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*Georgia State University*

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**GLUCOSE MODULATION OF THE SEPTO-HIPPOCAMPAL SYSTEM:  
IMPLICATIONS FOR MEMORY**

by

Desiree Lynne Krebs-Kraft

Under the Direction of Marise B. Parent

**ABSTRACT**

Extensive evidence suggests that glucose has both positive and negative effects on memory and these effects likely involve an influence on the brain. For instance, direct infusions of glucose into the septum (MS) or hippocampus can enhance or impair memory. The present set of experiments attempted to determine the different conditions that dissociate the memory-enhancing and -impairing effects of glucose in rats. Specifically, these experiments examined the effects of glucose in spontaneous alternation, a measure of spatial working memory and shock avoidance, an index of emotional long-term memory. The results showed that the memory-impairing effects of MS infusions of glucose are not concentration-dependent. These data also indicated that the memory-impairing effects of MS glucose elevations are specific to  $\gamma$ -aminobutyric acid (GABA) receptor activation but do not depend on increases in MS GABA synthesis or release. Importantly, we showed that the memory-impairing interaction between MS glucose and GABA agonists does not generalize to the hippocampus, suggesting the memory-modulating effects of glucose are brain

region-dependent. We showed further that these brain region-dependent effects of glucose are not due to difference in basal extracellular glucose levels. Moreover, these findings showed that the memory-enhancing effects of hippocampus glucose override the memory-impairing interaction between MS glucose and GABA. These findings are important because they are the first to show that the memory-modulating effects of glucose are both neurotransmitter- and brain region-dependent. Furthermore, these findings provide preliminary evidence suggesting that the memory-impairing effects of MS glucose may involve compromised hippocampal function. These data also suggest the memory-impairing effects of MS co-infusions of glucose with GABA agonists likely involve an influence on the GABAergic SH projection. Finally, these findings demonstrate the mnemonic and neurochemical consequences of glucose in the MS and hippocampus, two brain regions affected by normal aging, Alzheimer's disease, and diabetes.

**INDEX WORDS:** Septum, Hippocampus, Glucose, GABA, Acetylcholine, Spontaneous Alternation, Continuous Multiple Trial Inhibitory Avoidance (CMIA), Memory, Metabolism

**GLUCOSE MODULATION OF THE SEPTO-HIPPOCAMPAL SYSTEM:  
IMPLICATIONS FOR MEMORY**

by

Desiree Lynne Krebs-Kraft

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of  
Philosophy

in the College of Arts and Sciences

Georgia State University

2006

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Desiree Lynne Krebs-Kraft  
2006

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IMPLICATIONS FOR MEMORY**

by

DESIREE LYNNE KREBS-KRAFT

|                  |                     |
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|                  | Kim L. Huhman       |

Electronic Version Approved:

Office of Graduate Studies  
College of Arts and Sciences  
Georgia State University  
December 2006

## DEDICATION

I dedicate this dissertation to my brother, James E. Krebs, II. You gave me inspiration during all the times I thought I couldn't do something because I was threatened, intimidated, or at the limit of my physical, mental, or emotional limitations. You are so tough and have come so far, continue to be strong and refuse to be limited by your surroundings or situation. Let's make our own path and carve out our own destiny. Indeed, we will let our trials and tribulations motivate us, and through them we can reach greatness!

When things go wrong, as they sometimes will,  
When the road you're trudging seems all up hill,  
When the funds are low and the debts are high,  
And you want to smile, but you have to sigh,  
When care is pressing you down a bit,  
Rest, if you must-but don't you quit.

Life is queer with its twists and turns,  
As everyone of us sometimes learns,  
And many a failure turns about  
When he might have won had he stuck it out;  
Don't give up, though the pace seems slow  
You might succeed with another blow.

Often the goal is nearer than  
It seems to a faint and faltering man,  
Often the struggler has given up  
When he might have captured the victor's cup.  
And he learned too late, when the night slipped down,  
How close he was to the golden crown.

Success is failure turned inside out  
The silver tint of the clouds of doubt  
And you never can tell how close you are,  
It may be near when it seems afar;  
So stick to the fight when you're hardest hit  
It's when things seem worst that you mustn't quit.

**Faith by Wayne Miller**

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## LIST OF ABBREVIATIONS

|  |                          |
|--|--------------------------|
| <b>Medial Septum</b>   | <b>MS</b>                |
| <b>Extracellular Fluid</b>   | <b>ECF</b>               |
| <b>Spontaneous Alternation</b>   | <b>SA</b>                |
| <b>Continuous Multiple Trial Inhibitory Avoidance</b>                    | <b>CMIA</b>              |
| <b>Standard Error of the Mean</b>  | <b>SEM</b>               |
| <b>Interquartile Range</b>   | <b>IQ</b>                |
| <b>Septo-Hippocampal</b>   | <b>SH</b>                |
| <b>Acetylcholine</b>   | <b>ACh</b>               |
| <b>N-methyl-D-aspartate</b>  | <b>NMDA</b>              |
| <b>_ -amino-5-hydroxy-3-methyl-4-isoxazole propionic acid</b>            | <b>AMPA</b>              |
| <b>Long-term potentiation</b>  | <b>LTP</b>               |
| <b>Zero-net flux</b>   | <b>ZNF</b>               |
| <b>_ -aminobutyric acid</b>  | <b>GABA</b>              |
| <b>Phosphate buffered saline</b>   | <b>PBS</b>               |
| <b>Chlordiazepoxide</b>  | <b>CDP</b>               |
| <b>Benzodiazepine</b>  | <b>BZD</b>               |
| <b>Adenosine tri-phosphate</b>   | <b>ATP</b>               |
| <b>Potassium</b>   | <b>K<sup>+</sup></b>     |
| <b>Adenosine tri-phosphate-dependent potassium channels</b>              | <b>K<sup>+</sup>-ATP</b> |
| <b>(+)-10, 11-dihydro-5-methyl-5H-dibenzo[a,d]cyclohepten-5,10 imine</b> | <b>MK-801</b>            |

|  |                                      |
|--|--------------------------------------|
| <b>Acetylcholinesterase</b>                        | <b>AChE</b>                          |
| <b>Millimolar</b>                                  | <b>mM</b>                            |
| <b>Anterior to bregma</b>                          | <b>AP</b>                            |
| <b>Ventral to dura</b>                             | <b>DV</b>                            |
| <b>Phosphate-buffered saline</b>                   | <b>PBS</b>                           |
| <b>Seconds</b>                                     | <b>S</b>                             |
| <b>Minute(s)</b>                                   | <b>min(s)</b>                        |
| <b>Hour(s)</b>                                     | <b>hr(s)</b>                         |
| <b>Analysis of variance</b>                        | <b>ANOVA</b>                         |
| <b>GABA type A receptor</b>                        | <b>GABA<sub>A</sub></b>              |
| <b>GABA type B receptor</b>                        | <b>GABA<sub>B</sub></b>              |
| <b>Institutional Animal Care and Use Committee</b> | <b>IACUC</b>                         |
| <b>Artificial cerebrospinal fluid</b>              | <b>aCSF</b>                          |
| <b>Sodium chloride</b>                             | <b>NaCl</b>                          |
| <b>Potassium chloride</b>                          | <b>KCl</b>                           |
| <b>Calcium chloride</b>                            | <b>CaCl<sub>2</sub></b>              |
| <b>Magnesium chloride</b>                          | <b>MgCl<sub>2</sub></b>              |
| <b>Sodium phosphate monobasic</b>                  | <b>NaH<sub>2</sub>PO<sub>4</sub></b> |
| <b>Sodium phosphate dibasic anhydrous</b>          | <b>Na<sub>2</sub>HPO<sub>4</sub></b> |
| <b>Microliter</b>                                  | <b>μl</b>                            |
| <b>High-performance-liquid-chromatography</b>      | <b>HPLC</b>                          |
| <b>Milliliter</b>                                  | <b>ml</b>                            |

|  |                      |
|--|----------------------|
| <b>Tetrahydrofuran</b>                               | <b>THF</b>           |
| <b>Nitrogen</b>                                      | <b>N<sub>2</sub></b> |
| <b>Sodium</b>  | <b>Na</b>            |
| <b>Sodium-dependent high affinity choline uptake</b> | <b>SDHACU</b>        |
| <b>Ethanol</b>                                       | <b>EtOH</b>          |
| <b>Degrees Celsius</b>                               | <b>°C</b>            |
| <b>Degrees Fahrenheit</b>                            | <b>°F</b>            |
| <b>Gram</b>  | <b>G</b>             |
| <b>Microgram</b>                                     | <b>μg</b>            |
| <b>Milliamperes</b>                                  | <b>mA</b>            |
| <b>Centimeter</b>                                    | <b>Cm</b>            |
| <b>Millimeter</b>                                    | <b>Mm</b>            |
| <b>Milligram per kilogram</b>                        | <b>mg/kg</b>         |
| <b>Intraperitoneal</b>                               | <b>i.p.</b>          |
| <b>Intramuscular</b>                                 | <b>i.m.</b>          |
| <b>Subcutaneous</b>                                  | <b>s.c.</b>          |
| <b>Nanomole</b>                                      | <b>Nmol</b>          |
| <b>Tukey honestly significantly different</b>        | <b>Tukey HSD</b>     |
| <b>Hertz</b>   | <b>Hz</b>            |
| <b>Cubic centimeters</b>                             | <b>Cc</b>            |
| <b>Micrometers</b>                                   | <b>μm</b>            |
| <b>Acetyl coenzyme A</b>                             | <b>Acetyl coA</b>    |



|  |                                |
|--|--------------------------------|
| <b>Glucose hexokinase</b>                | <b>HK</b>                      |
| <b>Glucose-6-phosphate dehydrogenase</b> | <b>G<sub>6</sub>PDH</b>        |
| <b>Nicotinamide adenine dinucleotide</b> | <b>NAD<sup>+</sup> or NADH</b> |
| <b>Deionized water</b>                   | <b>dH<sub>2</sub>O</b>         |
| <b>Nanometer</b>                         | <b>Nm</b>                      |
| <b>Glucose-6-phosphate</b>               | <b>G<sub>6</sub>PD</b>         |

## CHAPTER 1

### GENERAL INTRODUCTION

Extensive evidence suggests that glucose is involved in the regulation of memory processes (Benton & Nabb, 2003; Corey-Bloom, 2002; Korol & Gold, 1998; Messier, 2004; Messier & Gagnon, 2000). More specifically, glucose has both positive and negative effects on memory. For example, glucose enhances memory (Lee, Graham, & Gold, 1988; Stefani, Nicholson, & Gold, 1999; Sunram-Lea, Foster, Durlach, & Perez, 2002) and reverses the memory deficits and cognitive dysfunction that are associated with Alzheimers's disease, aging, Down's syndrome, and schizophrenia (Craft, Murphy, & Wemstrom, 1994; Gold, 1995; Korol, 2002; Korol & Gold, 1998; Manning, Ragozzino, & Gold, 1993; Newcomer et al., 1999; Petit, 1988). In contrast, acute and chronic hyperglycemia are associated with memory dysfunction (Biessels et al., 1996; Convit, 2005; Elias et al., 1997; Flood, Mooradian, & Morley, 1990; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; McCall, 2002; Popovic, Biessels, Isaacson, & Gispen, 2001; Winocur et al., 2005). The mechanisms underlying the memory-modulating effects of glucose, particularly the conditions that differentiate the enhancing and impairing effects of glucose, are poorly understood.

The memory-modulating effects of glucose are mediated, at least in part, by the brain. Glucose readily crosses the blood-brain barrier via glucose transporters (Boado & Pardridge, 1990; Pardridge, Boado, & Farrell, 1990; Rahner-Welsch, Vogel, & Kuschinsky, 1995; Takata, Hirano, & Kasahara, 1997). Furthermore, training on an operant learning task is associated with increases in GLUT 1 transporter expression in the hippocampus, suggesting increased cellular metabolic demands and glucose utilization in brain areas important for memory processes (Choeiri, Staines, Miki, Seino, & Messier, 2005). Interestingly, extracellular fluid (ECF) levels of hippocampal glucose decrease with cognitive demand, and systemic infusions of glucose both enhance memory

and replenish these decreases in hippocampal glucose (McNay & Gold, 1999). These data suggest that during tasks that engage the hippocampus, peripheral glucose is preferentially directed to the hippocampus and enhances memory formation. Moreover, direct brain infusions of glucose affect memory. For example, infusions of glucose into the cerebral ventricular system enhance retention performance on a one-trial inhibitory avoidance task in rodents (Lee, Graham, & Gold, 1988). Direct injections of glucose into specific brain regions, including the septum (MS) or hippocampus, enhance spontaneous alternation (SA) performance (Ragozzino & Gold, 1995b; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Stefani & Gold, 1998; Stefani, Nicholson, & Gold, 1999), a measure of spatial working memory (Dember, 1989; Lalonde, 2002; Richman, Dember, & Kim, 1987). MS or hippocampal infusions of glucose also reverse drug-induced deficits in SA (Ragozzino & Gold, 1995b). MS co-infusions of glucose with muscimol potentiate memory deficits produced by MS  $\gamma$ -aminobutyric acid (GABA) receptor activation (Parent, Laurey, Wilkniss, & Gold, 1997), suggesting that the memory-impairing effects of glucose are also mediated, at least in part, in the brain.

