.....	44
REFERENCES.....	49

LIST OF FIGURES

Figure	Page
1. Track plots of distance traveled during open-field test.....	32
2. Distance traveled during open-field test	33
3. Distance traveled during open-field test based on test time segment	35
4. Average speed during open-field test	36
5. Average speed during open-field test based on test time segment	38
6. Number of line crossings during open-field test.....	40
7. Number of line crossings during open-field test based on test time segment.....	41
8. Representative photomicrographs of cresyl violet stained sections containing the hippocampal region.....	42

ABSTRACT

Stroke is a leading cause of death and ischemic stroke is the most common form. The deficits that follow ischemic stroke include memory and learning impairment. There are presently no treatments that can combat the effects of ischemia after the attack has occurred. Immediately following insult, locomotor activity increases in rodent models. The goal of the current research is to determine if methamphetamine administration following ischemic attack will have neuroprotective effects and prevent changes in locomotor behavior that are observed following insult. Ischemic insult was induced in gerbils by clamping the carotid arteries for 5 mins. Subjects in the sham surgical condition underwent similar surgical procedures, but the carotids were not clamped. Then, subjects were assigned randomly to saline or methamphetamine (5 mg/kg) injection groups. Drug treatment was administered within 2 mins of surgery. Measures of distance traveled, average speed, and number of line crossings were evaluated. Differences in levels of locomotion during the first and second halves of testing were also evaluated. Finally, sections containing the hippocampal CA1 region were rated on a 4-point scale for level of damage. Results show that subjects in the ischemic and saline condition traveled significantly further than those in the sham conditions and ischemic and methamphetamine condition. The speed of ischemic subjects treated with saline was significantly higher than ischemic subjects that received methamphetamine and sham conditions. Also, subjects in the ischemic and saline treatment group crossed more lines than sham and ischemic animals treated with methamphetamine. Analysis of cresyl violet-stained brain sections of ischemic animals treated with saline were rated as having less neuronal cell bodies in the CA 1 region. Ischemic and methamphetamine treated subjects' sections were similar to sham and saline treatment sections. These results suggest that methamphetamine, when injected after transient ischemic attack, may provide neuroprotection from damage that occurs to the CA1 region and prevent the impairments in locomotor behavior.

INTRODUCTION

Hippocampus

History of Discovery

The hippocampus, named by Giulio Cesare Aranzi (1564) for its exterior seahorse-like shape, is a structure located in each temporal lobe of the brain. Aranzi described the hippocampus as being associated with the sense of smell in 1587 (see Sano, 1997, for a review). This was widely accepted until Brodal (1947) demonstrated that behavioral conditioning of smell was not affected by hippocampal damage. The first suggestion that the hippocampus was involved in memory occurred around 1900. Vladimir Bekhterev described the first known link between the hippocampus and memory, based on case studies. The limbic system, named by Broca (1878), was initially linked to emotion in 1937 by James Papez, who suggested that emotional behavior is mediated by several cerebral structures, including the hippocampus, cingulate gyrus, thalamic nuclei and mammillary body. In 1952, the limbic system was expanded to include more structures and represents the current, complete system (MacLean, 1952).

Hippocampal Anatomy

The hippocampal formation is comprised of the dentate gyrus, Cornu Ammonis (fields CA1-CA3), the hilus (field CA4) and the subiculum. The hippocampus proper is

made up of fields CA1-CA3, but the formation refers to the collection of parts necessary for hippocampal function (Bayer, 1985). Therefore, the term “hippocampal formation” is frequently used to describe the processing pathways involved with information that the hippocampus receives.

The hippocampal formation receives information from several cortical regions. The perforant pathway projects from the entorhinal cortex and is considered the primary source of information to the hippocampus (Shepherd, 1998). The entorhinal cortex projects information to the hippocampus at different locations. Specifically, information is directed from the cortical region to the dentate gyrus and CA3 regions, while a secondary stream projects from the entorhinal cortex to the CA1 and subiculum regions. There is a specific, well-mapped, unidirectional pattern called the trisynaptic circuit in which hippocampal information is processed (Anderson, Holmqvist & Voorhoeve, 1966).

Information enters via the dentate gyrus from the entorhinal cortex then projects to the CA3 region via the mossy fiber pathway. Portions of the information are sent back to the dentate gyrus. The CA3 region is where the information is combined for processing, then sent to the CA1 region via Schaffer collateral fibers (Swanson, 1983). Therefore, information is processed at each step, starting at the dentate gyrus level, throughout each new level of the pathway (Amaral & Witter, 1989). The CA2 region does not redistribute processed information back to the beginning input step as the CA1 region does. Additionally, it is considerably more resistant to damage caused by oxygen deprivation than other CA regions. Finally, information that has been processed in the hippocampus

exits via projections to the cingulum bundle and fornix.

The CA1 region combines multiple streams of information derived from the CA3, entorhinal cortex and additional information from the thalamus. The information is then sent to the subiculum, CA3 and dentate gyrus inputs. The processes combine to form a system that merges information input into consolidated pieces. Finally, the CA1 region and entorhinal cortex send information to the subiculum, where it is distributed to other structures (See Mizuno & Giese, 2005). In addition to these two primary sources of input, there are several sub-level input systems that provide information to the hippocampus, including the amygdala, thalamus, raphe nuclei, tegmentum, central gray and the locus coeruleus. The input paths may provide the hippocampus with input necessary to create and recall emotional and spatial memories (e.g., Broadbent, Squire & Clark, 2004).

Relationship to Memory

The limbic system enhances the recall of memories linked to specific emotions in such a way that recalling the emotion or the memory enhances the likelihood that the other will be recollected as well. In this way, the hippocampus' role in the system has been implicated in memory consolidation (Eichenbaum & Cohen, 1992). Long term memories are considered either declarative (such as facts one can recall) or procedural (skills one acquires). The long-term memory system can store large quantities of information with potential for unlimited storage duration and is thought to be semantically encoded (Baddeley, 1966). Procedural memories are further classified as

being either semantic (such as word meanings, people's names) or episodic (events that one can recall happening in time) memories (Anderson, 1976). The hippocampus appears important in spatial navigation and declarative memory processes.

Environmental information is tied to specific orientations with the hippocampus during spatial memory encoding, creating a cognitive map (Tolman, 1948).

Episodic memory formation, such as memories about experienced events including time recognition is accepted to be a primary function of the hippocampal information processing system (Tulving, 1983). However, direct scientific support for this concept did not arise initially, possibly due to challenges associated with non-verbal laboratory animals. Recent evidence provided by work with Western Scrub-Jays demonstrates a memory for not only the placement of food caches, but the ability to discriminate which caches will perish sooner and thus time discrimination of those caches, suggesting that these animals demonstrate “what, where, and when” of episodic memory. It was concluded that the behavior represented “episodic-like” memory (Clayton & Dickinson, 1998). This evidence for episodic-like capabilities prompted scientists to re-evaluate ways to examine episodic memory in non-humans. Paradigms that emphasize the where/when phenomenon in animals such as rats have been successfully used to study this type of memory (Kart-Teke, De Souza Silva, Huston, & Dere, 2006; Eacott, Easton, & Zinkivskay, 2005).

There is some evidence suggesting that the hippocampus is responsible for storage and continued maintenance of declarative memories, such as those that are factual, as well as episodic memory formation (Eichenbaum, Otto, & Cohen, 1992). A different

hypothesis concerning the role of the hippocampus in memory is that it creates links between memories and emotion and after a period of “consolidation” the hippocampus no longer is involved in the process. In other words, after the information has been consolidated, it is sent as output to corresponding cortical areas where later retrieval does not involve the hippocampus. This would suggest that the hippocampus is not involved in the retrieval process, which must occur for pairing of new information into the old, retrieved form that is recalled during an event to create additional links based on new associations.

It is unclear as to which of these views of hippocampal processing roles is correct. Case studies of hippocampal damage demonstrate both antero and retrograde amnesia. Clinical findings may differ widely based upon variables, such as time, that are not controlled for in case-study fashion (Brna & Wilson, 1990). In the laboratory setting, rats trained in a standard Morris Water Maze task were found to perform poorly if hippocampal damage was introduced immediately after training, compared to rats that were spared damage until a later time, such as 8-12 wks after initial training (Sutherland & Arnold, 1987). These observations suggest that learned maze training was not impeded by the hippocampal damage after longer periods of time, supporting the concept that memories are stored elsewhere after consolidation by the hippocampus. Yet, not all memories are modified by the hippocampus. For example, studies have demonstrated that acquisition of new skills can occur in subjects whose hippocampus has been damaged suggesting that the formation of a procedural memory does not require the hippocampus (Backer, Cave, & Squire, 1991). Perhaps the most well known clinical case

study of hippocampal damage was that of “H.M.” who underwent a medio-temporal lobectomy to alleviate severe epilepsy, causing damage to his hippocampus. Although H.M. could not form spatial or episodic long-term memories, it was demonstrated that he could form procedural and semantic memories (Scoville & Milner, 1957).

Stroke

Historical Background

Stroke is the most common event that results in damage to cells within the Central Nervous System (CNS) (Johnston, Rothwell, Nguyen-Huynh, Giles, Elkins, Bernstein & Sidney, 2007). Hippocrates (460-379 B.C.) was one of the first to refer to the phenomenon of stroke that he termed as “apoplexy”, which means “struck down by violence” (Garrison, 1966). But the relationship between stroke and cerebral vasculature was not discovered until 1658, when Johann Jakof Wepfer reported that apoplexic death can be accompanied by bleeding in the brain. In addition, he suggested that if vessels were blocked within the brain, damage could be caused (see Librach, 1971).

Stroke Defined

Stroke is defined as a malfunction of blood flow through vessels of the brain. Also called “brain attacks”, strokes occur primarily because of either obstruction of the blood supply to a particular region of the brain or hemorrhage associated with ruptured blood

vessels. Stroke is the third-leading cause of death worldwide (American Heart Association, American Stroke Association, 2006). Approximately 700,000 people suffer a stroke annually, with approximately 70% of those being fatal. One person suffers a stroke every 45 secs and one dies every 3 mins. Although stroke is not the leading cause of death, it is the primary cause of long-term disability in the United States. These statistics suggest that stroke has a significant mortality rate. Of the survivors, many are left with serious debilitation.

Classification of stroke includes intracerebral hemorrhage, subarachnoid hemorrhage, and ischemic stroke. Intracerebral hemorrhage occurs when an artery ruptures, allowing blood to pool in the brain (Ay et al., 2005). Direct contact of blood to tissues caused by hemorrhage can lead to cell damage (Aschner & Kerper, 2000). Indirect damage may result from compression of tissue in the region of the bleed. Additional damage can result from a lack of blood flow to regions that are no longer perfused because of the hemorrhage. Subarachnoid hemorrhage is similar to intracerebral hemorrhage, except that the blood fills the subarachnoid space rather than cerebral tissues (Graham & Gennareli, 2000). Therefore, there is no direct contact toxicity to cerebral tissue, but the inability of blood to reach its original destination can result in cell death.

Ischemic stroke encompasses all non-bleed events that interfere with blood flow and/or oxygen to tissue, including the brain. This type of stroke is considered the most common form of attack, comprising roughly 80% of all strokes (Mohr, Caplan, & Melsk, 1978). Global ischemic events are ischemic attacks that affect the entire brain's ability to procure enough oxygen and energy to function properly. Although blood flow

is reduced to all parts of the brain during global ischemia, the hippocampus is the most sensitive to this type of insult. Focal ischemic events involve select areas of the brain afflicted with an ischemic insult. The location of deprivation will largely influence symptoms of the attack. Frequent symptoms include brief vision loss, speech difficulty, hemi paresis (single-sided limb weakness or numbness) and sometimes unconsciousness (Johnston et al., 2007). Regardless of onset location, focal ischemia is the most common human ischemic insult. A temporary insult is termed transient ischemia; real-world examples consist of victims that have been resuscitated after drowning, choking or environments that were oxygen-deprived. Ischemic attacks are viewed as warning signs of future cerebrovascular events, especially when ischemic attacks last longer than a few hrs. In fact, a scoring system called the “ABCD-squared” was created to predict the likelihood of future insult based on measures of certain factors including age, blood pressure, weakness and duration of attack, and diabetes diagnosis (Rothwell et al., 2005). Higher scores on this system indicate to medical staff that the chances of another attack is likely. The rating system has been used to determine what plan of action patients should pursue after the initial attack had been cared for in the emergency care setting.