The septo-hippocampal (SH) system (see Figure 1.1) is ideal to examine the central effects of glucose on memory because: 1) this brain system is involved in learning and memory processes (Farber, 1996; Harper, McLean, & Dalrymple-Alford, 1994; Izquierdo & Medina, 1993; Jarrard, 1993; Kornecook, Kippin, & Pinel, 1999; Ramos, 2002), 2) it has a well-characterized chemoanatomy (Amaral & Witter, 1995; Jakab & Leranth, 1995), and 3) both the memory-enhancing and -impairing effects of glucose can be demonstrated simultaneously in this system (Krebs & Parent, 2005a; Parent & Krebs, 2004; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997). The MS is connected to the hippocampus via the fimbria-fornix, and is composed primarily of cholinergic and GABAergic SH projection neurons (Kohler, Chan-Palay, & Wu,

1984; Lewis, Shute, & Silver, 1967; Rye, Wainer, Mesulam, Mufson, & Saper, 1984; see Figure 1.1). Although a putative glutamatergic projection has been recently proposed, very little is known about the connectivity or receptor make-up of the glutamatergic SH projection (Colom, Castaneda, Reyna, Hernandez, & Garrido-Sanabria, 2005; Sotty et al., 2003). The GABAergic septo-hippocampal (SH) projection synapses onto a GABA interneuron in the hippocampus (Freund & Antal, 1988). The GABA interneuron then synapses onto a pyramidal cell (Toth, Freund, & Miles, 1997). Therefore, the GABAergic afferents to the hippocampus produce a net disinhibition of pyramidal cells. Cholinergic SH projections project broadly to the hippocampus, synapsing onto pyramidal cells, dentate granule cells, and inhibitory interneurons (Frotscher & Leranth, 1985). Activation of the cholinergic SH projection excites pyramidal cells (Chabot, Massicotte, Milot, Trudeau, & Gagne, 1997; Izquierdo & Medina, 1993; Teyler, 1987; see Figure 1.1).

Extensive evidence support the hypothesis that the cholinergic SH projection is involved in memory. For example, a variety of studies have shown training-induced increases in hippocampal acetylcholine (ACh; Decker, Pellymounter, & Gallagher, 1988; Durkin, 1992a, 1994; Lebrun, Durkin, Marighetto, & Jaffard, 1990; Marighetto, Durkin, Toumane, Lebrun, & Jaffard, 1989; Marighetto, Micheau, & Jaffard, 1993, 1994; Toumane, Durkin, Marighetto, Galey, & Jaffard, 1988; Wenk, Hepler, & Olton, 1984). MS manipulations may affect memory through an influence on the hippocampal cholinergic neurotransmitter system. The MS is the primary source of ACh efferents to the hippocampus and modulates hippocampal ACh activity and memory (Chrobak, Stackman, & Walsh, 1989; Costa, Panula, Thompson, & Cheney, 1983). Neurochemical manipulations of the MS affect hippocampal ACh, and these changes are paralleled by alterations in memory performance (Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990; Durkin, 1992a; Herzog, Gahndi, Bhattacharya, & Walsh, 2000). For instance, MS GABA receptor activation

decreases hippocampal ACh. Moreover, only those doses of a GABA agonist that impair memory decrease hippocampal ACh (Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990).

Furthermore, MS GABA receptor activation increases the dose of ACh agonists needed in the hippocampus to reverse memory deficits (Farr, Uezu, Flood, & Morley, 1999). Manipulations of the hippocampus that enhance hippocampal ACh levels reverse the memory deficits produced by MS GABA receptor activation (Degroot & Parent, 2000, 2001; Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b).

More recent evidence, however, suggests the GABAergic SH projection may also be involved in memory. For instance, the GABAergic projection is likely important for the memory-impairing effects of MS GABA receptor activation because lesions of the cholinergic projection do not prevent the memory-impairing effects of muscimol (Pang, 1999; Pang, Nocera, Secor, & Yoder, 2001a). Furthermore, electrophysiological studies show that MS administration of muscarine, a cholinergic agonist with memory-enhancing actions, excites the GABAergic SH projection (Wu, Shanabrough, Leranth, & Alreja, 2000). Moreover, the muscarinic antagonist scopolamine, a drug that impairs memory when infused into the MS, decreases impulse flow in the GABAergic SH projection (Alreja et al., 2000).

There are multiple putative mechanisms that may underlie the memory-enhancing actions of glucose. For instance, evidence suggests that the memory-enhancing effects of glucose occur, in part, via insulin. Elevations in glucose lead to increased insulin levels and insulin administration enhances memory (Blanchard & Duncan, 1997; Craft et al., 1999; Park, Seeley, Craft, & Woods, 2000). Another line of evidence suggests that adenosine tri-phosphate (ATP)-dependent potassium (K<sup>+</sup>) channels (K<sup>+</sup>-ATP) are involved in the memory-enhancing effects of glucose. The opening of the K-ATP channels impairs memory (Rashidy-Pour, 2001; Stefani &

Gold, 1998, 2001a; Stefani, Nicholson, & Gold, 1999). Blocking K-ATP channels with glucose enhances memory and reverses memory deficits (Rashidy-Pour, 2001; Stefani & Gold, 1998, 2001a; Stefani, Nicholson, & Gold, 1999). Finally, glucose may affect memory via a product of its metabolism. Glucose administration may enhance memory by increasing ATP levels in brain regions important for learning and memory (da Silva, de Mendonca, & Ribeiro, 2005; Mori, Heuss, Gahwiler, & Gerber, 2001; Pankratov, Castro, Miras-Portugal, & Krishtal, 1998). In addition, glucose is the synthetic precursor for different neurotransmitter systems important for enhancing or impairing learning and memory (Dolezal & Tucek, 1981; Hertz & Dienel, 2002; Tucek, 1983).

The effects of glucose on neurotransmitter function may underline both the positive and negative effects of central glucose administration on memory (see Figure 1.2). For instance, it seems that glucose reverses deficits produced by drugs that modulate some neuroactive substances but not others. For example, glucose administration reverses deficits produced by the opiate agonist morphine, the cholinergic antagonist scopolamine, and the peptide galanin (Ragozzino, Arankowsky-Sandoval, & Gold, 1994; Ragozzino & Gold, 1995b; Ragozzino, Parker, & Gold, 1992; Rashidy-Pour, 2001; Stefani & Gold, 1998). Infusions of glucose, however, have no effect on the deficits produced by the noradrenergic antagonist propranolol (Lennartz, Hellems, Mook, & Gold, 1996) or the N-methyl-D-aspartate (NMDA) receptor blocker (+)-10,11-dihydro-5-methyl-5H-dibenzo[a,d]cycloheptene-5,10 imine (MK-801; (Roesler, Vianna, de-Paris, & Quevedo, 1999). Likewise, MS co-infusions of glucose do not reverse the memory-impairing effects of GABA agonists (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997). Furthermore, co-infusions of glucose with GABA agonists, at doses that do not affect memory alone, produce memory deficits (Parent & Gold, 1997; Shah & Parent, 2003, 2004).

Extensive evidence suggests that glucose enhances memory through a process that involves an increase in ACh synthesis or release, particularly in the hippocampus. For instance, ACh antagonists prevent the memory-enhancing effects of glucose (Kopf & Baratti, 1994). Co-infusions of sub-effective doses of glucose with an acetylcholinesterase (AChE) inhibitor enhance memory (Pavone, Capone, Battaglia, & Sansone, 1998). Glucose attenuates decreases in hippocampal ACh and SA performance deficits produced by systemic injections of ACh antagonists (Durkin, Messier, de Boer, & Westerink, 1992; Ragozzino, Arankowsky-Sandoval, & Gold, 1994). In addition, systemic administration of glucose augments hippocampal ECF ACh during performance on a spatial memory task in rodents (Ragozzino, Unick, & Gold, 1996). Moreover, hippocampal infusions of glucose mimic the effects of AChE inhibitors and reverse memory deficits produced by MS GABA receptor activation (Degroot & Parent, 2000, 2001; Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997). More directly, intra-hippocampal infusions of glucose also enhance SA scores and enhance ACh release in the hippocampus (Ragozzino, Pal, Unick, Stefani, & Gold, 1998b).

In contrast, glucose may impair memory through an influence on MS GABA receptor function. MS infusions of GABA<sub>A</sub> agonists impair memory (Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990; Chrobak, Stackman, & Walsh, 1989; Degroot & Parent, 2000, 2001; Farr, Uezu, Flood, & Morley, 1999; Izquierdo & Medina, 1991; Nagahara & McGaugh, 1992; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997). MS infusions of glucose potentiate these memory-impairing effects of GABA<sub>A</sub> agonists (Parent, Laurey, Wilkniss, & Gold, 1997). More interesting, MS co-infusions of glucose with the GABA receptor agonist muscimol, at doses that individually do not affect memory performance, produce memory deficits (Parent & Gold, 1997; Shah & Parent, 2003, 2004). Acute administration of large amounts of glucose and experimentally

induced chronic hyperglycemia increase GABA levels in the brain (Amoroso, Schmid-Antomarchi, Fosset, & Lazdunski, 1990; During, Leone, Davis, Kerr, & Sherwin, 1995; Fink & Gothert, 1993; Fink, Zentner, & Gothert, 1994; Ohtani, Ohta, & Sugano, 1997). Combined, these findings suggest that MS infusions of glucose impair memory by promoting MS GABA synthesis or release. It is possible that this glucose-induced release of GABA may summate with postsynaptic GABA receptor activation produced by GABA agonists to impair memory (see Figure 1.3).

In addition to being neurotransmitter-specific, the positive and negative effects of glucose on memory are likely to be dependent on brain region. Emerging evidence indicates that glucose is compartmentalized in the brain and that the effects of glucose on brain function are not similar throughout the brain. For example, under resting conditions ECF glucose concentrations are dissimilar between different brain areas important for learning and memory (Fellows, Boutelle, & Fillenz, 1992; Forsyth et al., 1996; Fray, Boutelle, & Fillenz, 1997; McNay, Fries, & Gold, 2000; McNay & Gold, 1999; McNay, McCarty, & Gold, 2001). More specifically, the ECF levels of glucose in the hippocampus are 1.0 -1.2 mM (McNay, Fries, & Gold, 2000; McNay & Gold, 1999; McNay, McCarty, & Gold, 2001), whereas the glucose levels in the striatum are estimated to be between 0.3- 0.71 mM (Fellows, Boutelle, & Fillenz, 1992; Forsyth et al., 1996; Fray, Boutelle, & Fillenz, 1997; Lowry, O'Neill, Boutelle, & Fillenz, 1998).

### **Specific Aims**

When combined together, the findings reviewed above suggest that the positive and negative effects of glucose on memory may also be mediated through regional variations in



glucose levels, glucose utilization, and neurotransmitter release. Accordingly, the goals of this dissertation were to investigate the following aims.

### Specific Aim 1

Although septal infusions of glucose produce memory deficits when combined with GABA agonists (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004; Krebs & Parent, unpublished findings), septal co-infusions of glucose typically reverse memory deficits produced by morphine (Ragozzino & Gold, 1995b; Ragozzino, Parker, & Gold, 1992). Combined, these findings suggest that the effects of glucose on memory are neurotransmitter-dependent. The doses of glucose (~ 18 nmol) that reverse memory deficits produced by morphine, (Ragozzino & Gold, 1995b; Ragozzino, Parker, & Gold, 1992) are smaller than the dose of glucose that typically produces memory deficits (33 nmol; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). The aim of this experiment; therefore, was to determine whether the dose of glucose that consistently impairs memory when co-infused with GABAergic drugs, also impairs memory when co-infused with the opiate morphine.

Previous studies suggest that the memory deficits produced by glucose are mediated through an interaction with the GABA neurotransmitter system. It is unknown; however, if the memory-impairing interaction between muscimol and glucose is specific to muscimol or can be generalized to other types of GABA<sub>A</sub> receptor activation. If GABA receptor activation is required for the memory-impairing effects of glucose, then co-infusions of glucose with other GABA receptor agonists should also impair memory. Benzodiazepines have a binding site on the GABA<sub>A</sub> receptor and enhance the inhibitory effects of GABA (Choi, Farb, & Fischbach, 1981; Duman, Sweetnam, Gallombardo, & Tallman, 1987). Activation of septal GABA<sub>A</sub> receptors by

benzodiazepines, such as chlordiazepoxide (CDP), also impair memory (Stackman & Walsh, 1995a). If the memory-impairing effects of glucose involve the potentiation of postsynaptic GABA receptor activation, then septal infusions of glucose will potentiate memory deficits produced by sub-effective doses of CDP as it does with muscimol. The goal of this experiment; therefore, was to determine whether co-infusions of glucose with CDP, at doses that have no effect on memory alone, produce memory deficits.

### Specific Aim 2

Elevations in glucose levels increase GABA levels in many brain areas (During, Leone, Davis, Kerr, & Sherwin, 1995; Fink & Gothert, 1993; Fink, Zentner, & Gothert, 1994). These findings raise the possibility that one mechanism through which septal infusions of glucose may impair memory is via glucose-induced increases in septal ECF GABA levels. These increases in septal GABA levels would then summate with the effects of the exogenous agonist (e.g., muscimol or CDP) to impair memory (see Figure 1.3). If the memory-impairing effects of septal infusions of glucose involve an increase in septal GABA synthesis or release, then septal infusions of glucose should increase septal ECF GABA levels. The present experiments tested whether increasing glucose in the septum would increase septal ECF GABA levels in rats performing on a memory task.

### Specific Aim 3

Septal infusions of glucose only impair memory when septal GABA receptors are activated; therefore, understanding how septal GABA receptor activation influences memory may help reveal the mechanisms underlying the memory-impairing effects of glucose. Although evidence supports the notion that septal infusions of muscimol impair memory through the cholinergic SH projection (Allen & Crawford, 1984; Moor, DeBoer, & Westerink, 1998; Walsh,

Stackman, Emerich, & Taylor, 1993), the possibility remains that the memory-impairing effects of septal GABA receptor activation also involve the GABAergic SH projection. In support of this possibility, GABA<sub>A</sub> receptors are present on both projection neurons (Gao, Hornung, & Fritschy, 1995), and lesions of the cholinergic SH projection do not prevent the memory-impairing effects of septal infusions of muscimol (Pang, 1999). The subsequent experiments; therefore, determined whether the memory-impairing effects of septal GABA receptor activation involve the GABAergic SH projection. The current experiment tested whether the memory-impairing effects of septal GABA receptor activation are prevented by blocking the activity of the GABAergic SH projection. Specifically, we determined whether hippocampal infusions of bicuculline, a GABA<sub>A</sub> antagonist, would prevent the memory-impairing effects of septal infusions of muscimol. Hippocampal infusions of bicuculline are expected to inhibit the GABA<sub>A</sub> receptors on both the inhibitory interneurons and pyramidal cells. Consequently, combining hippocampal infusions of bicuculline with septal infusions of muscimol should decrease the output of the the GABAergic SH projection (see Figure 1.1).

Septal infusions of the muscarinic antagonist scopolamine impair memory (Elvander et al., 2004; Givens & Olton, 1995; Gorman, Pang, Frink, Givens, & Olton, 1994; Pang, 1999). More recent findings show that scopolamine in the septum acts at the GABAergic rather than the cholinergic SH projection (Alreja et al., 2000). When two drugs act via a common mechanism such as the GABAergic projection to impair memory, then the effects of those two drugs should summate (Seeley & Moran, 2002). Specifically, doses of each drug that have no effect on memory alone, should summate to produce a memory deficit. If memory-impairing effects of septal GABA receptor activation involve an influence on the GABAergic SH projection, then co-infusions of muscimol with a drug that acts at the GABAergic SH projection should produce memory deficits.

The current study determined whether co-infusions of muscimol and scopolamine, at doses that have no effect on memory alone, should produce memory deficits.

#### Specific Aim 4

Septal co-infusions of glucose with muscimol *impair* memory (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). In contrast, hippocampal infusions of glucose *reverse* memory deficits produced by septal GABA receptor activation (Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997). The finding that hippocampal infusions of glucose prevent the memory-impairing effects of septal infusions of muscimol (Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997), suggests that septal GABA receptor activation impairs memory through an influence on the hippocampus. The effects of septal infusions of muscimol with glucose summate to produce memory deficits, suggesting that infusions of muscimol with glucose act on a common mechanism. Combined with previous findings showing that the memory-impairing effects of septal muscimol infusions involve an influence on the hippocampus, these results raise the possibility that the memory-impairing effects of septal infusions of glucose with muscimol may also involve an influence on the hippocampus. Therefore, the current experiment determined whether hippocampal infusions of glucose would prevent the memory-impairing effects of septal infusions of glucose. If the memory-impairing effects of septal infusions of glucose involve an influence on the hippocampus, then hippocampal infusions of glucose should reverse the memory deficits produced by septal co-infusions of glucose with muscimol.

#### Specific Aim 5

Although septal infusions of glucose typically reverse memory deficits produced by several drugs, this treatment exacerbates memory deficits produced by co-infusions of GABA receptor

agonists (Krebs & Parent, 2004; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Ragozzino & Gold, 1995b; Shah & Parent, 2003, 2004; Stefani & Gold, 1998). Collectively, these findings suggest that the effects of glucose on memory are neurotransmitter-dependent. The goal of the present set of experiments is to test whether the memory-impairing interaction between glucose and muscimol will generalize to other brain areas. The present experiment tested whether the memory-impairing effects of co-infusions of glucose with muscimol would be observed in the hippocampus, a brain region where glucose typically has positive effects on memory (Krebs & Parent, 2005a; Parent & Gold, 1997; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Ragozzino, Unick, & Gold, 1996). Previous findings from our lab suggest that the memory-enhancing effects of hippocampal infusions of glucose prevail over the memory-impairing effects of septal infusions of glucose (Krebs & Parent, unpublished findings). Combined together, these findings suggest elevating glucose within the hippocampus has positive consequences for memory. These results raise the possibility that the mnemonic effects of glucose may also be brain region-dependent. To test this hypothesis, we determined whether hippocampal infusions of glucose would reverse or exacerbate memory deficits produced by hippocampal co-infusions of the GABA receptor agonist muscimol. If the memory-impairing effects of glucose are brain-region dependent, then hippocampal co-infusions of glucose with muscimol would reverse the muscimol-induced memory deficits. If, however, the memory-impairing effects of glucose are only neurotransmitter specific, then hippocampal co-infusions of glucose with muscimol would produce memory deficits as it does in the septum.

#### Specific Aim 6

Previously it was assumed that ECF glucose levels were similar throughout the brain. In fact, it was thought that the brain had one compartment for glucose, which was at one steady-state

concentration (Sokoloff et al., 1977). More recent evidence indicates that ECF glucose concentrations vary by brain areas (Fellows, Boutelle, & Fillenz, 1992; Forsyth et al., 1996; Forsyth, 1996; Fray, Boutelle, & Fillenz, 1997; McNay & Gold, 1999; McNay, McCarty, & Gold, 2001). For example, ECF glucose levels differ between the hippocampus and striatum (Fellows, Boutelle, & Fillenz, 1992; Forsyth et al., 1996; Forsyth, 1996; Fray, Boutelle, & Fillenz, 1997; Lowry, O'Neill, Boutelle, & Fillenz, 1998; McNay, McCarty, & Gold, 2001). These findings raise the possibility that there may also be differences in ECF glucose levels in the septum versus the hippocampus. These differences could contribute to the differential effects of glucose on memory in the septum versus the hippocampus by changing the dose-response properties of glucose in these two brain areas. For example, if basal ECF glucose levels are higher in the septum than the hippocampus, then smaller increases in glucose may be required to produce memory deficits in the septum than in the hippocampus. If, however, the brain regions have similar ECF glucose levels, then the positive and negative effects of glucose on memory are due to some other difference between the brain regions. The goal of the present experiment; therefore, was to determine whether the ECF glucose concentrations between the septum and the hippocampus were different.

Rats (*Rattus norvegicus*) are a useful species with which to investigate the neurochemical correlates of learning and memory. Male Sprague-Dawley rats are used in this type of research because there is an extensive battery of standardized behavioral tests to assess learning and memory in rats and the neuroanatomical and neurochemical structure of the rat brain is the well-described. Furthermore, rats have been used to obtain most of the existing information regarding the neural mechanisms of learning and memory. Specifically, rats have been used to determine the neurochemical correlates of learning and memory processes. Consequently, using rats facilitates comparisons across studies and laboratories.

The SA and continuous multiple trial inhibitory avoidance (CMIA) tasks are useful to assess learning and memory because there is an extensive record of the effects of pharmacological manipulations on these forms of learning and memory, they are commonly used and well-characterized tasks, these types of learning and memory are affected by glucose manipulations in the SH system, they can be feasibly combined with *in vivo* microdialysis procedures, and they assess different types of learning and memory. Importantly, these tasks do not involve food deprivation or food reward, which is critical in the studies that manipulate brain glucose. SA is assumed to be a hippocampal-dependent measure of spatial working memory (Deacon, Bannerman, Kirby, Croucher, & Rawlins, 2002; Dember, 1989; Lalonde, 2002; Richman, Dember, & Kim, 1987; Stevens & Cowey, 1973). The underlying assumption is that in order to alternate successfully between locations the rat must remember its visits to previous places. This assumption is supported by the finding that SA is impaired by removing directional cues or by increasing the interval between arm choices (Dember, 1989; Lalonde, 2002; Richman, Dember, & Kim, 1987). CMIA is a measure of emotional long-term memory that is less dependent on spatial processes (McGaugh, 2004), but still involves the hippocampus (Lovely, Grossen, Moot, Bauer, & Peterson, 1971; Martinez, Quirarte, Diaz-Cintra, Quiroz, & Prado-Alcala, 2002). The motivational, temporal, and cognitive differences between the SA and CMIA tasks allow us to determine whether our manipulations are influencing memory rather than some other process such as attention or motivation that could influence performance in the memory task.

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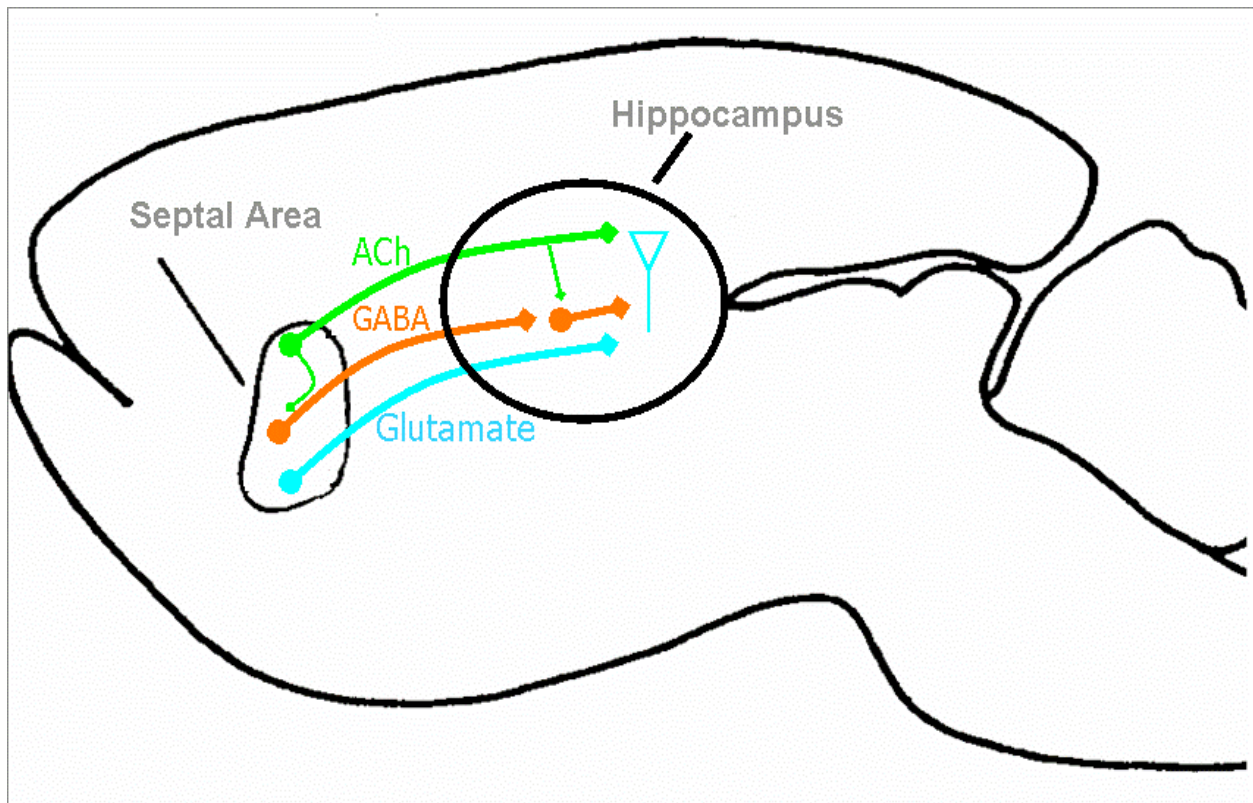


Figure 1.1. Schematic diagram of the simplified chemoanatomy of the septo-hippocampal system. The septo-hippocampal system contains cholinergic, GABAergic, and glutamatergic projection neurons.



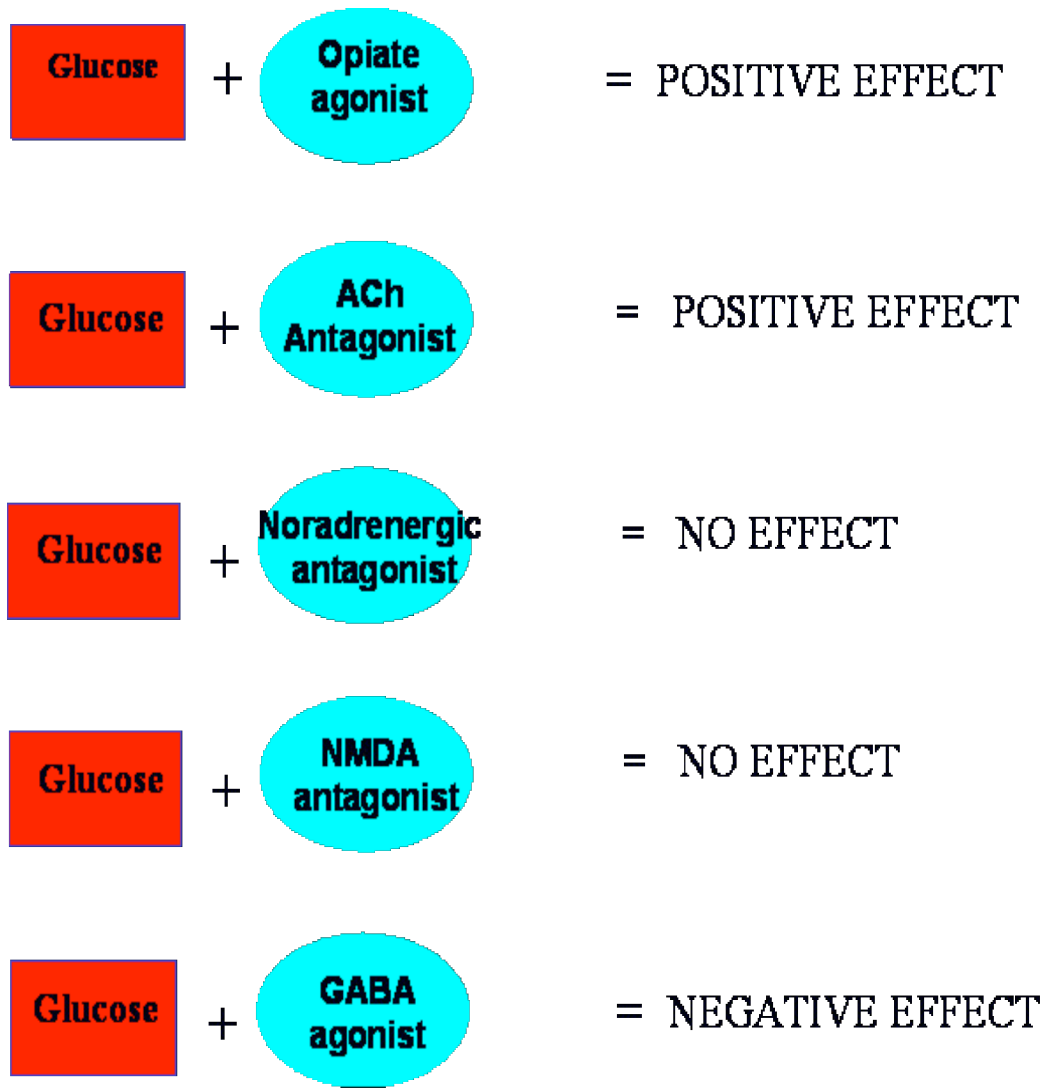


Figure 1.2. The effects of glucose on memory are neurotransmitter specific. Co-infusions of glucose with cholinergic or opiate drugs enhance memory or reverse memory deficits. Moreover, co-infusions of glucose with noradrenergic or NMDA antagonists do not affect memory. In contrast, co-infusions of glucose with GABAergic drugs impair memory.