The criteria that define transient ischemia have changed over the decades. A Committee formed by the National Institutes of Health in 1958 determined that the duration of a transient ischemic attack (TIA) could range from a few secs to several hrs, with the majority lasting several secs-10 mins (Winn, 2004). However, researchers using computed tomography (CT) to investigate patients originally diagnosed with transient ischemic attack reported that less than half were actually ischemic in nature. The

researchers then classified cases presented with attack durations of under 1 hr as ischemic, noting that ischemic attacks were likely to subside and were frequently recurrent (Ascheson & Hutchinson, 1964). In 1975, the National Institute of Neurologic Diseases and Stroke, based on the recommendations of the Committee on Cerebrovascular Diseases, re-defined transient ischemic attack duration as lasting up to 24 hrs. Transient ischemic attacks cause damage to many structures of the brain. The deleterious nature of an ischemic attack consists not only of the lack of oxygen and nutrients to the brain, but also prevents the removal of cellular waste and carbon dioxide (Hinkle & Bowman, 2003). Finally, without energy cellular function decreases to the point that the membrane cannot maintain ion gradients and the cell loses the ability to function.

Research continues with the aim of locating a mechanism for the cascade of cell death observed after transient ischemic insult. There are several steps after incursion of ischemic insult that are widely accepted (Siesjo, 1993). During stroke, loss of blood flow to cells creates a deficit in energy. Maintenance of a negatively-charged membrane gradient requires large amounts of energy; sodium-potassium pumps, for instance, are constantly working to push sodium out of the cell. If energy requirements are not met, the cell will not be able to maintain the negative gradient and depolarization will occur. A common excitatory neurotransmitter, glutamate, is released in sensitive brain regions. Introduction of large amounts of glutamate to cell cultures has been observed before cell death (Hansen, Hounsgaard, & Jahnsen, 1982).

Glutamate receptors in the surrounding areas are activated by large amounts of

neurotransmitter released by the pre-synaptic membrane. Specifically, NMDA receptors have been implicated in mediated cell death. Research involving the chemical blockage of these receptors has produced deficits in learning and have inhibited typical neuron damage associated with stroke (Wong et al., 1986). When NMDA receptors are activated, they allow a large influx of calcium into the cell. This process has been implicated in cellular death mechanisms, as manipulation that allows large amounts of calcium to enter the cell results in cellular death (Katchman & Hershkowitz, 1993). Intracellular levels of calcium have also been measured and observed to be high during the height of cell death (Krnjevic & Xu, 1989).

Processes that follow calcium influx into the cell via NMDA receptors have been widely debated. One enzyme that has been implicated in cell death is calcium calmodulin kinase II (CaMKII). This enzyme then translocates from the cytosol to the membrane region of cellular space. CaMKII phosphorylates other proteins as well as itself (Choi, 1988). Therefore, it can continue to phosphorylate as intracellular calcium declines. Eventually, however, phosphatase will strip the phosphate and reverse the compound. Research has implicated that CaMKII may be an integral part of the process, and that removal or manipulation may result in cell death (Limbrick, Churn, Sombati, & DeLorenzo, 1995). However, additional experiments have demonstrated that cells treated with a CaMKII inhibitor do not suffer damage and that the process for initial calcium influxes may be due to mitochondrial response to decreased cellular energy levels (Schinder, Olson, Spitzer, & Montal, 1996). Therefore, CaMKII may serve as a key component, but is not implicated as an isolated factor in cell death. The full process of

cell death is not clear at this time; further research in cascade steps is needed.

Models of Stroke

Animal models of transient ischemia are vital to furthering research in the field of stroke because human case studies are not practical and lack high levels of experimental control. Stroke has long been studied in a laboratory setting with the use of animal models (Sibbald, 2000). Lesion research, involving techniques such as cryogenic freezing, heating, chemical toxicity, and electrolytic procedures, allows further examinations of how incapacitation of specific brain regions impacts behavior. These procedures can mimic some of the deficits observed after stroke, but a model that specifically initiates stroke and the cascade of cell death is required for further understanding of how the steps of cell death are involved in damage to cerebral tissues. Specifically, CA1 region cells can be damaged irreparably so that it will not have the ability to return to normal function, known as an ischemic model of stroke (Gerriets et al., 2004). The ischemic model of stroke is considered a plausible model of human stroke. Researchers can then use the model to conduct experimental techniques and administer novel drugs to learn of the effects and possible relief that might be provided to humans in the future.

Temporary loss of blood flow can be produced using aneurysm clamps, which can be removed after a pre-determined amount of time. The key difference between permanent and transient methods to create stroke models is that the temporary blockage of blood supply is resolved and the original pathway unaltered after the procedure.

Animal models of ischemic stroke are vital to research furthering knowledge about the cascading death processes involved in stroke (Kirino, 1982).

Rodent Models of Ischemia

Gerbil Models

Gerbils have been used to model global cerebral ischemia because they lack a Circle of Willis structure, a circulatory “ring” that provides a common source for blood supply inside the brain (Willis, 1664). In humans and many mammals, this structure would serve as a way for blood to reach regions that were blocked from blood supply. Because gerbils lack this particular structure, they afford a simple model for producing ischemic stroke. Arterial occlusion is the only surgical procedure that must be carried out to result in stroke. Gerbils have been the subject of both temporary and permanent models (Uston, 2005). In fact, the first global ischemic stroke model was achieved by using a gerbil (Kirino, 1982). A common surgical procedure for temporary ischemic attack model is to clamp the carotid arteries. Although this model is considered one of the most simple for stroke demonstration, inherent behavior tendencies of gerbils can make testing difficult. The genetic sequence of gerbil DNA has not been mapped, therefore, investigation of possible mechanisms involved in cell death and their interaction with specific proteins would be difficult.

Rat Models

Rat models present another opportunity in stroke research. The process to create transient ischemia is more complicated than in the gerbil model. Rats have a Circle of Willis, which provides for other means of blood flow to reach areas that may be occluded during a transient ischemic insult (de Boorder et al., 2006). Complicated, additional surgical procedures, such as lowering blood pressure to induce hypotension are required to achieve a model in the rat. The genomic sequence of the rat has been mapped. This provides a powerful tool that allows researchers to delete or insert specific genes in the animal (Roberts, 2001). Genes code proteins that are created in the cell for vital function; alteration of the ability to create proteins can interfere with certain processes, allowing researchers to narrow the scope of focus. Experimenters can focus on one particular protein's role in the cascade effect that leads to cell death after stroke. Genetic sequencing also provides a way to observe the effects of over- and under-expression of certain proteins, which may be implicated by stroke research as an active part of the death process. For example, it cannot be assumed that an animal born with the inability to produce certain natural proteins would in all other ways be identical to the control group—a key assumption in experimental research. There is a strong possibility that altered animals rely on alternate protein expression or other systems to survive, or that they perhaps did not have the same experiences due to changes in capabilities. This question cannot be answered without launching a research investigation into the differences for each protein expression manipulation over a broad range of measures. Another problem with the use of transgenic models is that not all genes for protein expression can be

studied equally, as most protein alterations result in death and are not viable for study (Zambrowicz & Sands, 2003).

An alternative to transgenic models is the use of viral vectors as a means to silence certain gene expression (Fire et al., 1998). Researchers can remove genetic code from a simple virus and inject a new series of code that consist of the reverse (anti) thread of a targeted code for gene expression. When the targeted gene code is activated, it will read with the reverse, or compliment attached, which is deemed pathologic by the cell's mitochondria and therefore, will not result in creation of that protein.

A benefit of the rat model is that rats can provide additional behavioral task measurements, such as the Morris Water Maze Task (see Behavioral Paradigms) without food or water deprivation (Tecott & Nestler, 2004). Testing under other conditions, such as radial-arm mazes, can lead to performance changes in subjects (Pomple, Mullan, Bjugstad, & Arendash, 1999).

The process of producing a rat stroke model is complex; previous research using the rat model cite many unsuccessful attempts, often using another form of evaluation to determine which animals within the stroke condition received sufficient insult and pass criterion to be included in further testing (Katsumata, Kuroiwa, Shihong, Shu Endo, & Ohno, 2006). However, the genetic coding possibilities for protein expression manipulation make this model an attractive option in stroke research.

The majority of research involving transient ischemia is conducted with rodents. However, criticism of these animal models has been documented; some researchers pose that results gleaned from animal models are not valid comparisons to human stroke

occurrences due to the anatomical differences between species. Some cited differences involve capacity for memory, such as episodic memory, which may influence behavioral motivations and anatomical differences in the circulatory systems of the commonly researched rodents and humans (Croce, 1999). Nevertheless, animal models are an attractive alternative to human suffering that would otherwise take place if advancement in stroke science is to take place (MORI, 2002).

There are research benefits and costs involved with each of the rodent models for stroke research. Rats are complex models to create, while mouse and gerbil models limit the type of behavioral testing they can participate in after the model is created. Stresses of behavioral testing are also necessary to take into account when reviewing validity of stroke behavioral testing. Regardless of the animal model selected, there are several benefits and potential setbacks that must be considered during the design process. In summary, each rodent model has benefits and drawbacks. Gerbil models are simpler to create, while mouse and rat models can be genetically manipulated. Gerbil and mouse models have a limited capacity for behavioral task measures that the rat does not, but transient ischemic rat models are complicated surgical procedures and often result in lower model success rates.

Behavior Paradigms

Radial Arm Maze

An apparatus to measure spatial learning and memory was designed by Olton and

Samuelson (1976). The standard maze is composed of eight arms, or alleys, that extend from the center of a platform. Animals are required to remember which arms they visited previously for reward items such as food or water in order to obtain new rewards from arms that they had not entered. The motivation of this apparatus consists of reaching rewards quickly, as the animals are usually food or water deprived before testing, thus entering an arm that contains no more food or water reward wastes energy resources and further delays consumption of rewards. Olton and Samuelson discovered that rats, in particular, were able to remember (when unaltered) with 88% accuracy which arms they had previously entered. Further, after switching the intramaze cues, the researchers found that the animals re-entered previously visited arms, suggesting that they were relying on extramaze, rather than intramaze cues. Therefore, the radial arm maze has been a popular behavioral paradigm in researching spatial navigation. A recent evaluation of rat memory performance in the radial arm maze predicts there is a limited spatial memory for an average of 28 locations during a single behavioral test (Cole & Chappell-Stephenson, 2003). Another arm maze that is used to study working memory in animal paradigms is the T-maze, which consists a choice between two possible directions to obtain food rewards (Douglas & Pribram, 1966). In this maze, subjects must use prior knowledge of which arm they had previously visited and removed food from. This represents an either-or choice that in other aspects models the Radial-Arm maze, which can be made into a T-maze by closing off all but three arms to form the “T”.

Barnes Maze

The Barnes maze is similar to the Radial arm maze. However, there are typically twelve possible choices of direction. Carol Barnes (1979) of the University of Arizona designed this model in which holes are made available to the animal for escape. But, only one hole contains the target box. Animals must decide which hole to enter to avoid being out in the central, open area. The motivation is escape from punishment in the form of wind, loud or alarming sounds, harsh light etc. A noted problem with this apparatus is the aversive environment animals are subjected to during or while witnessing other animals perform before themselves being tested (King & Arendash, 2002). These conditions could lead to different results of a comparative analysis between groups (McLay, Freeman & Zadina, 1998).

Morris Water Maze

One of the most commonly used behavioral paradigms is the Morris Water Maze Task (Morris, 1984). It consists of a large circular tub of water and a hidden platform that is just below the surface in one location of the pool. The platform provides escape from swimming, which is a motivation to seek the platform out quickly. This particular paradigm is advantageous in that it eliminates the need for food or water deprivation, as the motivation to stop swimming is considered a sufficient reward. Often, the water maze is used to test animal models for spatial memory cues or patterns that involve working memory (Liang, Hon, Tyan, & Liao, 1994.) However, as mentioned previously,

some common rodent stroke models, such as mice and gerbils do not perform well in this task.