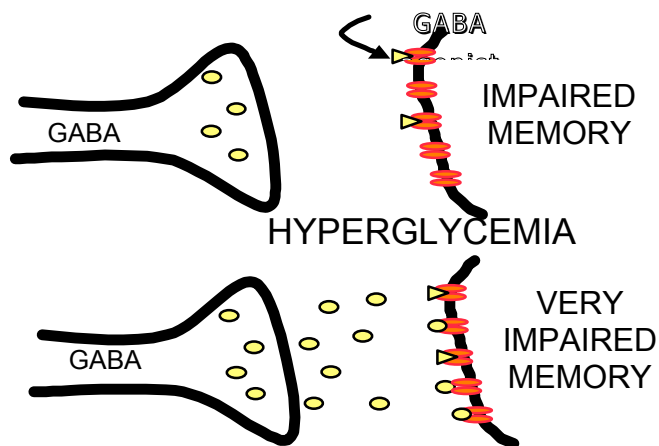


Figure 1.3. Schematic diagram of one possible mechanism through which glucose exerts its memory-impairing effects. In the normal glycemic state, MS infusions of GABA agonists bind to the postsynaptic receptor and impair memory. In the hyperglycemic state, glucose elevates MS GABA levels which combines with the memory-impairing actions of MS GABA agonist infusions to produce memory deficits.

## **CHAPTER 2**

### **THE MEMORY-IMPAIRING EFFECTS OF SEPTAL GLUCOSE INFUSIONS ARE NEUROTRANSMITTER-DEPENDENT, BUT NOT CONCENTRATION-DEPENDENT**

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### Abstract

Previous findings show that MS infusions of glucose reverse morphine-induced memory deficits; whereas, co-infusions of glucose with the GABA agonist muscimol into the medial septum (MS) impair memory. These positive versus negative effects of glucose might be due to the fact that higher doses of glucose are typically co-infused with muscimol than with morphine. To establish whether the effects of glucose on memory in the brain are concentration-dependent, we tested whether a dose of glucose that typically produces deficits when co-infused with GABA agonists would also impair memory when co-infused with the opiate morphine. To determine whether the memory-impairing effects of glucose are specific to the GABA<sub>A</sub> receptor, we tested whether glucose would produce memory deficits when co-infused with another GABAergic drug, the GABA<sub>A</sub> receptor modulator/benzodiazepine chlordiazepoxide (CDP). In Experiment 1, male Sprague-Dawley-derived rats were given MS infusions of phosphate-buffered saline (PBS; pH= 7.4), glucose (33 nmol), morphine (4 nmol), or a cocktail of morphine with glucose. In Experiment 2, rats were given MS infusions of vehicle (saline; pH = 3.0; 0.5 µl/1 min), PBS, glucose (33 nmol), CDP (15 nmol: spontaneous alternation [SA] or 30 nmol: SA or shock avoidance) or a cocktail of CDP with glucose. Fifteen minutes later, SA, a measure of spatial working memory, or shock avoidance, a measure of emotional long-term learning and memory, were assessed. The results of Experiment 1 showed that MS infusions of the higher dose of glucose with morphine did not produce memory deficits. The results of Experiment 2 indicated that MS infusions of either glucose or CDP alone did not affect SA scores. More importantly, the performance of rats given MS co-infusions of CDP with glucose was impaired. Therefore, these findings show that glucose interacts synergistically with the benzodiazepine CDP to impair memory. These results indicate that the memory-impairing effects of brain glucose

administration are not concentration-dependent. Furthermore, these data are consistent with the possibility that the memory-impairing effects of glucose occur via a process that involves the GABA<sub>A</sub> receptor.

**Key Words: Muscimol, GABA, Glucose, Hippocampus, Spontaneous Alternation, Inhibitory Avoidance**

## Introduction

Glucose typically enhances memory (Lee, Graham, & Gold, 1988; Stefani, Nicholson, & Gold, 1999; Sunram-Lea, Foster, Durlach, & Perez, 2002) and reverses the memory deficits associated with Alzheimers's disease, aging, Down's syndrome, and schizophrenia (Craft, Murphy, & Wemstrom, 1994; Gold, 1995; Korol, 2002; Korol & Gold, 1998; Manning, Ragozzino, & Gold, 1993; Newcomer et al., 1999; Petit, 1988). In contrast, acute and chronic hyperglycemia are associated with memory dysfunction (Biessels et al., 1996; Elias et al., 1997; Flood, Mooradian, & Morley, 1990; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; Hagemann, Sartory, Hader, & Kobberling, 2005; McCall, 2002; Popovic, Biessels, Isaacson, & Gispen, 2001; Winocur et al., 2005). The mechanisms underlying the memory-modulating effects of glucose, particularly the conditions that differentiate the memory-enhancing and -impairing effects of glucose, are poorly understood.

The memory-modulating effects of glucose are mediated, at least in part, by the brain. Glucose readily crosses the blood-brain barrier via glucose transporters (Pardridge, Boado, & Farrell, 1990; Rahner-Welsch, Vogel, & Kuschinsky, 1995; Takata, Hirano, & Kasahara, 1997). Extracellular levels of hippocampal glucose decrease with cognitive demand, and systemic infusions of glucose both enhance memory and replenish these decreases in hippocampal glucose (McNay & Gold, 1999). These data suggest that during tasks that engage the hippocampus, peripheral glucose is preferentially directed to the hippocampus and enhances memory formation. Moreover, direct brain infusions of glucose affect memory. For example, infusions of glucose into the cerebral ventricular system enhance retention performance on a one-trial inhibitory avoidance task in rodents (Lee, Graham, & Gold, 1988). Direct injections of glucose into specific brain regions, including the medial septum (MS) or hippocampus, enhance

spontaneous alternation (SA) performance (Ragozzino & Gold, 1995b; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Stefani & Gold, 1998; Stefani, Nicholson, & Gold, 1999), a measure of spatial working memory (Dember, 1989; Lalonde, 2002; Richman, Dember, & Kim, 1987). Septal (MS) or hippocampal infusions of glucose also reverse drug-induced deficits in SA (Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997; Ragozzino & Gold, 1995b; Stefani & Gold, 1998, 2001b). MS co-infusions of glucose with the  $\gamma$ -aminobutyric acid (GABA) receptor agonist muscimol produce memory deficits (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004), suggesting that the memory-impairing effects of glucose are also mediated, at least in part, in the brain.

The memory-modulating effects of central glucose administration appear to be neurotransmitter-dependent. For instance, glucose reverses deficits produced by some drugs but not others. Specifically, glucose administration reverses deficits produced by the opiate agonist morphine, the cholinergic antagonist scopolamine, or the peptide galanin (Ragozzino, Arankowsky-Sandoval, & Gold, 1994; Ragozzino & Gold, 1995b; Ragozzino, Parker, & Gold, 1992; Rashidy-Pour, 2001; Stefani & Gold, 1998). MS co-infusions of glucose do not reverse the memory-impairing effects of GABA agonists (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997). In contrast, co-infusions of glucose with GABA agonists impair memory. Specifically, MS infusions of glucose potentiate the memory-impairing effects of the GABA<sub>A</sub> agonist muscimol (Parent & Gold, 1997). More interestingly, MS co-infusions of glucose with muscimol, at doses that individually do not affect memory performance, produce memory deficits (Parent, Laurey, Wilkniss, & Gold, 1997; Shah & Parent, 2003, 2004).

The doses of glucose (~ 18 nmol) that reverse memory deficits produced by morphine and galanin (Ragozzino, Parker, & Gold, 1992; Stefani & Gold, 1998) are smaller than the dose of glucose that typically produces memory deficits (33 nmol; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). This raises the possibility that the impairing or enhancing effects of glucose are concentration-dependent. To test this, Experiment 1 determined whether the higher dose of glucose that impairs memory when co-infused with muscimol would also impair memory when co-infused with an opiate. On the other hand, the impairing effects of glucose may be specific to GABA receptor activation. To test this, Experiment 2 determined whether co-infusions of glucose with another GABA receptor agonist would impair memory. Collectively, the findings from these two experiments will dissociate whether the memory-impairing effects of glucose are concentration- or neurotransmitter-dependent.

## **Experiment 1**

### **Material and Methods**

The goal of Experiment 1 was to determine whether the memory-impairing effects of glucose are dose-dependent. Specifically, Experiment 1 tested whether the dose of glucose that consistently impairs memory when co-infused with the GABA agonist muscimol (Parent & Krebs, 2004; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004), also impairs memory when co-infused with the opioid morphine.

### **Subjects**

Sixty-three (n = 12-17 per group) male Sprague-Dawley-derived rats (Charles River, Wilmington, MA) were used for the SA task and 55 (n = 9-12 per group) were used for the



continuous multiple trial inhibitory avoidance (CMIA) task. CMIA training was given at least 3 days after SA was assessed. The two behavioral tasks were used because they both depend on the integrity of the SA (Krebs & Parent, 2005a, 2005c; Parent, Laurey, Wilkniss, & Gold, 1997), and because they assess different types of memory that vary in motivational, temporal, and cognitive demands. This allowed us to determine whether the manipulations affected memory rather than some process that influences performance on a memory task.

The rats weighed 200-250 g upon arrival and were housed individually in polycarbonate cages (20x40x20 cm) with corncob bedding. Furthermore, they were located in a temperature-controlled colony room (70-74°F) and kept on a 12 hour light-dark cycle (lights on at 7:00 a.m.). The rats had free access to food and water and were acclimated to lab conditions for approximately 1 week prior to surgery. The Georgia State University Institutional Animal Care and Use Committee (IACUC) approved all procedures involving rats.

## **Surgery**

Rats were placed in a clear, plastic gas induction chamber and anesthetized with 5% isoflurane gas (Baxter, Deerfield, IL) delivered in 1000 ml/min medical grade oxygen. After the rat was no longer ambulatory, it was removed from the chamber and placed on a face-mask that delivered 3% isoflurane gas. Rats were then given injections of atropine sulfate (0.4 mg/kg, ip, Baxter, Deerfield, IL) and penicillin (1500 units, im, Hanfords US Vet, Syracuse, NY). The future incision site was shaved with a #50 electric clipper blade (Oster) and betadine solution was applied to the surgical area. Anesthesia was maintained by delivering 1-3% isoflurane gas in 500 ml/min oxygen to the face-mask. The percentage of isoflurane gas given to the rats was adjusted (from 1-3%) to maintain a surgical plane of anesthesia as determined by the toe pinch

test. A stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) that was equipped with an anesthesia mask was used to implant one 22-gauge stainless-steel injection guide cannulae (Plastics One, Inc., Roanoke, VA) aimed at the SA (0.5 mm anterior to bregma, 4.9 mm ventral to dura; Paxinos & Watson, 1998). The incision site was anesthetized with a 2% lidocaine/.001% epinephrine cocktail (0.5-2.0 cc, sc, Abbott Labs, Chicago, IL). After the incision, the 2% lidocaine/.001 % epinephrine cocktail solution (.05- 1.0 cc) was applied topically to the skull to facilitate viewing lambda and bregma. The cannulae were secured to the skull with three jeweler's screws and a cranioplastic cement and acrylic mixture (Duralay, Worth, IL). A dummy cannula (Plastics One, Roanoke, VA) was inserted to keep the cannulae free of debris. Immediately after surgery, the rats were given an injection of 0.9 % sterile saline (3.0 cc, sc) and the non-steroidal anti-inflammatory analgesic flunixin meglumine (2.5 mg/kg, ip, Fort Dodge Animal Health, Fort Dodge, IA) and then wrapped with a paper towel and kept under a warm lamp (60 W) until recovery from anesthesia. Two days following surgery, the patency of each cannula was checked and betadine was applied to the surgical wound. If signs of infection were evident, the rats were anesthetized with isoflurane gas (5%) delivered in 1000 ml/min of oxygen and given an additional injection of penicillin (1500 units im).

### **Drug Preparation and Intracranial Infusions**

Two days prior to behavioral testing, the experimenter handled each rat for 2 minutes. Before and after all handling and behavioral testing, the rats were allowed a minimum of 30 minutes to habituate to the laboratory environment. Behavioral testing was conducted between 7:00 a.m. and 7:00 p.m. Fifteen minutes prior to behavioral testing, different groups of rats were given MS infusions of vehicle (0.5  $\mu$ l, 0.5  $\mu$ l/ min; phosphate-buffered saline [PBS]; pH = 7.4),

glucose (33 nmol), morphine (4 nmol: SA and CMIA or 8 nmol: CMIA; Sigma) or morphine combined with glucose in one solution. The drugs that were to be combined in the same solution were prepared at double the desired concentration and then combined, thereby reducing the concentration of each by half. Drug treatments were counterbalanced over the course of the day. The 33 nmol dose of glucose was selected because it produces memory deficits when infused with the GABA receptor agonist muscimol in the MS (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). The dose of morphine was selected based on pilot data suggesting that 4 nmol dose of morphine is the maximum sub-effective dose (i.e., highest dose that does not cause a statistically significant deficit) for SA and CMIA, and the 8 nmol dose of morphine was chosen because it produces avoidance retention deficits.

Drug solutions were prepared on the day of testing. The drugs were infused through a 28-gauge injection needle that extended 1.0 mm beyond the guide cannulae. The needle was connected to a 25  $\mu$ l Hamilton syringe by polyethylene tubing (PE-50), and the infusions were delivered using an infusion pump (Harvard Apparatus 11). Following the completion of the injections, the needle was left in place for 1 minute to facilitate drug diffusion.