Open Field Apparatus

The open field apparatus was originally designed to evaluate emotionality in rats by preventing escape from an unknown environment (Prut, 2003). Described by Hall (1934), the paradigm originally had a brightly lit circular platform with high walls and was used to determine which rats ate and which did not (i.e., stressed animals). Current open field paradigms are diverse in shape, and some contain objects or paths within the apparatus, such as passageways, novel and old objects, etc (Takahashi & Kalin, 1989). The procedure of introducing novel items and comparing exploration is known as novel object response (Belzung, 1999).

Recordings of horizontal locomotion, such as the number of lines crossed per test are common measurements. Also, vertical activities, such as rearing, leaning and grooming can be observed (Matto & Lembit, 1999). Additional exploration behavior measurements can be quantified as time spent within a central, open area of an open field compared to the time spent in the wall areas, or outermost zones (Basso, Beattie, & Bresnahan, 1995). Limitations of the open-field apparatus include the inability to test other aspects of hippocampal function, such as working memory and cue memory for spatial navigation. However, the field can be modified easily to perform such memory tasks, as evidenced by novel object recognition paradigms (Save, Poucet, Foreman, & Buhot, 1992) that consist of an open-field with novel and familiar objects placed within the field.

Neuroprotection and Deficits

General Deficits

As mentioned previously, the financial burden of stroke is substantial. After a transient ischemic attack has been resolved, the chances of a recurrent episode are high and close medical observation or treatments must be accommodated to reduce the chances of another attack. In addition, the initial attack may result in damage so severe that previously independent individuals may require respite care, estimated at 75% (Bonita, Solomon, & Broad, 1997). After the initial insult, there can be lingering or permanent effects, often depending on the length and magnitude of the attack.

Hyperactivity

Hyperactivity, defined as an increased speed and/or distance traveled, is commonly observed in subjects proceeding ischemic damage (Gerhardt & Boast, 1988; Mileson & Schwartz, 1991; Babcock, Baker, & Lovec, 1993; Nurse & Corbett, 1995). Chandler, DeLeo, and Carney (1985) induced ischemic attacks in animal subjects and observed increased locomotor activity immediately following the procedure. Interestingly, hippocampal cell death occurs much later, 2-3 days, following insult. This observation supports the implication that the death process of these cells is an extended, perhaps related process (Kirino, 1982; Foreman, 1983).

A transient ischemic gerbil model resulted in higher locomotor activity than controls for up to 7 days post-ischemia (Wang & Corbett, 1990). Within the same study, gerbils that were exposed to a novel open-field apparatus for 5 days before ischemic insult did not demonstrate this increase in locomotor activity, implicating the activity increase as an inability to form spatial maps, rather than simple insult-induced hyperactivity.

Treatments

Hypothermia. Hypothermic treatment has been demonstrated to protect CA1 region cells during 5 min ischemic insult in the gerbil (Babcock et al., 1993). Hypothermia was induced via neurotensin to lower subjects' rectal temperatures below 37-38 °C and either subjected to ischemic insult or sham surgery. Animals in the non-hypothermic and ischemic group demonstrated increased locomotor activity, while hypothermic ischemic subjects' activity was similar to controls; histological data supports the behavioral assessment. Another benefit of hypothermic treatment is the potential to increase tolerance to future ischemic episodes (Pedersen, Thorbjornsen, Klepstad, Sunde, & Dale, 2007). There are limitations to hypothermic therapy, however. Treatment must be administered by trained, professionals in the location of designated acute care facilities. A more recent observation is that during hypothermic treatment, patients undergo certain biochemical changes; metabolic systems are altered slightly by the reduction of heat and energy within the normal optimal range for cellular activity, including breakdown systems, such as bile-production, liver and kidney waste-transport pathways (Pedersen, et al.). Because little is known about drug metabolic reactions in hypothermic states, typical

medications are still prescribed by physicians. Slight metabolic changes can result in the drugs having no effect or staying in the system for unexpected amounts of time, leading to possible confounding medical problems (Pedersen, et al.). Although success has been clear when hypothermic medications are administered within a reasonable window of ischemic insult, more research on potentials of hypothermic treatment should be a focal point of ongoing research.

Thrombolytics and Vasodialators. Pharmaceutical treatments for ischemic insult are few. A review of therapies demonstrating success in treatment after insult concluded that thrombolysis and tirilazad, a free radical regulation agent, are the only two established methods (Perel et al., 2007). Thrombolysis is the procedure of breaking down a blood clot, also referred to as lysis or clot busting, by way of proteins that normally activate plasmin (Mielke, Wardlaw, & Liu, 2004). Although intracranial hemorrhage events are a potential effect, the overall effectiveness of the drug to stabilize acute ischemic stroke is 24%, based on post mortem protein assays of tissue plasminogen levels (Perel, et al).

Another post-ischemic treatment is Dipyridamole (Aggrenox). Dipyridamole causes vasodilation, by inhibiting platelet accumulation, which aides in clot deterioration and speeds ischemic attack relief (Cori, Fata-Hartley, & Palmenberg, 2005). Another drug to consider in the antiplatelet group is Clopidogrel (Plavix) which is used for many arterial and vascular diseases (Chan et al., 2005). This drug is used for post-ischemic therapy and other blood-thinning treatments; it was the international second-highest selling pharmaceutical product in 2005. Finally, the common, over-the-counter product that many adults consume even before they show signs of ischemic insult is aspirin, another

blood-thinning drug. These anti-platelet pharmaceuticals are the most frequent treatment after ischemic insult occurs in an attempt to thwart another, or prevent longer-lasting cerebrovascular attacks.

Free Radical Regulators. Researchers have documented the release or increase of free radicals in arteries following ischemic insult (Flamm, Demopoulos, Seligman, Poser, & Ransohoff, 1978). Free radicals remove oxygen from domestic proteins and enzymes, resulting in unstable cellular mechanisms. A drug showing great promise in regulation of free radicals, NXY-059, was investigated by AstraZeneca researchers in 2005. However, replication of those results during late-phase trials did not provide statistically significant results, and the drug company withdrew FDA petitions (Lees et al., 2006). A review of therapies demonstrating success in treatment after insult reported tirilazad as one of only two established methods of stroke intervention in phase-III trials (Perel, et al, 2007). Tirilazad inhibits lipid peroxidation, which is the oxygen-based degradation of fats by free radicals. This drug was found, serendipitously, to demonstrate neuroprotective properties (Tirilazad, 2000). The review, conducted by Perel, et al, denotes the quality of the experiment as “poor”. However, the results demonstrate a 29% decrease in infarction volume after Tirilazad implementation. The manufacturer’s self-reported analysis contains documentation that treatment animal death rates were high, but behavioral tests indicated a 48% increase in neurobehavioral scores. Unfortunately, information documenting the specific behavioral tests conducted to arrive at the improved scores was not provided (Perel, et al; Tirilazad).

Amphetamines Lazar Edeleanu (1887) synthesized amphetamines at the University of Berlin. The compound was previously linked to a plant derivative of ephedrine, purified two years earlier by Nagayoshi Nagai (1885). The first documented medicinal use of amphetamines was the drug Benzedrine, used to alleviate fatigue in pilots and infantry as well as relief from asthma (Schube & Raskin, 1940). However, the drug was limited to prescription use in 1959 by the FDA following amphetamine class's high-addiction rate.

Amphetamines have been implicated as facilitators of recovery after stroke in clinical patients (Walker-Batson, Smith, Curtis, Unwin, & Greenlee, 1995). However, their effectiveness was only demonstrated when physical therapy was part of the treatment plan. The evidence suggests that motor skill recovery involved with these drugs may actually be the result of facilitation or generation of alternate, new sensory motor information pathways.

In primate research, physical therapy was facilitated by D-amphetamine and new synaptic activity was observed in most cases (Stroemer, Kent, & Hulsebosch, 1998). A recent review of amphetamine treatment in stroke recovery determined that the success of individual treatment studies was high (Martinsson, Hardemark, & Eksborg, 2007). Although death rates and dependence requirements did not subside with treatment, it was determined that the studies included more devastating baseline attack rates, a common problem in exploratory treatment enrollment. Heart rate and blood pressure vitality statistics rose significantly in all studies, however physicians reported an overall improvement from baseline to the last follow up when rated on motor function ability. It

is important to note that many of the studies involved did not use similar baseline/follow up procedures or did not list parameters for these milestones in treatment.

Methamphetamine. Nagayoshi Nagai was the first person to synthesize methamphetamine from ephedrine in 1893. Later, crystallized methamphetamine synthesis was conducted by Akira Ogata (1919) by way of red phosphorus and iodine reduction of ephedrine. Methamphetamine was known soon after its discovery as an aid for alertness; the German military put it in chocolate bars known as *fliegerschokolade*, or flyer's chocolate, when given to pilots and *panzerschokolade*, or tanker's chocolate" when distributed to tank crews during WWII (Heston & Heston, 1979). Adolph Hitler was also reportedly given daily injections of methamphetamine by Theodore Morrell, his personal physician. Commercial use of methamphetamine developed in the 1950's, in the form of prescription drug Pervitin. Prescriptions for Pervitin treated narcolepsy, alcohol abuse, Parkinson's disease, depression and even obesity (Feldman, Meyer & Quenzer, 1997). Prescription use of methamphetamine reached an all-time high in 1967 with an estimated 31 million filled prescriptions during that year.

Methamphetamine derivatives can be ingested many ways, but regardless of method, it enters the brain rapidly, causing a release of norepinephrine, dopamine and serotonin (Rothman & Bauman, 2002). The drug interferes with normal CNS regulation of heart rate, body temperature, blood pressure, appetite, attention, mood and alertness.

When high levels of methamphetamine were administered to animal subjects, an increase in harmful free radicals was observed in the cortex (Giovanni, Liang, Hastings, & Zigmond, 1995). Further, a case study identified methamphetamine use with chronic

cerebrovascular disease and delayed ischemia (Ohta, Mori, Yoritaka, Okamoto, & Kishida, 2005). While this case might seem isolated, a similar case was reported in 2004 when an incarcerated male died after inhalation of methamphetamine immediately following a prolonged abstinence (McGee, McGee, & McGee, 2004). Autopsy reports yielded evidence of massive cerebrovascular event as the cause of death.

Cerebrovascular effects on retrovirus-infected mice revealed that methamphetamine treatment counterbalanced effects of murine-virus induced dilated cardiomyopathy (DCM) (Yu, Montes, Larson, & Watson, 2002). Methamphetamine, through its action of vasoconstriction, is in direct opposition to DCM's effect on vascular structures. This appears to be the first documentation of extended treatment using methamphetamine that yields a positive outcome. The mechanism underlying documented cases of methamphetamine benefit in cerebrovascular distress is unclear. To date, there have been no investigations into the effects of methamphetamine on transient cerebral ischemia

STATEMENT OF PUPOSE

The goal of this experiment was to determine if methamphetamine injection following transient cerebral ischemic insult is neuroprotective. Subjects underwent occlusion of carotid arteries for 5 mins or sham surgery, followed by either injections of 5 mg/kg methamphetamine or saline solution. Forty-eight hrs post-surgery, subjects were tested in an open field apparatus and locomotion was recorded. Twenty-one days after testing, histological evaluation of the hippocampus was conducted.

It was hypothesized that subjects in the stroke + methamphetamine conditions would demonstrate locomotor patterns similar to sham surgery-treatment animals. Subjects in the saline + stroke conditions would demonstrate higher levels of locomotion, such as distance traveled, average speed, and number of lines crossed than all other animals in the experiment. In addition, it was hypothesized animals in the stroke + methamphetamine condition would not exhibit damage to the CA1 region.

METHODS

Subjects

Twenty six male gerbils weighing ~80 gms were used as subjects. The subjects were allowed unrestricted access to standard food pellets and water. Animals were maintained in separate solid-bottom cage housing in a room that was temperature (23°C) and light cycle (12-hour light/dark) controlled. All animal procedures described herein were approved by the Montana State University Institutional Animal Care and Use Committee.

Surgery

Subjects were randomly assigned to drug and surgical treatment. A total of 12 subjects received methamphetamine treatment (5 ischemic, 7 sham) and 14 subjects received saline as a control (8 ischemic, 6 sham). Subjects were induced and maintained with isoflurane. A homothermic blanket (Harvard Apparatus, South Natick, USA) was used to maintain animal rectal temperatures at 37°C. The carotid arteries were exposed via a midline incision made to the ventral surface of the neck. Arteries were isolated and clamped for 5 min using 85-gm pressure aneurysm clamps. Sham subjects underwent the identical procedure, except carotid arteries were not clamped. Following the occlusion period, clamps were removed and the incision sutured.

Drug Treatment

Subjects were administered 5 mg/kg methamphetamine (Sigma) or saline vehicle i.p. within 5 mins of reperfusion. Subjects were placed in separate solid-bottom cages that contained small amounts of bedding and were observed during recovery from anesthesia (60 mins) and monitored for another hour before their cages were removed from the surgical room and placed in the housing room.

Behavioral Testing

Behavioral testing was conducted 48 hrs following surgery. Subjects were placed in the center of an open field maze measuring 77 cm x 77 cm containing 15 cm-high walls. The apparatus was marked into 9 squares. Testing lasted 10 mins. Behavioral data were collected using a digital camera (Logitech, Inc., CA) connected to a computer and tracking program (ANY-maze; Stoelting, IL).

Histology

Approximately 19 days after behavioral testing, subjects were euthanized with CO₂ and perfused transcardially with phosphate buffered solution followed by 4% paraformaldehyde. Brains were removed and stored until processing. Brain tissue was coated with a gelatin/yolk protein mixture, consisting of 1:5 parts gelatin to yolk and submerging in 4% paraformaldehyde. Following 3 days in this mixture, the tissue was sectioned with the aid of a vibratome. Sections (50 μm) were collected through the

region of the hippocampus, mounted on slides and stained with cresyl violet. The slides were then evaluated by 2 individuals blind to the condition using a 4-point rating scale (Babcock, Baker, and Lovec, 1993). Sections were assigned a score of 0 (4-5 compact layers of normal neuronal bodies present) 1 (4-5 compact layers present with some altered neurons) 2 (sparse neuron bodies with glial cells or spaces between them) or 3 (total absence or minimal neuronal bodies with intense gliosis of the CA1).

Experimental Design

Locomotor activity was collected for 10 mins in an open-field test 48 hrs post-surgery. Separate 2 x 2 ANOVA were conducted to evaluate the effects of surgery (ischemic or sham) and drug treatment (methamphetamine or saline) on the following dependent measures: distance traveled (m), speed (m/sec), and line crossings (number of times subject crossed one of the 9 border squares of the bottom of the apparatus). Line crossing has previously been used in our laboratory as a measure of locomotor activity level before software programs that track the actual distance traveled became available. To investigate possible changes in these dependent measures during the testing interval, data were separated into two segments (min 1-5 and 6-10) and reanalyzed using a 2 (surgery) x 2 (drug) x 2 (time) mixed model ANOVA. It was hypothesized that overall, subjects would score lower on locomotion behavior measures during the second half of the testing time. In addition, locomotor measures (distance traveled, speed, line crossing) for the ischemic + saline animals would be higher than ischemic animals treated with methamphetamine and all sham conditions. Histological rating scores were evaluated

using a Kruskal-Wallis analysis of ranks test. It was hypothesized that the methamphetamine would reduce the amount of neuronal damage in ischemic animals compared to ischemic animals treated with saline.

RESULTS

Open-Field TaskDistance Traveled

Subjects were observed during a 10 min open-field test and data collected using an automated tracking program. A sample track plot from each treatment condition is shown in Figure 1. Ischemic animals treated with saline traveled a greater distance relative to all sham conditions as well as ischemic treated with methamphetamine. Subjects in the ischemic surgical and saline condition ($M = 120.77$, $SE = 19.90$) on the average traveled further than those in the ischemic 5 mg/kg methamphetamine condition ($M = 67.43$, $SE = 6.00$).

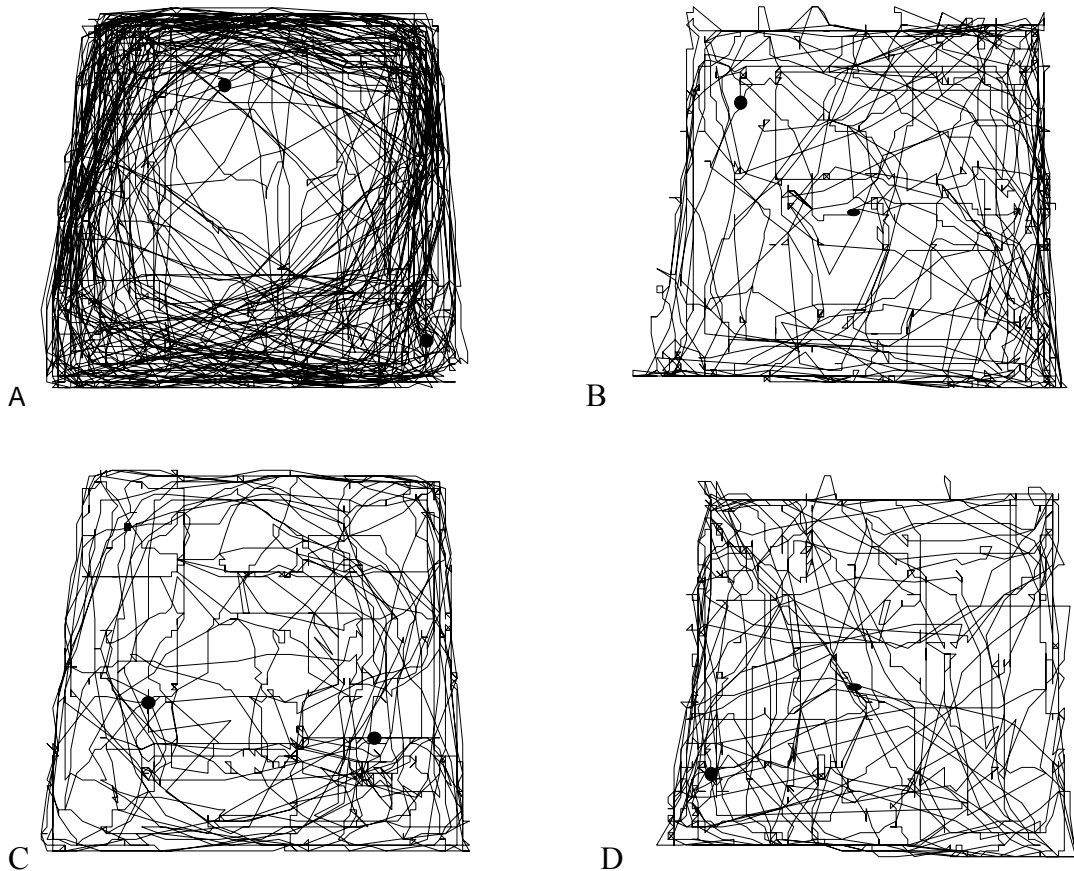


Figure 1. Track plots of distance travelled during open-field test. Samples of plot data for each of the four treatment assignment groups is shown. The dark lines depict the progress of subjects during 10 mins open-field test. Panel A. represents an ischemic + saline condition. Panel B. represents an ischemic + methamphetamine condition. Panel C. represents a sham + saline condition and panel D. represents a sham + methamphetamine condition.

An ANOVA was conducted to evaluate the effects of surgery and drug on distance traveled during the 10 min open-field task. The results indicated a nonsignificant main effects for surgery, $F(1, 22) = 1.08, p = .31, \text{partial } \eta^2 = .05$, and drug, $F(1, 22) = 3.20, p = .09, \text{partial } \eta^2 = .13$. However, a significant interaction between surgery and drug, $F(1, 22) = 4.18, p = .05, \text{partial } \eta^2 = .16$ was revealed.

An independent-samples t test was conducted to evaluate the hypothesis that within the surgical condition ischemia, subjects in saline drug treatment would have higher scores for distance traveled when compared to ischemic treated with methamphetamine subjects. The test was significant, $t(11) = 1.80, p < .05$, which supported the research hypothesis. Figure 2 depicts the distance traveled for the treatment conditions.

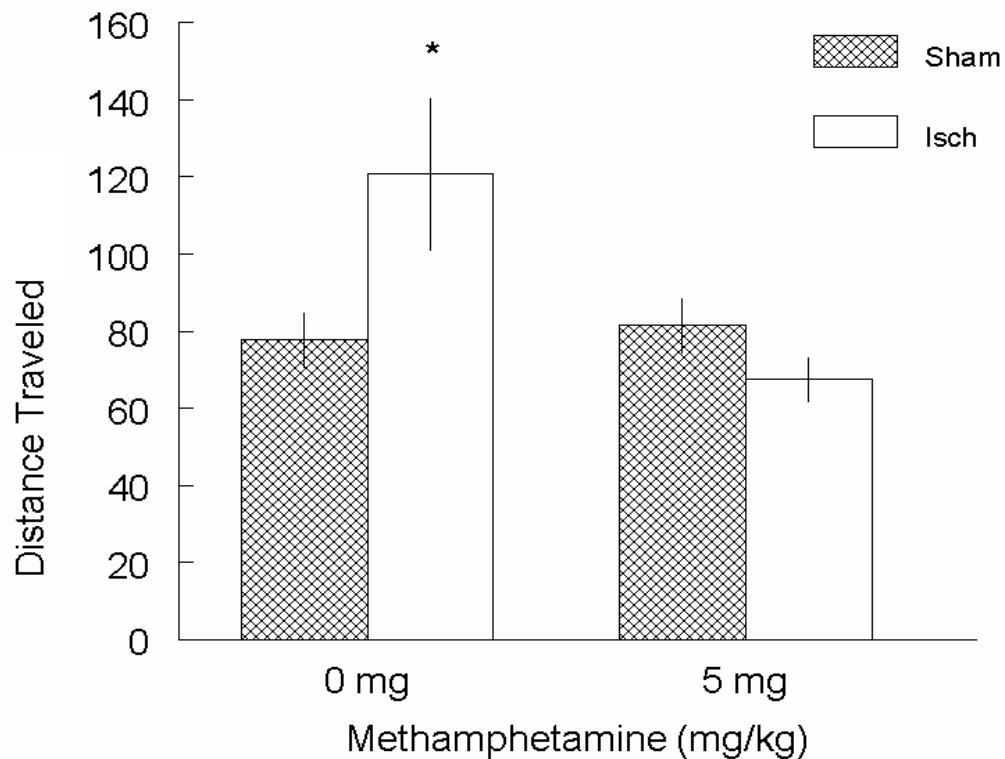


Figure 2. Distance traveled during open-field test. Locomotor activity 48 hrs following transient cerebral ischemia (Isch) or sham. Animals received methamphetamine (5 mg/kg) or saline (0 mg) within 5 mins of surgery. Ischemic animals treated with methamphetamine exhibited activity levels that were similar to sham treatment subjects. Ischemic + saline treated animals demonstrated significantly higher activity levels for distance traveled than subjects in all other treatment conditions ($p < .05$).

A mixed model ANOVA was conducted to evaluate the effects of the first and second half of testing time. Segment 1 refers to the first 5 mins of testing and Segment 2 describes the second 5 mins of testing time. Analysis revealed that distance traveled during the second segment was significantly less, irrespective of the treatment condition; all treatment subjects traveled less distance during the latter half of testing than during the first half.

There was a main effect for scores of the dependent variable distance traveled for segment, $F(1,22) = 22.84, p < .01$. A comparison of means for the first 5 min segment ($M = 50.54, SD = 20.98$) and last 5 min segment ($M = 39.47, SD = 19.29$) indicates that on average, all subjects in the study traveled more distance during the first 5 min of testing than the last. Figure 3 shows the relationship of treatment conditions based on time segments for distance traveled.