### **Spontaneous Alternation**

SA is assumed to be a hippocampal-dependent measure of spatial working memory (Deacon, Bannerman, Kirby, Croucher, & Rawlins, 2002; Dember, 1989; Lalonde, 2002; Pacteau, Einon, & Sinden, 1989; Richman, Dember, & Kim, 1987; Stevens & Cowey, 1973; Will, Deluzarche, & Kelche, 1983). The underlying assumption is that in order to alternate successfully between locations the rats must remember its visits to previous places. This assumption is supported, in part, by data showing that SA is impaired by removing directional

cues or by increasing the interval between arm choices (Dember, 1989; Lalonde, 2002; Richman, Dember, & Kim, 1987). Fifteen minutes after the drug injections, SA performance was assessed by placing each rat in a Y-maze composed of three equally spaced arms (60°; 60 cm long x 17.5 cm high). The floor of each arm was composed of stainless steel (3.5 cm wide) and the top (14 cm wide) was covered with a translucent plexiglass lid. All rats were placed in the same starting arm of the Y-maze and allowed to explore the maze freely for 8 minutes. The experimenter, who was blind to drug treatment, recorded the sequence and number of arms the rats entered during the 8 min. period. The maze was cleaned with 70% ethanol after each rat. The number of arms each rat entered was used as a measure of activity. A percent alternation score was computed for all rats that entered at least 10 arms. An alternation was defined as entering three different arms consecutively. The percent alternation score was computed by dividing the number of alternations each rat made by the number of arms entered minus two (i.e., the number of alternations possible) and then multiplying that resulting quotient by 100.

### **Continuous Multiple Trial Inhibitory Avoidance**

CMIA training was given a minimum of 3 days after SA testing. The avoidance apparatus consisted of a trough-shaped alley (91 cm long, 15 cm high, 20 cm wide at the top, and 6.4 cm wide at the bottom) that was divided into a lighted (31 cm long) and a dark (60 cm long) compartment by a retractable guillotine door. The dark compartment had a metal floor through which shock could be delivered. A 15-watt lamp was placed over the lighted compartment and was the only source of illumination in the room. The table underneath the avoidance apparatus was lined with bench paper and the apparatus was cleaned with 70% ethanol after each rat was trained or tested.

For the training the rat was placed in the lighted compartment with its head facing away from the door. Once the rat turned around to face the door or after 12 seconds (s) passed, the retractable door was opened and the rat was allowed to cross over to the dark (shock) compartment. After the rat crossed with all four paws, the rat was given a footshock (1.2 mA) until it returned to the lighted compartment (maximum 4 s). This sequence constituted one training trial. Training continued until the rat remained in the lighted compartment for 100 consecutive s or for a maximum of 5 trials. The rat was not removed from the avoidance apparatus between trials. The number of trials needed to reach the criterion was recorded and used as a measure of acquisition.

Retention of the training was tested 48 hours (+/- 2 hrs) later. For the retention test, the rats were placed in the lighted compartment of the avoidance chamber with their heads facing away from the closed door. After each rat turned to face the door or 12 s passed, the door was opened and the latency (s) to cross over to the dark (shock) compartment was recorded and used as a measure of retention. Each rat was given a maximum of 600 s to enter the dark compartment during the retention test. Footshock was not delivered on the retention test.

## **Histology**

After behavioral testing, the rats were euthanized with an overdose of sodium pentobarbital (Sleepaway; 400 mg/kg, ip, Fort Dodge, Fort Dodge, IA) and perfused intracardially with 0.9% saline followed by 10% formalin. Their brains were stored in a 10% formalin solution for at least 2 days before sectioning. All brains were sectioned on a cryostat (Leica CM 30510 S) and 45-60  $\mu$ m sections were taken through the MS and hippocampal cannulae tracts. The brain sections were stained with thionin and an unbiased observer

determined the cannulae placement using a light microscope (Olympus BX41). Acceptable medial MS cannulae placement was defined as injection sites located within the MS, but not within the lateral septum or the ventral diagonal band of Broca. Moreover, the cannula must not have penetrated the fimbria. Only rats with acceptable cannulae placements were included in the statistical analyses.

### **Statistical Analysis**

The data were expressed as means and standard errors of the mean (S.E.M.) and analyzed using a 1-way analysis of variance (ANOVA) and Tukey post hoc tests where appropriate. The acquisition and retention latency data were not normally distributed due to the fact that several of the rats reached the maximum trials to criterion and 600 s retention latency cut-off. Consequently, these data were expressed as medians and inter-quartile ranges (I.Q.) and the non-parametric Kruskal-Wallis and Mann-Whitney U tests were used to detect differences between treatment groups. An alpha level of 0.05 was used as the criterion for statistical significance. Bonferonni corrections were used for the Mann-Whitney U tests based on the number of planned comparisons.

### **Results**

Figure 2.1 shows the approximate location of drug infusions in Experiment 1. As Figure 2.2 shows, the drug infusions into the MS did not significantly affect SA performance [ $F(3, 62) = 0.36$ ;  $p > .05$ ] or the number of arms that the rats entered in the maze [ $F(3, 62) = 1.54$ ;  $p > .05$ ].

Figure 2.3A demonstrates that MS drug infusions did significantly affect trials to criterion during CMIA training [ $F(4, 55) = 10.77$ ;  $p < .05$ ]. After Bonferonni correction ( $p < .007$ ), the post-hoc tests revealed no significant differences between any of the groups on the

number of trial to criterion during CMIA training. Figure 2.3B illustrates that the pre-training drug infusions into the MS significantly affected subsequent CMIA retention [ $\chi^2(4,55) = 11.47$ ;  $p < .05$ ]. MS infusions of morphine dose-dependently impaired memory. Specifically, rats that were given MS infusions of 8 nmol of morphine had significantly shorter retention latencies than did those rats that were given MS infusions of vehicle [ $U(1,21) = 28$ ;  $p < .007$ ]. MS infusions of morphine (4 nmol) or glucose alone did not significantly impair CMIA retention. The retention latencies of rats that were given MS infusions of 4 nmol of morphine [ $U(1,22) = 40$ ,  $p > .007$ ] or glucose [ $U(1,19) = 48$ ;  $p > .007$ ] were not statistically different from those of rats given vehicle. MS infusions of glucose did not produce deficits when combined with morphine. The retention latencies of rats that were given MS infusions of morphine (4 nmol) with glucose did not significantly differ from those of rats that were given MS infusions of vehicle [ $U(1,22) = 65$ ;  $p > .007$ ].

### Discussion

The findings of the present experiment demonstrate that MS co-infusions of glucose, at a dose of glucose that typically produces memory deficits when combined with the GABA agonist muscimol, does not produce memory deficits when combined with morphine. This pattern was observed in a spatial working memory task and in an emotional, long-term memory task. These findings are consistent with previous evidence suggesting that the memory deficits associated with glucose are not due to hyperosmolarity (Shah & Parent, 2003, 2004). These findings rule out the possibility that the positive and negative effects of glucose on memory in the same brain region are concentration-dependent. Combined with previous findings (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004), these data suggest that the memory-impairing effects of glucose may be specific to GABA receptor activation.

## Experiment 2

### Materials and Methods

It is unknown if the memory-impairing interaction between muscimol and glucose is specific to muscimol or can be generalized to other drugs that affect the GABA<sub>A</sub> receptor. If GABA receptor activation is required for the memory-impairing effects of glucose, then co-infusions of glucose with other GABA receptor agonists should also impair memory.

Benzodiazepines have a binding site on the GABA<sub>A</sub> receptor and enhance the inhibitory effects of GABA (Choi, Farb, & Fischbach, 1981; Duman, Sweetnam, Gallombardo, & Tallman, 1987). Activation of MS GABA<sub>A</sub> receptors by benzodiazepines, such as chlordiazepoxide (CDP), also impair memory (Stackman & Walsh, 1995a, 1995b). The goal of Experiment 2 was to determine whether MS infusions of glucose would potentiate memory deficits produced by co-infusions of sub-effective doses of CDP as they do with muscimol.

The same procedures were used as in Experiment 1 with the following exceptions: Fifteen minutes prior to behavioral testing, different groups of rats were given MS infusions of vehicle (0.5  $\mu$ l, 0.5  $\mu$ l/ min; saline, pH = 3.0), phosphate-buffered saline (PBS; pH = 7.4), glucose (33 nmol), CDP (15 nmol: SA or 30 nmol: SA and CMIA, Sigma) or CDP combined with glucose in one solution. The doses of CDP were selected based on pilot data suggesting that 15 nmol of CDP is the maximum sub-effective dose (i.e., highest dose that does not cause a statistically significant deficit) for SA, and the 30 nmol dose of CDP was chosen to produce SA deficits. Although our pilot data indicated that a large range of doses of CDP (15-200 nmol) did not produce shock avoidance retention deficits, the 30 nmol dose of CDP was chosen for the



CMIA task because it was the dose that had a tendency to produce a mild deficit. CDP could only go into solution at low pH values (pH = 3.0); therefore, infusions of PBS (pH = 7.4) served as a pH control. Eighty-two (n = 11-17 per group) rats were used for the SA task and 56 (n = 9-15 per group) were used for the CMIA task.

## Results

Drug infusions into the MS [ $F(5, 81) = 3.91$ ;  $p < .05$ ] significantly affected SA performance (see Figure 2.4A). Consistent with previous research, MS infusions of CDP dose-dependently impaired spatial working memory performance (Herzog, Gahndi, Bhattacharya, & Walsh, 2000; Stackman & Walsh, 1992, 1995a, 1995b; Tonkiss et al., 2000; Tonkiss, Trzcinska, Shultz, Vincitore, & Galler, 2000). Specifically, the percent alternation scores of rats given CDP (30 nmol) were significantly lower than of those rats given MS infusions of vehicle ( $p < .05$ ). MS infusions of glucose or CDP (15 nmol) alone did not significantly affect SA performance. The percent alternation scores of rats given glucose or CDP (15 nmol) or glucose alone did not differ significantly from those of rats given MS infusions of vehicle ( $p > .05$ ). Low pH did not likely contribute to the memory-impairing effects of CDP because the percent alternation scores of rats given low pH saline (pH = 3) were not significantly different from those of rats given MS infusions of PBS ( $p > .05$ ). Interestingly, the findings showed that MS co-infusions of glucose with CDP (15 nmol), at doses that produced no affect alone, significantly impaired SA performance. Specifically, the percent alternation scores of rats given glucose with CDP (15 nmol) in the same solution were significantly lower than those of rats given MS infusions of vehicle ( $p < .05$ ). The same drug infusions into the MS did not significantly affect the number of arms that the rats entered in the maze ( $[F(5,81) = .98$ ;  $p > .05$ ]; see Figure 2.4B).

Drug infusions into the MS significantly affected the number of trials to criterion in CMIA training ( $[F(4,55) = 11.750; p < .05]$ ; see Figure 2.5A). After Bonferonni correction ( $p < .017$ ), the post-hoc tests revealed no significant differences between any of the groups on the number of trial to criterion during CMIA training. Figure 2.5B shows that drug infusions into the MS also did not significantly affect avoidance retention latencies [ $F(4, 55) = 3.421; p > .05$ ].

### **General Discussion**

The present findings show that the same dose of glucose that produced memory deficits when combined in the GABA agonist muscimol does not produce deficits when combined with the opiate morphine. These findings are consistent with previous research showing that MS co-infusions of glucose do not potentiate, but rather reverse deficits produced by impairing doses of the opiate morphine (Ragozzino & Gold, 1995b; Ragozzino, Parker, & Gold, 1992). Importantly, the present findings show that MS co-infusions of glucose do impair memory when combined with the benzodiazepine agonist CDP. The memory-impairing interaction between glucose and CDP was observed in the spatial working memory task, but not in the emotional, long-term memory task. Collectively, these results suggest that the memory-impairing effects of MS infusions of glucose are not concentration-dependent, but are rather neurotransmitter-specific. In particular, these data suggest that MS infusions of glucose impair memory through an interaction with the GABA neurotransmitter system.

The finding that MS co-infusions of glucose impair memory when combined with GABAergic drugs but not with other types of memory-impairing drugs suggests that the effects of glucose on memory are neurotransmitter-specific (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Ragozzino & Gold, 1995b; Shah & Parent, 2003, 2004; Stefani, Nicholson,

& Gold, 1999). Extensive evidence suggests that glucose enhances memory through a process that involves an increase in acetylcholine (ACh) synthesis or release. For instance, hippocampal infusions of glucose reverse memory deficits and enhance ACh in the hippocampus while rats are performing on a memory task (Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Ragozzino, Unick, & Gold, 1996). In addition, MS infusions of the opiate morphine decrease hippocampal markers of ACh such as extracellular ACh and high affinity choline uptake (Ragozzino & Gold, 1995b), suggesting that the memory-impairing effects of MS infusions of morphine are mediated via an influence on the hippocampal cholinergic neurotransmitter system. In contrast, MS infusions of glucose may impair memory when combined with GABA agonists by modulating MS GABA levels. Elevating glucose levels increases GABA levels in the brain (Amoroso, Schmid-Antomarchi, Fosset, & Lazdunski, 1990; During, Leone, Davis, Kerr, & Sherwin, 1995; Fink & Gothert, 1993; Fink, Zentner, & Gothert, 1994; Ohtani, Ohta, & Sugano, 1997; Schmid-Antomarchi, Amoroso, Fosset, & Lazdunski, 1990), raising the possibility that MS infusions of glucose impair memory by promoting MS GABA synthesis or release. An alternate possibility is that glucose may both enhance and impair memory via its effects on ACh. Specifically, research has shown that MS administration of the cholinergic agonist oxotremorine can produce memory deficits (Elvander et al., 2004; Pang & Nocera, 1999), and striatal administration of oxotremorine impairs long-term potentiation (LTP; (Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998). This raises the possibility that MS infusions of glucose impair memory by increasing MS ACh release. Consequently, to dissociate these hypotheses future experiments should test the effects of MS infusions of glucose on MS extracellular GABA and ACh levels.