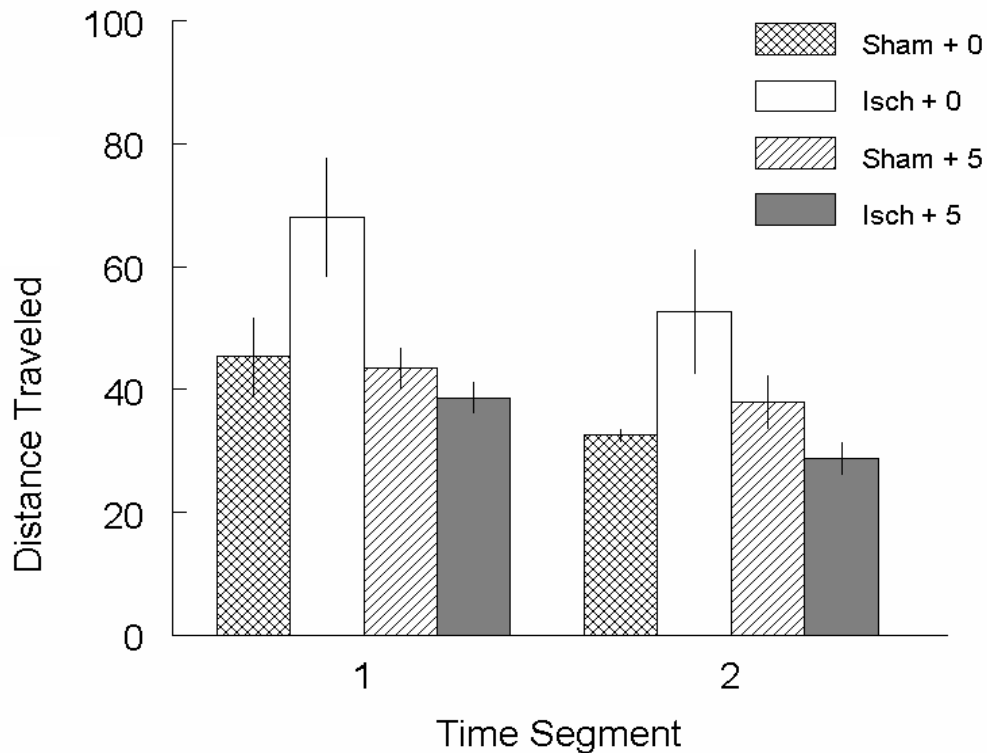


Figure 3. Distance traveled during open-field test based on test time segment. Locomotor activity 48 hrs following transient cerebral ischemia (Isch) or sham. Animals received methamphetamine (5 mg/kg) or saline (0 mg) within 5 mins after surgery. The 10 min test duration was divided in half for analysis of behavior over time in the apparatus. Subjects traveled more distance during the first 5 mins of testing than the last 5 mins, regardless of treatment condition ($p < .05$).

Speed

The average speed (m/sec) traveled during the open-field test was measured.

Animals in the ischemic + saline treatment condition traveled at a higher rate of speed than subjects in all other conditions. Subjects in the ischemic surgical and saline conditions ($M = .20$, $SE = .03$) traveled at higher speeds than those in the ischemic + 5 mg/kg methamphetamine condition ($M = .11$, $SE = .01$).

An ANOVA was conducted to evaluate the effects surgery and drug had on the

average speed subjects traveled at during the 10 min open-field task. Results indicated a nonsignificant main effect for surgery, $F(1, 22) = 1.08$, $p = .31$, partial $\eta^2 = .05$, a nonsignificant main effect for drug, $F(1, 22) = 3.16$, $p = .09$, partial $\eta^2 = .13$, and a significant interaction between surgery and drug, $F(1, 22) = 4.15$, $p = .05$, partial $\eta^2 = .16$. An independent-samples t test was conducted to evaluate the hypothesis that within the surgical condition ischemia, subjects in saline drug treatment would have higher speed scores when compared to ischemic methamphetamine subjects. The difference was significant, $t(11) = 1.80$, $p < .05$. Figure 4 illustrates the relationship between surgical and drug condition measures of speed.

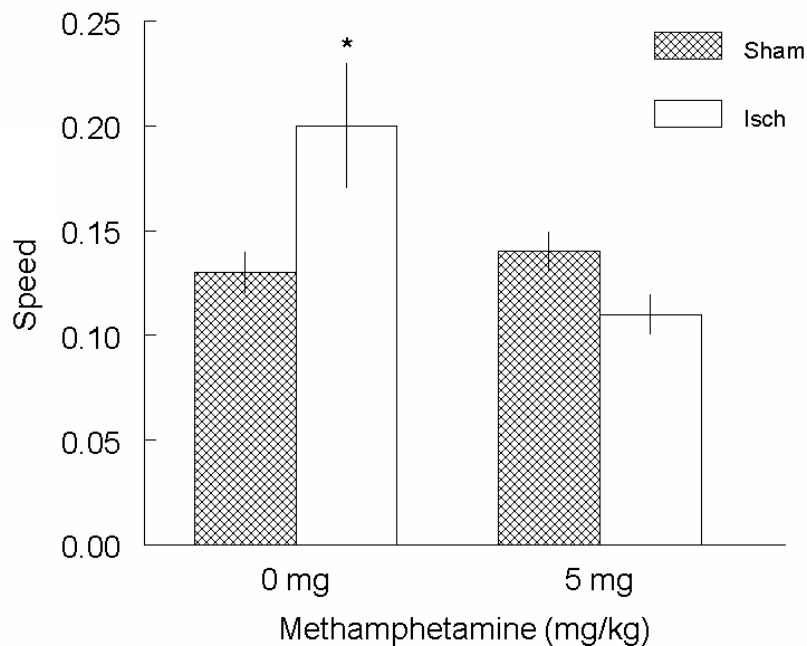


Figure 4. Average speed during open-field test. Locomotor activity 48 hrs following transient cerebral ischemia (Isch) or sham. Animals received methamphetamine (5 mg/kg) or saline (0 mg) within 5 mins of surgery. Ischemic animals treated with methamphetamine exhibited activity levels that were similar to sham treatment subjects. Ischemic + saline treated animals demonstrated significantly higher activity levels for average speed (m/sec) than subjects in all other treatment conditions ($p < .05$).

A mixed model ANOVA was conducted to evaluate the differences between the first and second 5 min segments of the testing period in relation to average speed measures. For all treatment conditions, subjects traveled at a faster rate during the first compared to the last 5 min segment of the test.

There was a main effect for average speed traveled for segment, $F(1,22) = 23.07, p < .01$. A comparison of means for the first 5 min segment ($M = .17, SD = .07$) and last 5 min segment ($M = .13, SD = .06$) indicates that on average, all subjects in the study traveled at higher speeds during the first 5 min of testing than for the last 5 min. Figure 5 illustrates the average speed measures for treatment groups based on test time segments.

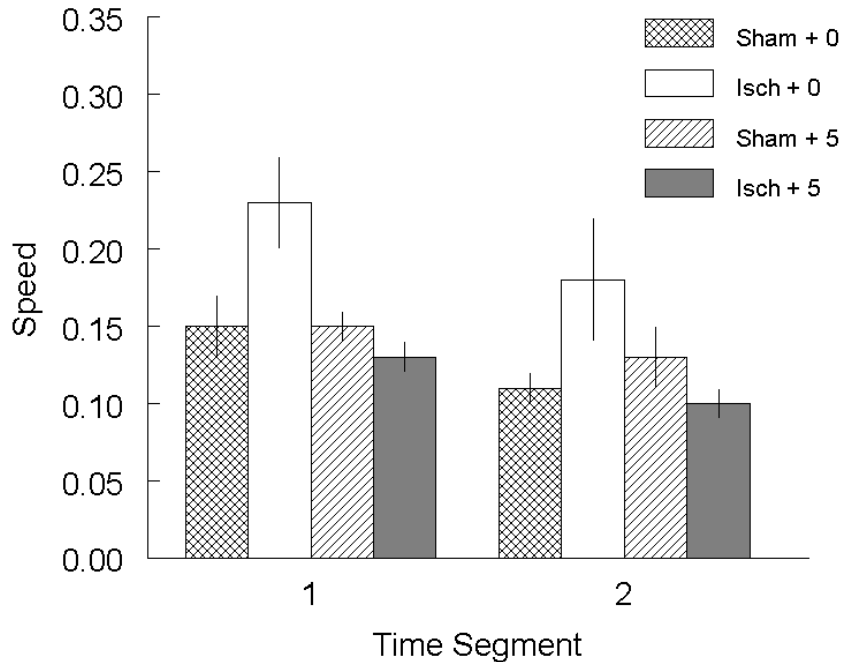


Figure 5. Average speed during open-field test based on test time segment. Locomotor activity measurement of speed (m/sec) 48 hrs following transient cerebral ischemia (Isch) or sham. Animals received methamphetamine (5 mg/kg) or saline (0 mg) within 5 mins of surgery. The 10 min test duration was divided in half for analysis of behavior over time in the apparatus. Subjects traveled at a higher average speed during the first 5 mins of testing than the last 5 mins, irrespective of treatment condition ($p < .05$).

Line Crossings

Line crossing measures were collected by dividing the apparatus into 9 equal squares and drawing the grid onto the visual representation of the open-field apparatus. To generate line crossing data, each time gerbils crossed one of the lines it was scored. The tallied number of times that lines were crossed for the duration of the test period was collected for each trial. Ischemic + saline treated subjects averaged more line crossings

than ischemic + methamphetamine as well as all sham treatment groups. These results coincide with data collected for distance traveled (Figure 2), indicating that the measures are comparable. Subjects in the ischemic surgical and saline conditions ($M = 669.50$, $SE = 85.07$) on the average crossed more lines than those in the ischemic 5 mg/kg methamphetamine condition ($M = 389.00$, $SE = 24.50$).

An ANOVA was conducted to evaluate the effects surgery and drug had on the number of lines crossed during the open-field task. The results indicated a nonsignificant main effect for surgery, $F(1, 22) = 1.12$, $p = .30$, partial $\eta^2 = .05$, a significant main effect for drug, $F(1, 22) = 5.67$, $p < .05$, partial $\eta^2 = .21$, and a significant interaction between surgery and drug, $F(1, 22) = 4.73$, $p < .05$, partial $\eta^2 = .18$. The animals treated with saline ($M = 584.21$, $SD = 214.33$) crossed more lines than animals treated with methamphetamine ($M = 429.08$, $SD = 84.38$).

An independent-samples t test was conducted to evaluate the interaction between the surgical condition and drug. Ischemic + saline treated subjects crossed more lines than ischemic + methamphetamine treated animals, $t(11) = 1.80$, $p < .05$. Figure 6 depicts the number of line crossings for treatment groups during the open-field test.

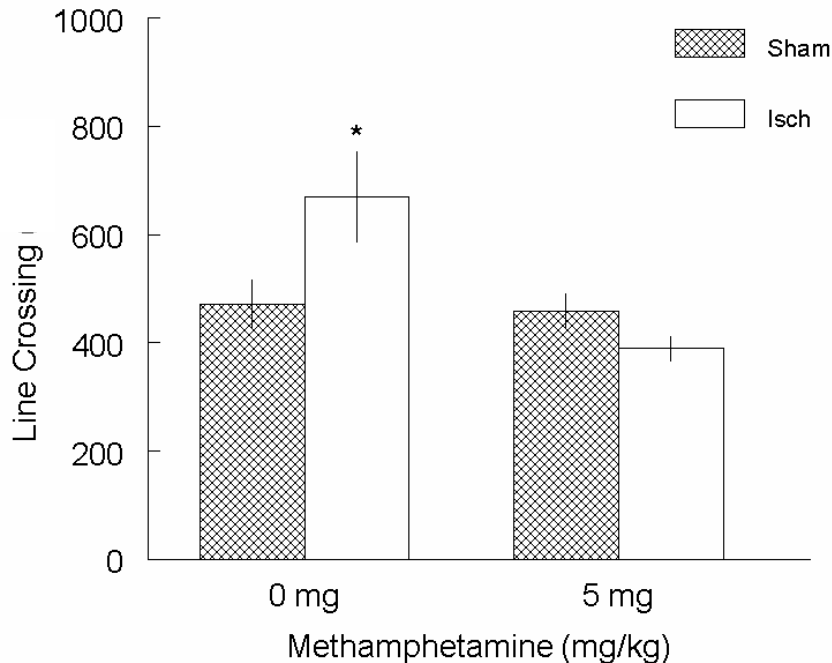


Figure 6. Number of line crossings during open-field test. Locomotor activity was measured for the number of lines crossed 48 hrs following transient cerebral ischemia (Isch) or sham. Animals received methamphetamine (5 mg/kg) or saline (0 mg) within 5 mins of surgery. Ischemic animals treated with methamphetamine exhibited activity levels that were similar to sham treatment subjects. Ischemic + saline treated animals demonstrated significantly more line crossing activity than subjects in all other treatment conditions ($p < .05$).