These results are also consistent with previous research showing that systemic and MS infusions of CDP impair spatial working memory (Farber, 1996; Stackman & Walsh, 1992,

1995a, 1995b; Tonkiss, Trzcinska, Shultz, Vincitore, & Galler, 2000). To our knowledge, there are not any studies showing that MS infusions of CDP impair shock avoidance (although systemic infusions do impair shock avoidance; Tohyama, Nabeshima, Ichihara, & Kameyama, 1991). It is not clear why CDP has these task-dependent effects on memory. One possibility is that shock avoidance memory can be based on the formation of different types of associations mediated by different brain regions. That is, when MS function is impaired by morphine, other brain areas may mediate the spared retention that is observed. For instance, avoidance retention can reflect stimulus-stimulus associations mediated by the hippocampus (Compton, 1993; Compton, Griffith, McDaniel, Foster, & Davis, 1997; Eichenbaum, 1992; Eichenbaum, Fagan, Matthews, & Cohen, 1988; Eichenbaum, Matthews, & Cohen, 1989; Eichenbaum, Stewart, & Morris, 1991; Hirsh, 1974; Sutherland & Rudy, 1989), stimulus-affect associations mediated by the amygdala (Compton, 1995; Izquierdo & Medina, 1997; McDonald & White, 1995a; McGaugh, McIntyre, & Power, 2002; Salinas & White, 1998), or stimulus-response associations mediated by the striatum (Kirkby, Polgar, & Coyle, 1981; Packard, Hirsh, & White, 1989; Packard & McGaugh, 1996; Packard & White, 1990; Petri & Mishkin, 1993; Phillips & Carr, 1987; Salinas & White, 1998; Squire & Butters, 1984). It is not clear, though, why CDP does not produce deficits when MS infusions of morphine or muscimol do.

In summary, MS co-infusions of glucose produce memory deficits when combined with drugs that affect MS GABA receptors, but not when combined with drugs that affect opioid receptors. Specifically, MS co-infusions of glucose with the GABA<sub>A</sub> modulator CDP, at doses that individually have no effect on memory, produce spatial working memory deficits. In contrast, MS co-infusions of the same concentration of glucose with the opiate morphine do not produce spatial working memory deficits or emotional, long-term memory deficits. Collectively,

these findings suggest that the memory-impairing effects of glucose in the MS are not concentration-dependent but are rather specific to GABA receptor activation.

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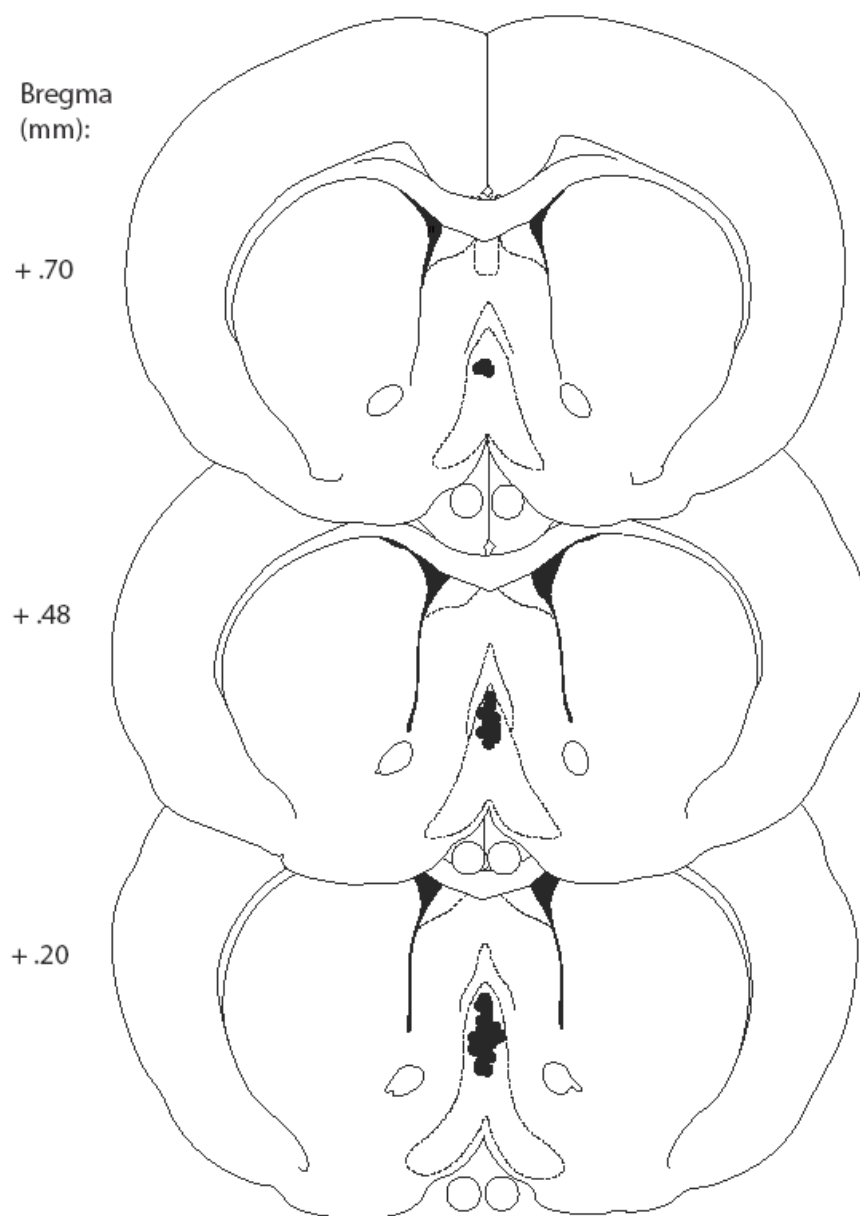


Figure 2.1. Schematic illustration of coronal sections of the rat brain showing the approximate location of MS infusion sites in Experiment 1. Atlas plates were adapted from Paxinos and Watson (1998).

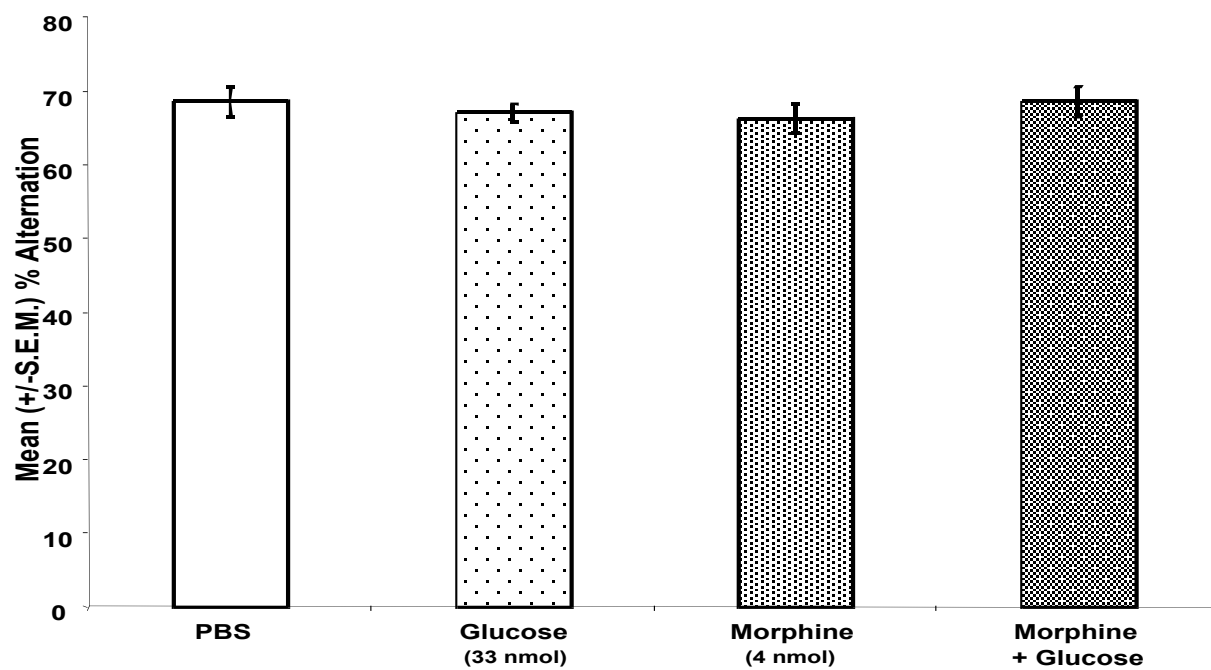


Figure 2.2A. MS co-infusions of glucose with morphine did not significantly decrease mean ( $\pm$  S.E.M.) percent alternation scores ( $p > .05$  vs. PBS).

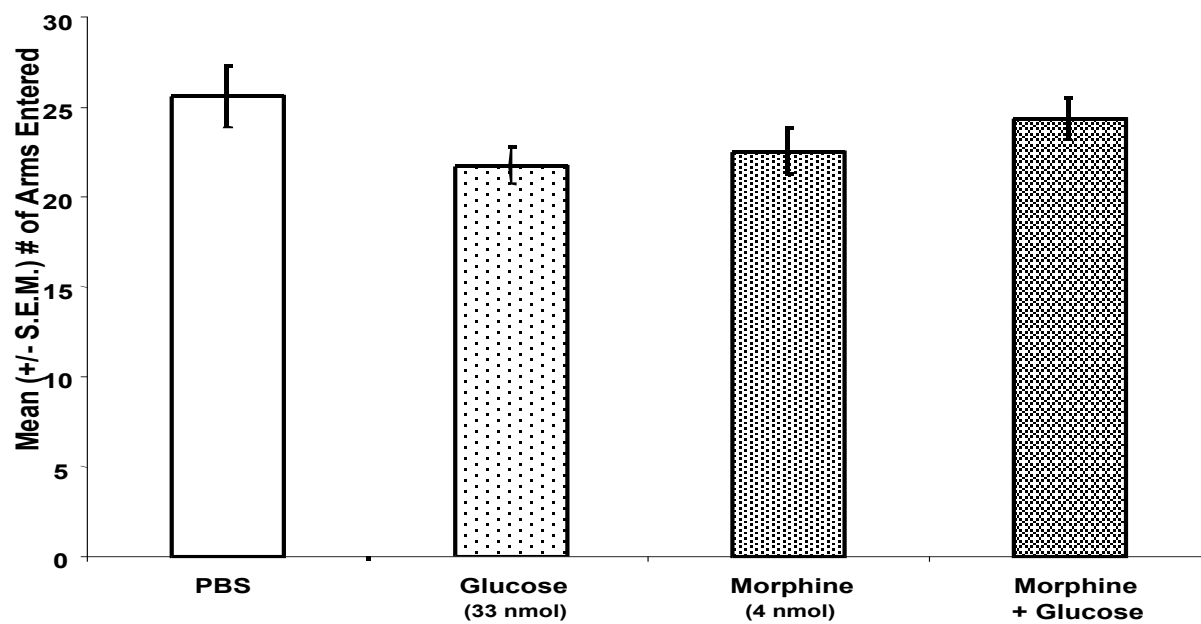


Figure 2.2B. There were no significant effects of any of the manipulations on the mean (+/- S.E.M.) number of arm entries ( $p > .05$  vs. PBS).

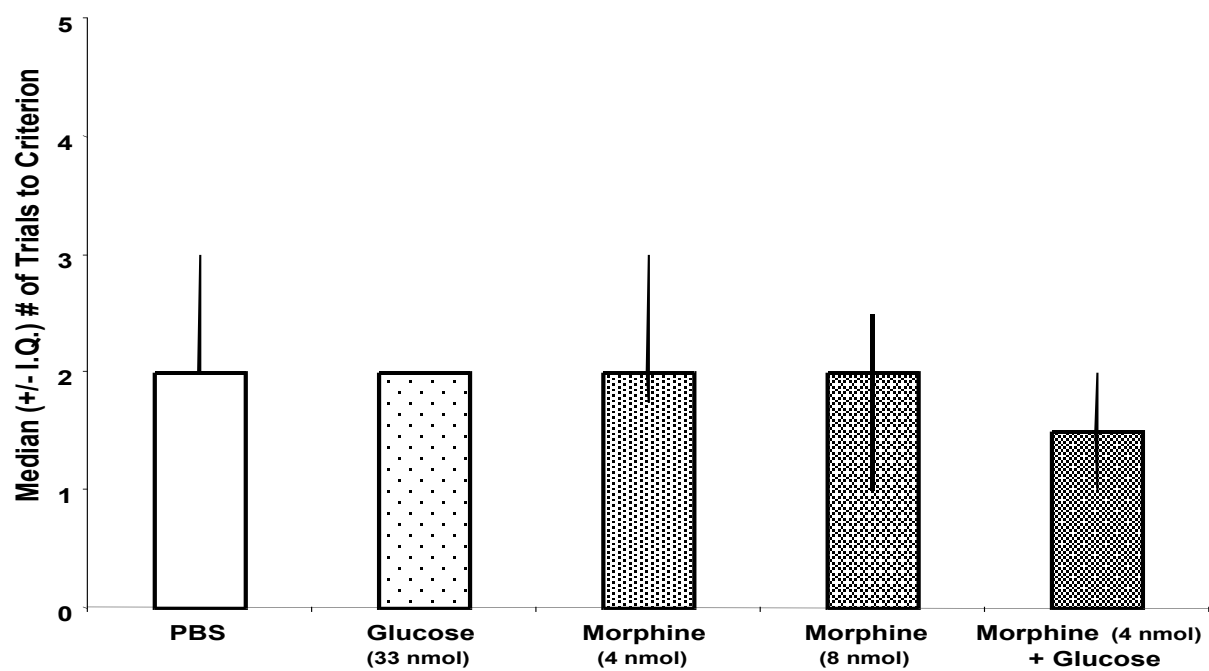


Figure 2.3A. There were no significant effects of any of the manipulations on the mean ( $\pm$  S.E.M.) number of trials to criterion ( $p > .05$  vs. PBS).

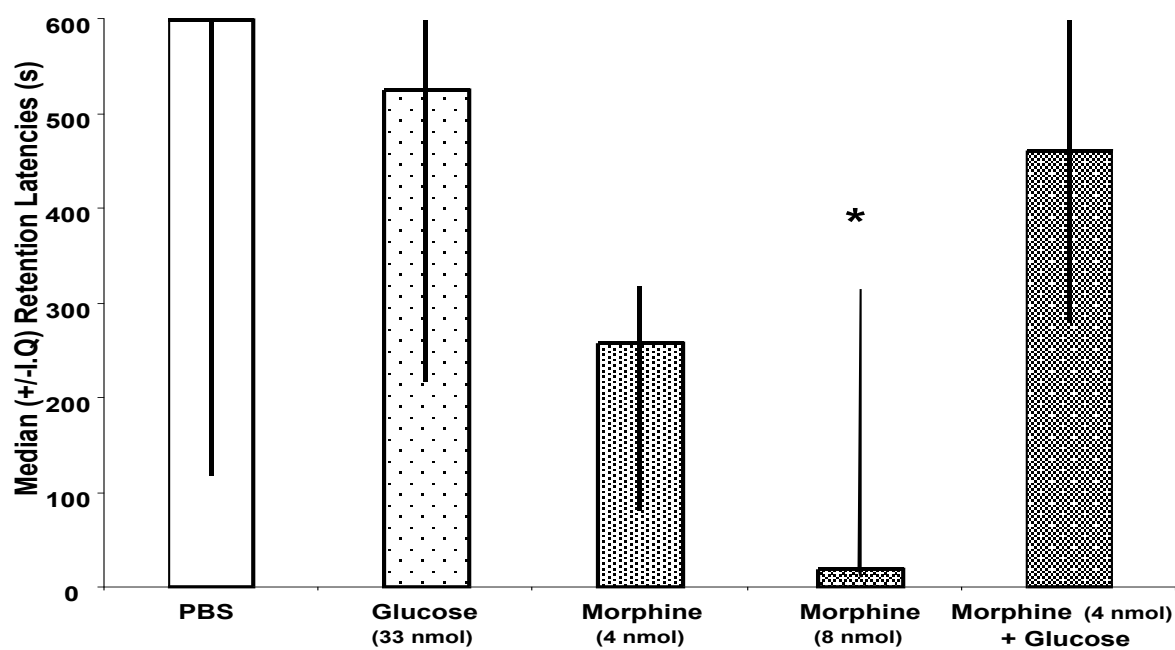


Figure 2.3B. MS infusions of morphine (8 nmol) decreased median (+/- I.Q.) retention latencies (\* $p < .05$  vs. PBS). MS infusions of glucose did not produce deficits when combined with subeffective doses of morphine ( $p > .05$  vs. PBS).

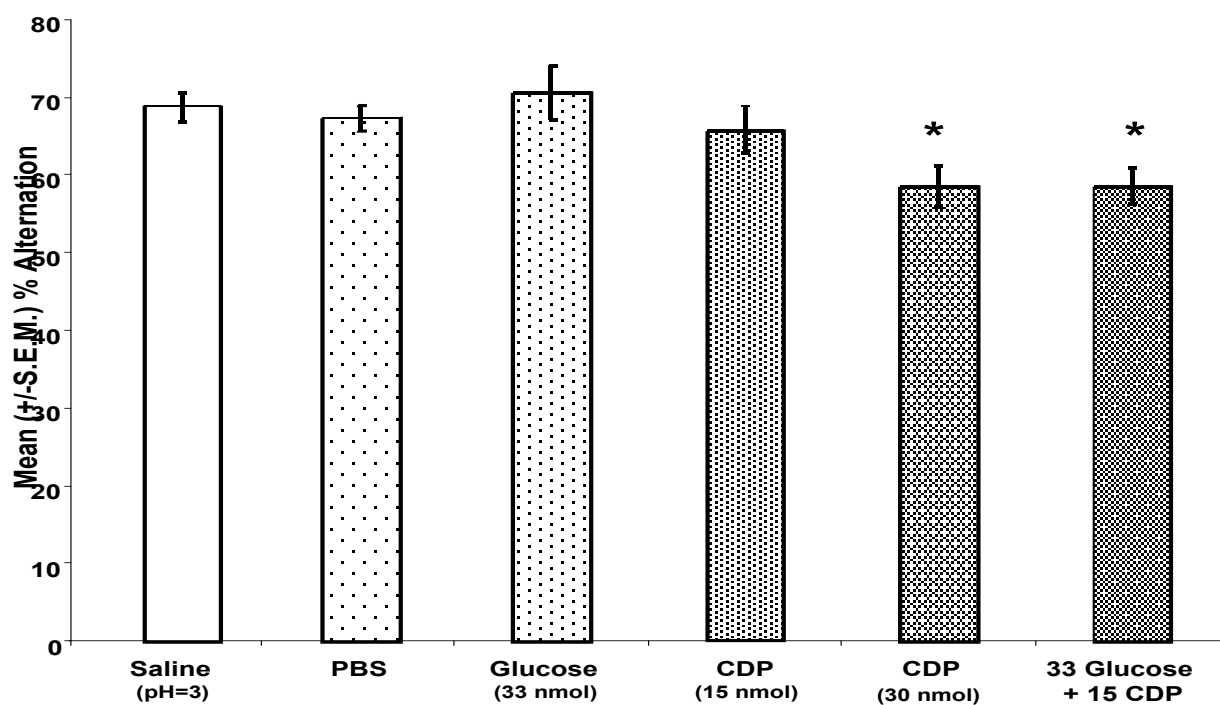


Figure 2.4A. MS infusions of CDP (30 nmol) significantly decreased mean ( $\pm$  SEM) percent alternation scores ( $*p < .05$  vs. saline). More importantly, MS co-infusions of CDP (15 nmol) with glucose, at doses that had no affect on their own ( $p > .05$  vs. saline), significantly decreased mean percent alternation scores ( $*p < .05$  vs. saline).

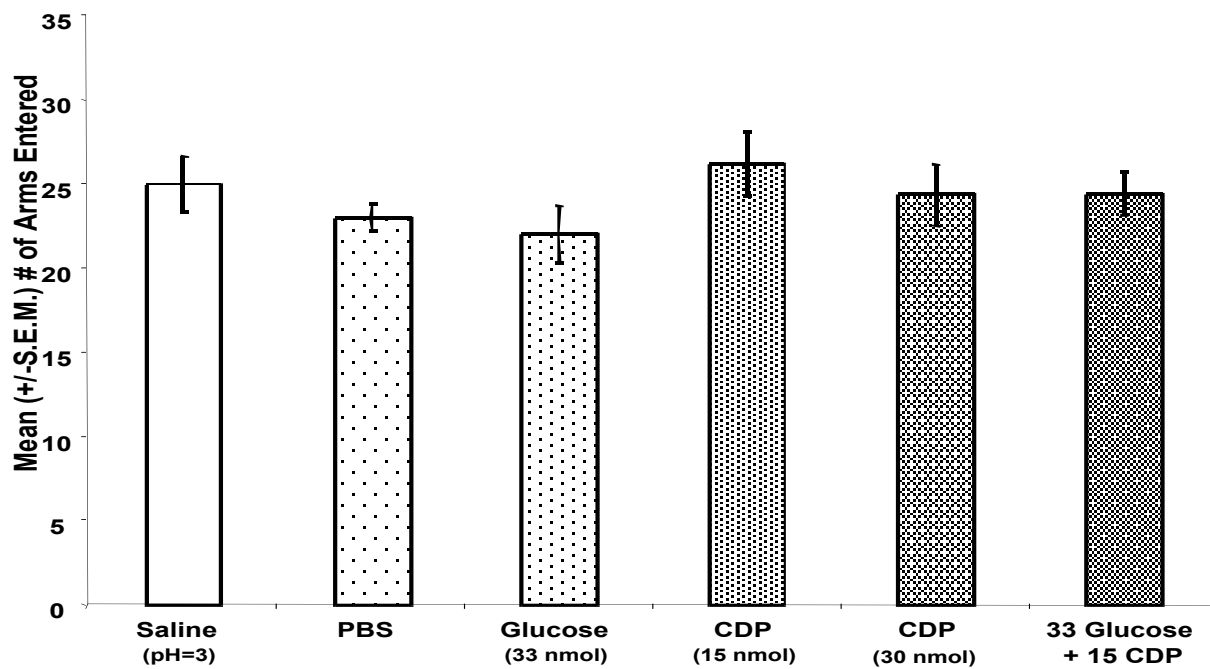


Figure 2.4B. MS infusions of CDP did not significantly impair mean ( $\pm$ -S.E.M.) number of arm entries ( $p > .05$  vs. saline).

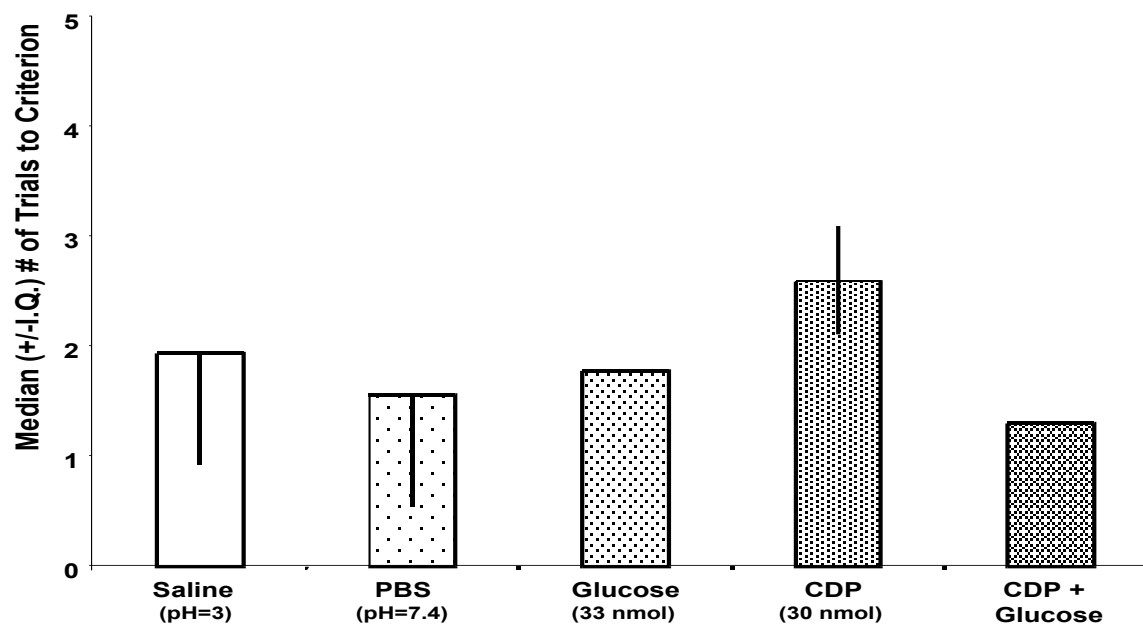


Figure 2.5A. MS infusions of CDP did not significantly impair median (+/-I.Q.) trials to criterion ( $p > .05$  vs. saline).



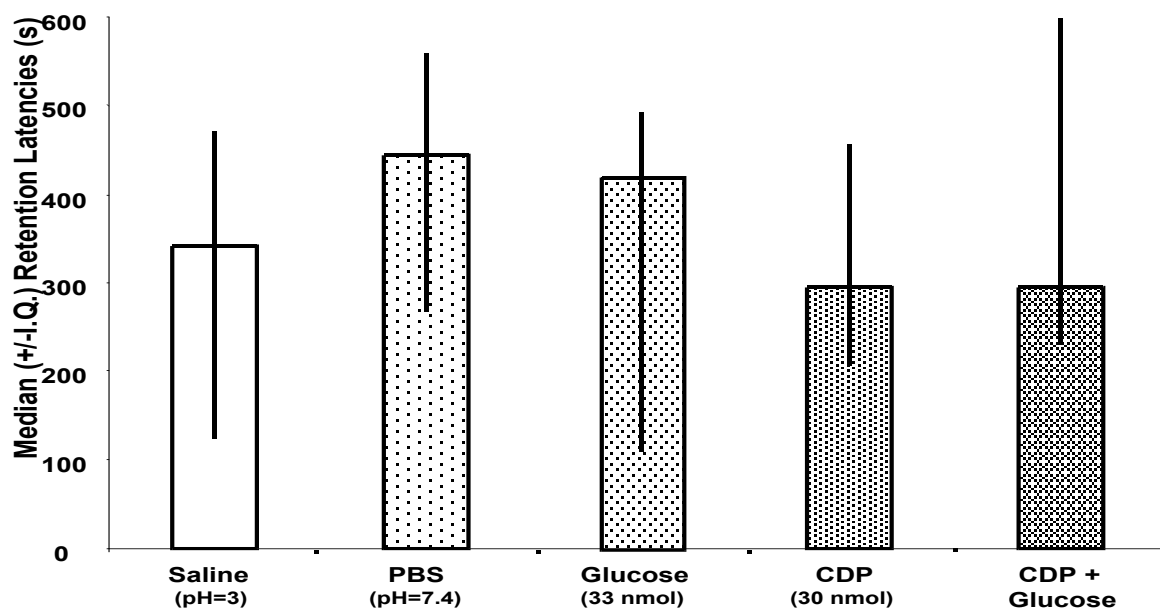


Figure 2.5B. MS infusions of CDP did not significantly impair median (+/-I.Q.) retention latencies ( $p > .05$  vs. saline).