A mixed model ANOVA was conducted to evaluate the effects of the first and second segments of the 10 min testing period. All treatment groups performed more line crossings during the first 5 min segment than the last 5 min segment of testing. All treatment groups displayed statistically similar patterns of reduced line crossing during the last 5 mins of the test.

There was a main effect for the dependent variable lines crossed based on segment, $F(1,22) = 25.33, p < .01$. A comparison of means for the first 5 min segment ($M = 289.27, SD = 98.80$) and last 5 min segment ($M = 223.35, SD = 92.65$) indicates that on

average, all subjects in the study crossed more lines of the apparatus flooring during the first 5 min of testing than the last. Figure 7 shows the relationship between drug and surgery treatment groups during the first and last 5 min time segments of the open-field test.

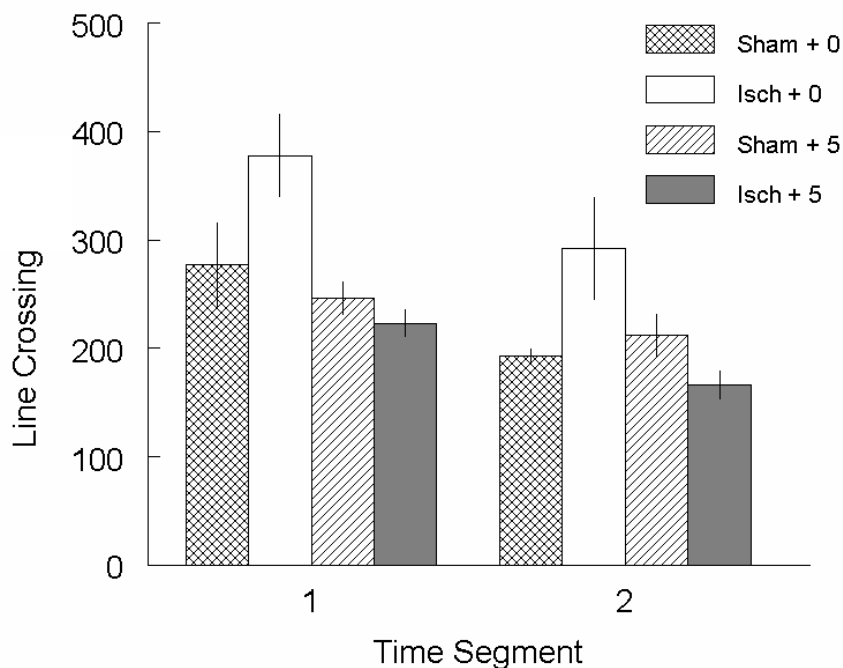


Figure 7. Number of line crossings during open-field test based on test time segment. Locomotor activity 48 hrs following transient cerebral ischemia (Isch) or sham. Animals received methamphetamine (5 mg/kg) or saline (0 mg) within 5 mins after surgery. The 10 min test duration was divided in half for analysis of behavior over time in the apparatus. Subjects crossed more lines during the first 5 mins of testing than the last 5 mins, regardless of treatment condition ($p < .05$).

Histology

Brain sections were mounted on slides and stained with cresyl violet. Independent raters that were blind to the treatment conditions of each sample viewed the CA1 region

and assigned a rating of 0 – 3 for level of neuronal cell body damage (Babcock, et al., 1993). Sample photomicrographs of stained sections from each condition are depicted in Figure 8.

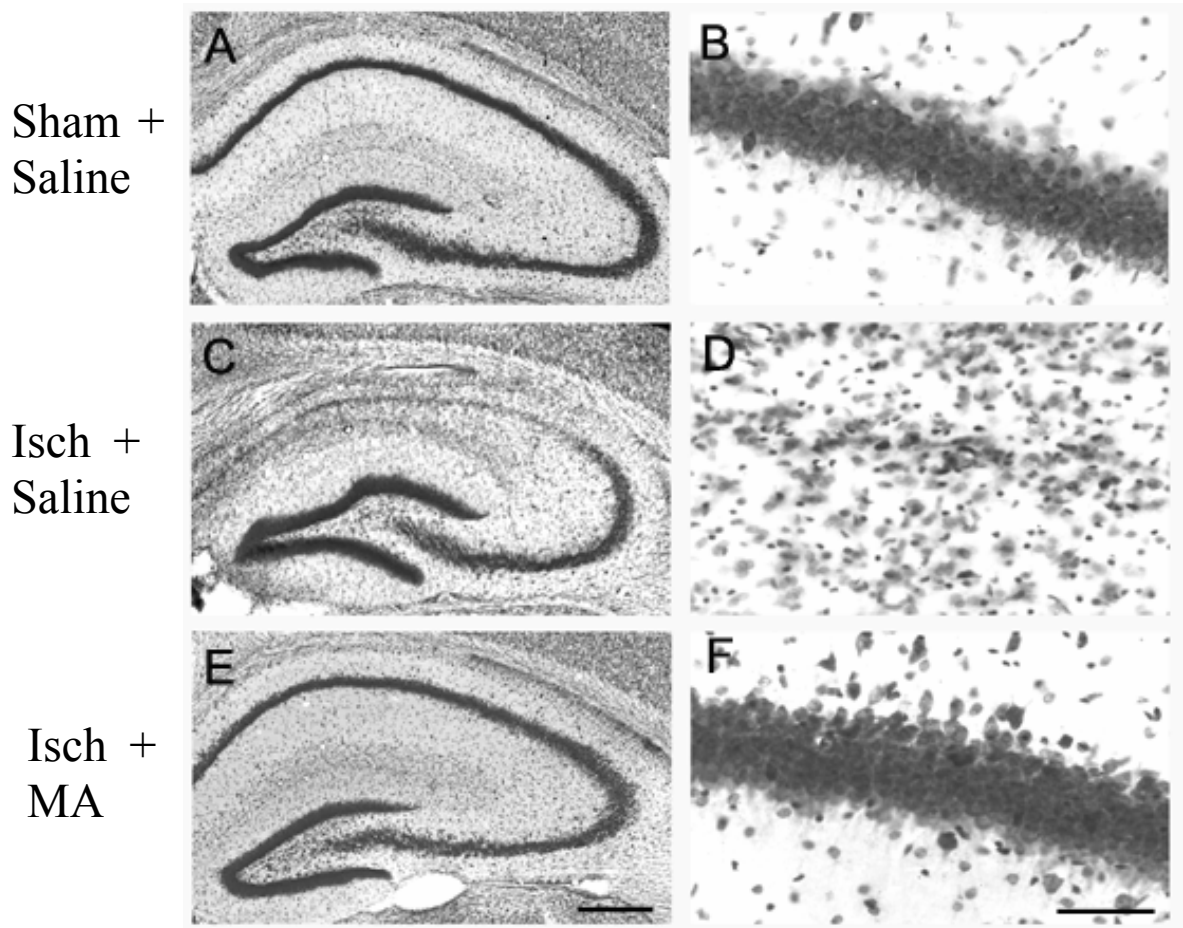


Figure 8. Sample photomicrographs of cresyl violet stained sections containing the hippocampal region. Tissue was collected 21 dys post surgery. Panels A and B are animals from the treatment group sham surgery and saline. Panels C and D are animals from the treatment group ischemic surgery and saline. Panels E and F are animals from the treatment group ischemic surgery and methamphetamine. Scale bars = 200 μ m (E) and 60 μ m (F).

Sham animals injected with methamphetamine were not evaluated in the histological procedures; this group served only as a control during behavioral testing. Ischemic + saline and ischemic + methamphetamine groups, as well as sham and methamphetamine subjects, were evaluated for neuronal cell body damage.

Data were analyzed using the Kruskal-Wallis analysis of ranks. The ratings indicated no significant differences between damage ratings of ischemic + methamphetamine and sham + saline treated subjects. Kruskal-Wallis rank order analysis of the rankings yielded $p < .05$. However, raters gave higher scores (more damage) to ischemic + saline treated animals. Of the two ischemic groups, only those treated with saline were rated as having damage to the CA1 region. The results indicate that there was a low rating of damage and no statistical difference in ratings between ischemic and sham treated animals injected with methamphetamine after surgical assignment.

DISCUSSION

Previous studies have demonstrated that transient ischemic attack induces behavioral changes. Specifically, an increase in locomotor activity has been observed soon after insult in animal models (Chandler, DeLeo, & Carney, 1985). In addition, pyramidal cells of the CA1 region are reduced or damaged following attack (Kirino, 1982). Wang and Corbett (1990) found that ischemic damage to the CA1 region of the hippocampus correlated with an increase in locomotor activity. Previous research has established locomotor activity as a good predictor of delayed cell death following ischemic insult. In the current study, a 5 min bilateral carotid vessel occlusion in the gerbil was used to model transient ischemia in order to study the neuroprotective efficacy of methamphetamine. As previous studies have shown, ischemic treatment animals injected with saline demonstrated significantly higher activity levels for distance traveled and other related measures when compared to controls (Wang & Corbett, 1990). The present study also evaluated ischemic subjects treated with methamphetamine and both sham condition subjects.

Locomotion behavior was also examined by dividing the testing time into two equal segments of 5 mins each. Irrespective of treatment, it was predicted that subjects would be more active during the first 5 mins of testing in a novel environment than the last 5 mins because normal exploration patterns of rodents suggest that they will demonstrate more locomotion during the exploration phase of testing and gradually, locomotion will reduce with repeated exposure or longer durations (Markgraf et al., 1994). Locomotor activity levels were consistently lower during the last 5 mins of testing time than the first

half of testing. Locomotion activity levels decreased as predicted.

In previous research studies, methamphetamine increased locomotor activity (Parker, Coughenour, & McLean, 1976). Therefore, the condition of sham treated subjects injected with methamphetamine was necessary to rule out any excitatory effects from the drug. The effects of methamphetamine on locomotion was anticipated to diminish within the 48 hrs post-surgery rest period prior to the behavioral test. Sham treatment results support the expectation that excitation effects of methamphetamine on locomotion were not observed during testing 48 hrs following treatment.

Procedures described by previous researchers in which subjects were pre-exposed to the open-field at various times before treatment conditions were not implemented in the current study. The testing apparatus was novel when testing began, therefore, animals did not have an opportunity to explore the new location until their locomotor behaviors were recorded. Pre-exposure would represent a possible confound as research demonstrates that the timing of exposure, from days to hrs before treatment, and the number of times subjects are introduced to the apparatus preceding treatment may impact results during open-field testing (Cohn & Coryslehta, 1993). The decision to omit pre-exposure in the current experimental procedure was made to eliminate further confounds such as amount of practice and learning, which were not among the behavioral indices presently investigated.

Histological procedures were used to investigate the amount of neuronal damage and to relate the outcome with locomotor behavior. Twenty-one days after surgery, subjects were perfused and brain slices were mounted and stained with cresyl violet. Two independent raters that were blind to the conditions of the samples rated the

sections based on level of damage to neuronal cell bodies in the CA1 region. As predicted, there were large amounts of damage in ischemic + saline treated subjects and no damage observed in the ischemic treatment injected with methamphetamine or sham treated subjects injected with saline. Measures of locomotor activity and hippocampal CA1 region damage ratings represent converging evidence for neuroprotection; subjects demonstrating higher activity levels also were rated as having more cell damage. These findings support the hypothesis and previous research results on cell damage and behavior (Wang & Corbett, 1990).

Ischemic insult can lead to locomotion hyperactivity, followed later by cell death of hippocampal CA1 region neuronal cells. Methamphetamine following insult appears to prevent the onset of locomotor pathology as well as cell death. These findings do not appear to follow convention in stroke research involving methamphetamine. However, previous work focused on other models of stroke, such as permanent occlusion (Wang et al., 2001). Also, other studies involving methamphetamine introduced the drug before insult and on repeated occasions (Baldwin, Colado, Murray, Desouza, & Green, 1993). The mechanism of methamphetamine neuroprotection is not known. It is possible that administration following ischemia allows methamphetamine to inhibit some part of the cell death cascade that is already in motion. Another possible explanation for neuroprotection might be that the stimulation of neurotransmitter release caused by methamphetamine may play a role in the alteration of synaptic transmission. Given the 21-day time span before histological evidence was collected, it is conceivable that alternate pathways for neuron connectivity were strengthened. In this scenario, the cells that would normally have died after insult might have been replaced by new, or

alternate neurons that were strengthened by the drug's effects and no change in the number of neurons would be detected. This is unlikely, because only one injection was administered, providing a small window of opportunity for the drug to induce alternate synaptic strengthening of neurons.

Methamphetamine is known to release large amounts of dopamine. It is possible that the strong excitation response to dopamine may interfere with cell death cascade processes. Perhaps the disruption affects the ability of NMDA receptors to close after large influxes of calcium, implicated as an important step in cell death (Bonfoco, Krain, Ankarcrona, Nicotera, & Lipton, 1995). High levels of CaMKII have been observed to facilitate cell death processes. It is a possibility that methamphetamine interferes with the cell death cascade after calcium has bound to cal-modulin to produce CaMKII (Colbran, 1992). If this were the case, methamphetamine's high level of cell excitation could block the cells' ability to shut off calcium flow- an indicated "off switch" that cells initiate in the cell death process. By way of excitation, methamphetamine could thus temporarily extinguish a cell's ability to stop calcium influx, which is an observed phenomenon that occurs during cell death.

During the cascade of cell death, another observation is that homeostatic levels of nitrogen and oxygen falter (Beckman, 1991). During cell death, this regulatory upset allows other products, such as free radicals, to remain in cells rather than be ionized and carried away as waste. Research indicates that the presence of methamphetamine changes cell production levels of nitrogen regulators within the cell. Perhaps there is a process in which methamphetamine influences the balance, despite reduced oxygen supply to the cell. Further studies into the steps of cell death cascade effects, past

calcium influx steps, are necessary to determine if methamphetamine is involved at that particular level. In addition, research continues on the role that prions and free radicals have on the process of cell death. Research based on the abuse of methamphetamine has yielded extensive knowledge about excitatory effects of dopamine receptors during chronic usage, but non-abuse studies should continue in order to clarify clinical effects of the substance.

The results of this experiment indicate novel uses for methamphetamine. One of the most appealing aspects of using this drug for ischemic treatment is that it can be administered after an attack has occurred – a frequent scenario in emergency ischemic care. This is controversial, due to previous research using different administration designs as well as the fact that methamphetamine is a highly addictive drug that is involved in a staggering amount of violence and death in rural North America. Future work in this line of research should involve replicating the results of this experiment, as well as testing with other animal models, using different time lines for administration and conducting a variety of behavioral tests. Current work on such models and their involvement in transient ischemic stroke is underway.

Due to potential effectiveness post-ischemia, methamphetamine should be pursued as a candidate for clinical trials. However, considerably more information is needed to better determine how long after an insult the drug can still be administered with successful neuroprotection. One pharmacological goal would be to explore further the mechanisms by which methamphetamine protects hippocampal neurons, to determine if modifications could be made that would reduce or eliminate the well-known recreational side effects on patients that may one day be part of a treatment trial.

REFERENCES

- Andersen, P., Holmqvist, B., & Voorhoeve, P.E. (1966). Entorhinal activation of dentate granule cells. *Acta Physiologica Scandinavica*, *66*, 448-460.
- Anderson, J. R. (1976). *Language, Memory and Thought*. Mahwah, NJ: Erlbaum.
- Aschner, M. & Kerper, L.E. (2000). Transport of metals in the nervous system. In R.K. Zalups & D.J. Koropatnick (Eds.), *Molecular biology and toxicology of metals* (pp. 276-299). New York, NY: Taylor and Francis.
- Ay, H., Furie, K. L., Singhal, A., Smith, W. S., Sorensen, A.G., & Koroshetz, W.J. (2005). An evidence-based causative classification system for acute ischemic stroke. *Annual Neurology* *58*, 688-697.
- Babcock, A. M., Baker, D.A., Hallock, N.L., Lovec, R., Lynch, W.C., & Peccia, J.C. (1993). Induced-hypothermia prevents hippocampal neuronal damage and increased locomotor activity in ischemic gerbils. *Brain Research Bulletin*, *32*, 373-378.
- Backer Cave, C., Squire, L. R. (1991). Equivalent impairment of spatial and nonspatial memory following damage to the human hippocampus. *Hippocampus*, *1*, 329-340.
- Baddeley, A. D. (1966). The influence of acoustic and semantic similarity on long-term memory for word sequences. *Quarterly Journal of Experimental Psychology*, *18*, 302-309.
- Baldwin, H.A., Colado, M.I., Murray, T.K., DeSouza, R.J., & Green, A.R. (1993). Striatal dopamine release *in vivo* following neurotoxic doses of methamphetamine and effect of the neuroprotective drugs, chlormethiazole and dizocilpine. *British Journal of Pharmacology*, *108*, 590-596.
- Barnes, C. A. (1979). Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. *Journal of Comparative Physiology and Psychology*, *93*, 74-104.
- Basso, D.M., Beattie, M.S., & Bresnahan, J.C. (1995). A sensitive and reliable locomotor rating-scale for open-field testing in rats. *Journal of Neurotrauma*, *12*, 1-21.
- Bayer, S. (1985). *Hippocampal region*. In G. Paxinos (Ed.) *The rat nervous system*, volume 1: Forebrain and midbrain (pp. 335-352). New York: Academic Press.

- Beam, T.R., & Allen, J.C. (1977). Blood, brain, and cerebrospinal fluid concentrations of several antibiotics in rabbits with intact and inflamed meninges. *Antimicrobial Agents and Chemotherapy*, *12*, 710-716.
- Beckman, J.S. (1991). The double-edged role of nitric-oxide in brain-function and superoxide-mediated injury. *Journal of Developmental Physiology*, *15*, 53-59.
- Belzung, C. (1999). Measuring exploratory behavior: Handbook of molecular genetic techniques for brain and behavior research. In W. E. Crusio & R. T. Gerlai (Eds.), *Techniques in the Behavioral and Neural Sciences* (pp. 739-749). Amsterdam: Elsevier.
- Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P., & Lipton, S.A. (1995). Apoptosis and necrosis- 2 distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric-oxide superoxide in cortical cell-cultures. *Proceedings of the National Academy of Sciences of the United States of America*, *92*, 7162-7166.
- Bonita, R., Solomon, N., & Broad, J.B. (1997). Prevalence of stroke and stroke-related disability. Estimates from the Auckland stroke studies. *Stroke*, *28*, 1989-1902.
- Brna, T. G., & Wilson, C.C. (1990). Psychogenic amnesia. *American Family Physician*, *4*, 229-234.
- Broadbent, N.J., Squire, L.R., & Clark, R.E. (2004). Spatial memory, recognition memory and the hippocampus. *Proceedings of the National Academy of Sciences, USA*, *101*, 14515-14520.
- Broca, P. (1878). Anatomie comparee des circonvolutions cerebrales: le grand lobe limbique dans le serie des mammiferes. *Reviews in Anthropology*, *1*, 385-498.
- Brodal, A. (1947). The hippocampus and the sense of smell. *Brain*, *70*, 179-222.
- Capecchi, M.R. (1989). The new mouse genetics: Altering the genome by gene targeting. *Trends in Genetics*, *5*, 70-76.
- Chan, F.K., Ching, J.Y., Hung, L.C., Wong, V.W., Leung, V.K., Kung, N.N., Hui, A.J., Wu, J.C., Leung, W.K., Lee, V.W., Lee, K.K., Lee, Y.T., Lau, J.Y., To, K.F., Chan, H.L., Chung, S.C., & Sung, J.J. (2005). Clopidogrel versus aspirin and esomeprazole to prevent recurrent ulcer bleeding. *New England Journal of Medicine*, *352*, 238-244.
- Choi, D.W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron*, *1*, 623-634.

- Chandler, M.J., DeLeo, J., & Carney, J.M. (1985). An unanesthetized-gerbil model of cerebral ischemia-induced behavioral changes. *Journal of Pharmacology*, *14*, 137-146.
- Chemerinski, E., & Levine, S.R. (2006). Neuropsychiatric disorders following vascular brain injury. *The Mount Sinai Journal of Medicine*, *73*, 1006-1012.
- Cohn, J. & Coryslehta, D.A. (1993). Subsensitivity of lead-exposed rats to the accuracy-impairing and rate-altering effects of MK-801 on a multiple schedule of repeated learning and performance. *Brain Research*, *600*, 208-218.
- Colbran, R.J. (1992). Regulation and role of brain calcium calmodulin-dependent protein kinase-II. *Neurochemistry International*, *21*, 469-497.
- Cole, M.R., & Chappell-Stephenson, S. (2003). Exploring the limits of spatial memory using very large mazes. *Learning and Behavior*, *31*, 349-368.
- Croce, P. (1999). *Vivisection or science? An investigation into testing drugs and safeguarding health*. London: Zed Books.
- de Boorder, M.J., van der Grond, J., Van Dongen, A.J., Klijn, C.J., Jaap, K.L., Van Rijk, P.P., & Hendrikse, J.S. (2006). Measurements of regional cerebral perfusion and carbon dioxide reactivity: Correlation with cerebral collaterals in internal carotid artery occlusive disease. *Journal of Neurology*, *253*, 1285-1291.
- Douglas, R.J., & Pribram, K.H. (1966). Learning and limbic lesions. *Neuropsychologia*, *4*, 197-220.
- Eichenbaum, H., Otto, T., & Cohen, N.J. (1992). The hippocampus-What does it do? *Behavioral and Neural Biology*, *57*, 2-36.
- Fata-Hartley, C.L., & Palmenberg, A.C. (2005). Dipyridamole reversibly inhibits mengovirus RNA replication. *Journal of Virology*, *79*, 11062-11070.
- Feldman, R.S., Meyer, J.S., & Quenzer, L.F. (1997). *Principles of neuropsychopharmacology*. Sunderland, Massachusetts: Sinauer Associates.
- Fire, A., Xu, S., Montgomery, M., Kostas, S., Driver, S., & Mello, C. (1998). Po10t and specific genetic interference by double-stranded RNA in caenorhabditis elegans. *Nature*, *391*, 806-811.
- Flamm, E.S., Demopoulos, H.B., Seligman, M.L., Posner, R.G., & Ransohoff, J. (1978). Free radicals in cerebral ischemia. *Stroke*, *9*, 445-447.

- Foreman, N.P. (1983). Head-dipping in rats with superior collicular, medial frontal cortical and hippocampal lesions. *Physiology and Behavior*, *30*, 711-717.
- Garrison, F.H. (1966). *History of Medicine*. Philadelphia: W.B. Saunders Company.
- Gerriets, T., Stolz, E., Walberer, M., Muller, C., Rottger, C., Kluge, A., Kaps, M., Fisher, M., & Bachmann, G. (2004). Complications and pitfalls in rat stroke models for middle cerebral artery occlusion: A comparison between the suture and the macrosphere model using magnetic resonance angiography. *Stroke*, *35*, 2372-2377.
- Giovanni, A., Liang, L.P., Hastings, T.G., & Zigmond, M.J. (1995). Intraventricular salicylate- Impact of methamphetamine. *Journal of Neurochemistry*, *64*, 1819-1825.
- Graham, D.I., & Gennareli, T.A. (2000). Pathology of brain damage after head injury. In P. Cooper & G. Golfinos (Eds.), *Head injury* (pp. 212-257). New York: Morgan Hill.
- Hall, C.S. (1934). Emotional behavior in the rat: Defecation and urination as measures of individual differences in emotionality. *Journal of Comparative Psychology*, *18*, 385-403.
- Hansen, A.J., Hounsgaard, J., & Jahnsen, H. (1982). Anoxia increases potassium conductance in hippocampal nerve-cells. *Activa Physiologica Scandanavia*, *115*, 301-310.
- Heston, L.L., & Heston, R. (1979). *The medical casebook of Adolf Hitler: His illnesses, doctors and drugs*. New York: Stein and Day.
- Hinkle, J., & Bowman, L. (2003). Neuroprotection for ischemic stroke. *Journal of Neuroscience and Nursing*, *35*, 114-118.
- Johnston, S., Rothwell, P., Nguyen-Huynh, M., Giles, M., Elkins, J., Bernstein, A., & Sidney, S. (2007). Validation and refinement of scores to predict very early stroke risk after transient ischemic attack. *Lancet*, *369*, 283-292.
- Katchman, A.N., & Hershkowitz, N. (1993). Early anoxia-induced vesicular glutamate release results from mobilization of calcium from intracellular stores. *Journal of Neurophysiology*, *70*, 1-7.
- Katsumata, N., Kuriowa, T., Ishibashi, S., Li, S., Endo, S., & Ohno, K. (2006). Heterogeneous hyperactivity and distribution of ischemic lesions after focal cerebral ischemia in Mongolian gerbils. *Neuropathology*, *26*, 283-292.

- King, D.L., & Arendash, G.W. (2002). Behavioral characterization of the Tg2576 transgenic model for Alzheimer's disease through 19 months. *Physiology and Behavior*, *75*, 627-642.
- Kirino, T. (1982). Delayed neuronal death in the gerbil hippocampus following ischemia. *Brain Research*, *239*, 57-69.
- Krnjevic, K, and Xu, Y. Z. (1989). Dantrolene suppresses the hyperpolarization or outward current observed during anoxia in hippocampal-neurons. *Canadian Journal of Physiology and Pharmacology*, *67*, 1602-1604.
- Lees, K.R., Davalos, A., Davis, S.M., Diener, H., Grotta, J., Lyden, P., Shuaib, A., Ashwood, T., Hardemark, H., Wasiewski, W., Emeribe, U., & Zivin, J.A. (2006). SAINT I investigators additional outcomes and subgroup analyses of NXY-059 for acute ischemic stroke in the SAINT I trial. *Stroke*, *37*, 2970-2978.
- Leppavuori, A., Pohjasvaara, T., Vataja, R. (2003). Generalized anxiety disorders three to four months after ischemic stroke. *Cerebrovascular Disease*, *16*, 257-264.
- Liang, K.C., Hon, W., Tyan, Y.M., & Liao, W.L. (1994). Involvement of hippocampal NMDA and AMPA receptors in acquisition, formation and retrieval of spatial memory in the Morris water maze. *Chinese Journal of Physiology*, *37*, 201-212.
- Limbrick, D.D., Churn, S.B., Sombati, S., & DeLorenzo, R.J. (1995). Inability to restore resting intracellular calcium levels as an early indicator of delayed neuronal cell death. *Brain Research*, *690*, 145-156.
- Librach, I.M. (1978). Johan Jakob Wepfer (1620-1695). *Medical History*, *15*, 314-315.
- Maclean, P.D. (1952). Some psychiatric implications of physiological studies on frontotemporal portion of limbic system (visceral brain). *Electroencephalography and clinical neurophysiology*, *4*, 407-418.
- Markgraf, C.G., Green, E.J., Watson, B., McCabe, P.M., Schneiderman, N., Dietrich, W.D., & Ginsberg, M.D. (1994). Recovery of sensorimotor function after distal middle cerebral-artery photothrombotic occlusion in rats. *Stroke*, *25*, 153-159.
- Martinsson, L., Hardemark, H., & Eksborg, S. (2007). Amphetamines for improving recovery after stroke. *Cochrane Database of Systematic Reviews*, (Publication Number CD002090. DIO: 10.1002/14651858).
- Matto, V., Allikmets, L. (1999). Acute and chronic citalopram treatment differently modulates rat exploratory behavior in the exploration box test: No evidence for increased anxiety or changes. *Pharmacology*, *58*, 59-69.

- McCann, U.D., Ridenour, A., Shaham, Y., & Ricaurte, G.A. (1994). Serotonin neurotoxicity after (+/-) 3,4-methylenedioxymethamphetamine (MDMA Ecstasy)-A controlled-study in humans. *Neuropsychopharmacology*, *10*, 129-138.
- McGee, S.M., McGee, D.N., McGee, M.B. (2004). Spontaneous intracerebral hemorrhage related to methamphetamine abuse: Autopsy findings and clinical correlation. *The American Journal of Forensic Medicine and Pathology*, *25*, 334-337.
- McLay, R.N., Freeman, S.M., & Zadina, J.E. (1998). Chronic corticosterone impairs memory performance in the Barnes maze. *Physiology and Behavior*, *63*, 933-937.
- Meilke, O., Wardlaw, J., & Liu, M. (2004). Thrombolysis (different doses, routes of administration and agents) for acute ischemic stroke. *Cochrane Database of Systematic Reviews*, (Publication Number CD000514. DIO: 10.1002/14651858).
- Mizuno, K., & Giese, K.P. (2005). Hippocampus-dependent memory formation: Do memory type-specific mechanisms exist? *Journal of Pharmacological Sciences*, *98*, 191-197.
- Mohr, J.P., Caplan, L.R., & Melski, J.W. (1978). The Harvard Cooperative Stroke Registry: A prospective registry. *Neurology*, *28*, 754-762.
- MORI. (2002). The use of animals in medical research. *Research study conducted for the Coalition for Medical Progress*. London: Market & Opinion Research International.
- Morris, R. (1984). Developments of water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, *11*, 47-60.
- Ohta, K., Mori, M., Yoritaka, A., Okamoto, K., & Kishida, S. (2005). Delayed ischemic stroke associated with methamphetamine use. *Journal of Emergency Medicine*, *28*, 165-167.
- Olten, D.S., & Samuelson, R.J. (1976). Remembrance of places passed: Spatial memory in rats. *Journal of Experimental Psychology: Animal Behavior Processes*, *2*, 97-116.
- Papez, J.W. (1937). A proposed mechanism of emotion. *Journal of Neuropsychiatry and Clinical Neuroscience*, *7*, 103-112.
- Parker, R.B., Coughenour, L.L., & McLean, J.R. (1976). Inhibition of spontaneous and methamphetamine-induced locomotor activity in mice. *Federation Proceedings*, *35*, 785-785.

- Pedersen, T.F., Thorbjornsen, M.L., Klepstad, P., Sunde, K., & Dale, O. (2007). Therapeutic hypothermia-pharmacology and pathophysiology. *Tidsskr Nor Laegeforen*, *127*, 163-166.
- Perel, P., Roberts, I., Sena, E., Wheble, P., Briscoe, C., Sandercock, P., Macleod, M., Mignini, L.E., Jayaram, P., & Khan, K.S. (2007). Comparison of treatment effects between animal experiments and clinical trials: Systematic review. *British Medical Journal: Clinical Research Edition*, *334*, 197-206.
- Pompl, P.N., Mullan, M.J., Bjugstad, K., & Arendash, G.W. (1999). Adaptation of the circular platform spatial memory task for mice: Use in detecting cognitive impairment in the APP (SW) transgenic mouse model for Alzheimer's disease. *Journal of Neuroscience Methods*, *87*, 87-95.
- Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*, 3-33.
- Roberts, L. (2001). Controversial from the start. *Science*, *291*, 1182-1188.
- Rothman, R.B., & Baumann, M.H. (2002). Therapeutic and adverse actions of serotonin transporter substrates. *Pharmacology and Therapeutics*, *95*, 73-85.
- Rothwell, P.M., Giles, M.F., Flossmann, E., Lovelock, C.E., Redgrave, J.N., Warlow, C.P., & Mehta, Z. (2005). A simple score (ABCD) to identify individuals at high early risk of stroke after transient ischemic attack. *Lancet*, *366*, 29-36.
- Sano, K. (1997). Hippocampus and epilepsy surgery. *Epilepsia*, *38*, 4-8.
- Save, E., Poucet, B., Foreman, N., Buhot, M. (1992). Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal-formation. *Behavioral Neuroscience*, *106*, 447-456.
- Schinder, A.F., Olson, E.C., Spitzer, N.C., & Montal, M. (1996). Mitochondrial dysfunction is a primary event in glutamate neurotoxicity. *Journal of Neuroscience*, *16*, 6125-6133.
- Schube, P.G., & Raskin, N. (1940). The neuropathology of Benzedrine poisoning. *Psychiatric Quarterly*, *14*, 47-52.
- Shepherd, G.M. (1998). *The synaptic organization of the brain*. New York: Oxford University Press.
- Sibbald, W.J. (2000). An alternative pathway for preclinical research in fluid management. *Critical Care*, *4*, 8-15.

- Siesjo, B.K. (1993). Basic mechanisms of traumatic brain damage. *Annual Review of Emergency Medicine*, 22, 959-969.
- Siesjo, B.K., Zhao, Q., Palhmark, K., Siesjo, P., Katsura, K., & Folbergrova, J. (1995). Glutamate, calcium, and free radicals as mediators of ischemic brain damage. *Annual Thoracic Surgery*, 59, 1316-1320.
- Stroemer, R. P., Kent, T.A., & Hulsebosch, C.E. (1998). Enhanced neocortical neural sprouting, synaptogenesis, and behavioral recovery with D-amphetamine therapy after neocortical infarction in rats. *Stroke*, 29, 2381-2393.
- Sutherland, R. J., & Arnold, K. (1987). Temporally graded loss of memory after hippocampal damage. *Neuroscience*, 22, 87-96.
- Swanson, L.W. (1983). The hippocampus and the concept of the limbic system. In W. Seifert (Ed.), *Neurobiology of the hippocampus* (pp. 1-19). London: Academic Press.
- Takahashi, L. K., & Kalin, N.H. (1989). Role of corticotropin-releasing factor in mediating the expression of defensive behavior. In R.J. Blanchard, P.F. Brain, D.C. Blanchard, & S. Parmigiani (Eds.), *Ethoexperimental approaches to the study of behavior, NATO ASI Series* (pp. 580-594). Boston: Kluwer Academic Publishing.
- Tecott, L.H., & Nestler, E.J. (2004). Neurobehavioral assessment in the information age. *Nature and Neuroscience*, 7, 462-466.
- The Tirilazad International Steering Committee (2001). Tirilazad for acute ischemic stroke: Tirilazad Mesylate in acute ischemic stroke. A systematic review. *Stroke*, 31, 2257-2265.
- Tolman, E.C. (1948). Cognitive maps in rats and man. *Psychological Review*, 55, 189-208.
- Uston, C. (2005). Dr. Thomas Willis' famous eponym: The circle of Willis. *Journal of the History of Neurosciences*, 14, 16-21.
- Walker-Batson, D., Smith, P., Curtis, S., Unwin, H., & Greenlee, R. (1995). Amphetamine paired with physical therapy accelerates motor recovery after stroke-further evidence. *Stroke*, 26, 2254-2259.
- Wang, D., & Corbett, D. (1990). Cerebral ischemia, locomotor activity and spatial mapping. *Brain Research*, 533, 78-82.

- Wang, Y., Hayashi, T., Chang, C.F., Chiang, Y.H., Tsao, L., Su, T.P., Borlongan, C., & Lin, S.Z. (2001). Methamphetamine potentiates ischemia/reperfusion insults after transient middle cerebral artery ligation. *Stroke*, *32*, 775-782.
- Wong, E.H., Kemp, J.A., Priestley, T., Knight, A.R., Woodruff, G.N., & Iverson, L.L. (1986). The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proceedings of the National Academy of Sciences of the United States of America*, *83*, 7104-7108.
- Willis, T. (1664). *Cerebri Anatome: cui accessit nervorum descriptio et usus*. London.
- Yu, Q., Montes, S., Larson, D.F., & Watson, R.R. (2002). Effects of chronic methamphetamine exposure on heart function in uninfected and retrovirus-infected mice. *Life Sciences*, *71*, 953-965.
- Zambrowicz, B.P., & Sands, A.T. (2003). Knockouts model the 100 best-selling drugs—will they model the next 100? *Nature Reviews: Drug Discovery*, *2*, 38-51.