**CHAPTER 3**

**SEPTAL INFUSIONS OF GLUCOSE DO NOT INCREASE SEPTAL  
EXTRACELLULAR GABA LEVELS BUT DO IMPAIR MEMORY WHEN CO-  
INFUSED WITH A GABA RECEPTOR AGONIST**

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### Abstract

Septal co-infusions of glucose with  $\gamma$ -aminobutyric acid (GABA) receptor agonists or modulators produce memory deficits. Elevating glucose increases brain GABA levels, raising the possibility that septal glucose infusions impair memory by increasing septal GABA synthesis or release. To test this hypothesis, Experiment 1 determined whether septal infusions of glucose, at a dose that impairs memory when co-infused with muscimol, would increase septal extracellular (ECF) GABA levels. Using *in vivo* microdialysis, we tested the effects of infusing glucose into the septum on septal ECF GABA concentrations in rats performing in a spontaneous alternation (SA) task. Male Sprague-Dawley-derived rats were given septal infusions of vehicle (phosphate buffered saline [PBS]), glucose (33 nmol), the GABA receptor agonist muscimol (0.10 nmol), or a cocktail composed of muscimol with glucose 16 min prior to assessing SA. Co-infusions of muscimol with glucose, at doses that had no effect on their own, decreased percent alternation scores; in contrast, none of the infusions significantly affected septal ECF GABA levels. Experiment 2 tested whether perfusions of glucose (6.6 mM) at longer durations or perfusing potassium (50 mM K<sup>+</sup>), a manipulation known to affect GABA release, would increase septal ECF GABA concentrations. The results showed that septal perfusions of glucose did not significantly increase ECF GABA levels, whereas septal perfusions of K<sup>+</sup> did. These findings suggest that the memory-impairing effects of septal infusions of glucose are not likely mediated via an increase in septal GABA release.

**Key Words:** Muscimol, GABA, Glucose, Spontaneous Alternation, Septum, Memory

## Introduction

Although glucose typically has positive effects on memory (Craft, Murphy, & Wemstrom, 1994; Gold, 1995; Korol, 2002; Korol & Gold, 1998; Manning, Ragozzino, & Gold, 1993; Messier, 2004; Newcomer et al., 1999; Petit, 1988), glucose can also produce memory deficits (Flood, Mooradian, & Morley, 1990; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; Popovic, Biessels, Isaacson, & Gispen, 2001; Ryan & Williams, 1993; Winocur et al., 2005). For instance, acute and chronic hyperglycemia are associated with memory dysfunction (Biessels et al., 1996; Convit, 2005; Elias et al., 1997; Flood, Mooradian, & Morley, 1990; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; Hagemann, Sartory, Hader, & Kobberling, 2005; McCall, 2002; Popovic, Biessels, Isaacson, & Gispen, 2001; Winocur et al., 2005). The mechanisms underlying the memory-modulating effects of glucose, particularly the conditions that differentiate the enhancing and impairing effects of glucose, are poorly understood. It is clear, though, that these effects of glucose are mediated, at least in part, by the brain (Choeiri, Staines, Miki, Seino, & Messier, 2005; Krebs & Parent, 2005a; McNay & Gold, 1999; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Stefani & Gold, 1998; Stefani, Nicholson, & Gold, 1999). Moreover, extensive evidence suggests that glucose enhances memory through a process that involves an increase in acetylcholine (ACh) synthesis or release in the brain, particularly in the hippocampus (Degroot & Parent, 2000, 2001; Durkin, Messier, de Boer, & Westerink, 1992; Kopf & Baratti, 1994; Pavone, Capone, Battaglia, & Sansone, 1998; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b). In contrast, there is some limited evidence suggesting that the memory-impairing effects of glucose may involve the septal GABA system. For instance, we have shown that septal infusions of glucose exacerbate the memory deficits produced by GABA receptor agonists and

interact with sub-effective doses of these agonists to impair memory (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). The pharmacological interaction between glucose and GABA agonists is synergistic, such that doses of glucose and GABAergic drugs that individually have no effect produce memory deficits when the two are co-infused (Parent & Gold, 1997; Shah & Parent, 2003, 2004). The memory-impairing interaction between glucose and GABA agonists in the septum occurs with multiple GABAergic drugs: the GABA<sub>A/C</sub> agonist muscimol (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004), the benzodiazepine (BZD) GABA<sub>A</sub> receptor modulator chlordiazepoxide (CDP; Krebs & Parent, 2004), and the GABA<sub>B</sub> agonist baclofen (Erickson, Watts, & Parent, 2006). Furthermore, these negative effects of glucose with GABA on memory are not likely due to extracellular (ECF) hyperosmolarity, because equiosmolar concentrations of other sugars do not produce memory deficits when combined with muscimol (Shah & Parent, 2003, 2004).

The synergistic interaction between elevated glucose and GABA receptor activation in the septum suggests that both are acting on a common mechanism to impair memory (Seeley & Moran, 2002). One candidate is glucose metabolism, because GABA agonists inhibit brain glucose metabolism (Ishizuka et al., 1989; Ito et al., 1994). This possibility seems unlikely, though, because both morphine and muscimol inhibit glucose metabolism (Cohen, Kimes, & London, 1991; Ito et al., 1994), yet glucose potentiates deficits produced by muscimol, but prevents deficits produced by morphine. A second possibility is that the synergistic interaction is somehow due to decreases in the membrane potential produced by the various GABA agonists. This is also not likely because glucose reverses deficits produced by galanin and morphine (Ragozzino & Gold, 1995b; Ragozzino, Parker, & Gold, 1992; Stefani & Gold, 1998), two

peptides that also hyperpolarize neurons (Hokfelt et al., 1999; Inturrisi, 2002). A third candidate mechanism is the septal GABA neurotransmitter system. The fact that glucose impairs memory when it is combined with GABA agonists that act at different GABA receptor subtypes provides a clue regarding the locus of the interaction. The GABA<sub>A</sub> receptor is linked to a chloride-gated ionophore and is located postsynaptically; in contrast, GABA<sub>B</sub> receptors are localized pre-or post-synaptically and are coupled to calcium or potassium channels via G-proteins and second messenger systems (Martin & Olsen, 2000). Therefore, it is unlikely that the effects of glucose on memory in the presence of GABA agonists are due to an effect of glucose on GABA receptor function. Rather, a more likely hypothesis is that glucose influences the neurotransmitter that binds to these different receptor subtypes.

We hypothesize that glucose increases septal GABA synthesis or release, and that this increase in ECF GABA summates with the effects of the sub-effective doses of the GABA agonists to produce memory deficits. This hypothesis is supported by the fact that acute administration of large amounts of glucose and experimentally induced chronic hyperglycemia increase GABA levels in the brain (Amoroso, Schmid-Antomarchi, Fosset, & Lazdunski, 1990; Fink & Gothert, 1993; Fink, Zentner, & Gothert, 1994; Ohtani, Ohta, & Sugano, 1997; Schmid-Antomarchi, Amoroso, Fosset, & Lazdunski, 1990). If the memory-impairing effects of septal infusions of glucose involve an increase in septal GABA release, then septal infusions of glucose should increase septal ECF GABA levels. The present set of experiments tested whether septal infusions of glucose, at a dose that typically impairs memory when co-infused with muscimol, would increase septal ECF GABA levels.

## **Experiment 1**

The goal of Experiment 1 was to determine whether septal infusions of glucose would increase septal ECF GABA levels. To date, the memory-impairing effects of septal infusions of glucose are observed only when the glucose is co-infused with muscimol. This suggests that the effects of glucose on ECF GABA levels may depend on the presence of muscimol. Therefore, we also tested whether the effects of septal infusions of glucose septal ECF GABA levels would vary as a function of the presence or absence of muscimol.

### **Materials and Methods**

#### **Subjects**

Twenty-four male Sprague-Dawley derived rats ( $n = 5-8$  per group) weighing 200-250 g upon arrival (Charles River, Wilmington, MA) were used. The rats were housed individually in polycarbonate cages (20x40x20 cm) with corncob bedding on a 12 hour light-dark cycle (lights on at 7:00 a.m.) in a temperature-controlled colony room (70-74 °F). Animals had free access to food and water. The rats were acclimated to laboratory conditions for approximately 1 week prior to surgery. The Georgia State University Institutional Animal Care and Use Committee (IACUC) approved all procedures involving rats.

#### **Surgery**

Prior to surgery, rats were placed in a clear, plastic gas induction chamber and anesthetized with 5% isoflurane (Baxter, Deerfield, IL) delivered in 1000 ml/min medical grade oxygen. After the rat was no longer ambulatory, it was removed from the chamber and placed on a face-mask that delivered 3% isoflurane in 1000 ml/min of oxygen. Rats were then given injections of atropine sulfate (0.4 mg/kg, ip, Baxter, Deerfield, IL) and penicillin (1500 units, im, Hanfords US Vet, Syracuse, NY). The future incision site was shaved with a #50 electric clipper

blade (Oster) and betadine solution was applied to the surgical area. The percentage of isoflurane given to the rats was adjusted from 1-3% to maintain a surgical plane of anesthesia as determined by the toe pinch test. A stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) that was equipped with an anesthesia mask was used to implant one 15 mm inert, biocompatible plastic dialysis probe guide cannulae (Bioanalytical Systems, Inc., Roanoke, VA) aimed at the medial septum (0.5 mm anterior to bregma and 4.9 mm ventral to dura; Paxinos & Watson, 1998). The incision site was anesthetized locally with a 2% lidocaine/.001% epinephrine cocktail (0.5-2.0 cc, sc, Abbott Labs, Chicago, IL). After the incision, the 2% lidocaine/.001 % epinephrine cocktail solution (.05- 1.0 cc) was applied topically to the skull to facilitate seeing lambda and bregma. The cannula was secured to the skull with three jeweler's screws and cranioplastic cement and a stylet was inserted to keep the cannula free of debris. Immediately after surgery, the rats were given an injection of 0.9 % sterile saline (3.0 cc, sc) and the non-steroidal anti-inflammatory flunixin meglumine (2.5 mg/kg, ip, Fort Dodge Animal Health, Fort Dodge, IA), and then wrapped with a paper towel and kept under a warm lamp until recovery from anesthesia. Two days following surgery, the patency of each cannula was checked and betadine was applied to the surgical wound. If signs of infection were evident, the rats were anesthetized with isoflurane (5%) gas delivered in 1000 ml/min of oxygen and given an additional injection of penicillin (1500 units im).

### ***In Vivo* Microdialysis Procedures**

Each rat was handled for 2 minutes on two separate occasions prior to microdialysis procedures. Each rat participated in two microdialysis sessions separated by at least 4 days. Before and after all handling and *in vivo* microdialysis, the rats were allowed a minimum of 30 minutes to habituate to the laboratory environment. *In vivo* microdialysis procedures were



conducted between 7:00 a.m. and 7:00 p.m. On the day of microdialysis, the rat was placed in a round Plexiglas containment bowl (BAS) and attached to a tether (BAS) that permitted him to move in the microdialysis containment bowl. Following a 1-hour habituation period, a polyacrylonitrile microdialysis probe (320  $\mu\text{m}$  OD; BAS) that extended 2 mm beyond the guide cannula was inserted into the guide cannula. The dialysis probe was equipped with an injection guide, which allowed for both injections into and sampling from the septal brain region. The probe length allowed for sampling from the medial septal area and possibly from the ventral diagonal band of Broca. Using a microinfusion pump (BAS), the probe was perfused at the rate of 2  $\mu\text{l}/\text{min}$  with artificial cerebrospinal fluid (aCSF; mM: NaCl 145.0, KCl 3.0,  $\text{CaCl}_2$  1.5,  $\text{MgCl}_2$  1.0,  $\text{NaH}_2\text{PO}_4$  2.0,  $\text{Na}_2\text{HPO}_4$  2.0, dextrose 1.0; pH 7.3; filtered [0.2  $\mu\text{M}$ ] and degassed). The concentration of glucose was selected based on preliminary zero net flux dialysis data suggesting the ECF glucose concentrations in the septum are approximately 1 mM (Krebs & Parent, unpublished data). The flow rate of 2  $\mu\text{l}/\text{min}$  was chosen to permit the collection of sample volumes needed to assay amino acids (i.e., 15  $\mu\text{L}$ ), while also allowing for sample periods that approximated the duration used to test the rats in previous behavioral experiments (i.e., 8 min; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997). After a 2-h stabilization period, three baseline samples were collected every 8 min. Then the rats were given septal infusions of vehicle (0.5  $\mu\text{l}$ , 0.5  $\mu\text{l}/\text{min}$ ; phosphate buffered-saline [PBS]), glucose (33 nmol), muscimol (0.1 nmol), or co-infusions of glucose with muscimol combined in one solution. The drugs that were combined in the same solution were prepared at double the desired concentration and then combined reducing the concentration of each by half. The dose of glucose was chosen based on previous research showing that this dose impairs memory when

combined with the GABA agonist muscimol (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). The dose of muscimol was selected based on previous findings showing that this dose does not significantly impair memory when infused alone into the septum, but does significantly impair memory when combined with glucose (Parent & Krebs, 2004). SA was assessed 2 sample periods (i.e., 16 min) following the drug infusions. The rats were placed in a 3-arm maze at the onset of the third sample period after the injection and remained in the maze for the duration of the sample period (i.e., 8 min). Three more samples were collected following the manipulations. The samples were kept on dry ice during the experiment and then transferred to a -80 °C freezer for storage until analysis with high-performance-liquid-chromatography (HPLC). Half-way through each sample period, the overall motor activity of the rat was rated using a 5-point scale (0 = no obvious movement, 1 = head movement, 2 = head and fore-limb movement, 3 = infrequent movement of all 4 limbs [e.g., burrowing into bedding], and 4 = movement of all four paws with locomotion and/or rearing; Moore, Sarter, & Bruno, 1993).

### **Spontaneous Alternation**

Spontaneous alternation (SA) is assumed to be a hippocampal-dependent measure of spatial working memory (Johnson, Olton, Gage, & Jenko, 1977; Lalonde, 2002; Richman, Dember, & Kim, 1987; Stevens & Cowey, 1973). The underlying assumption is that in order to alternate successfully between locations the rats must remember its visits to previous places. This interpretation is supported by the finding that SA is impaired by removing directional cues or by increasing the interval between arm choices (Lalonde, 2002; Richman, Dember, & Kim, 1987). Sixteen minutes after the drug injections, SA performance was assessed by placing each

rat in a Y-maze composed of three equally spaced arms (60°; 61 cm long x 20 cm high) separated by a circular center with a diameter of 43.5 cm. The 3-arm maze was used for the microdialysis experiments in order to parallel our previous research (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). Each arm was composed of black plexiglass (15.5 cm wide) and the top was left uncovered. All rats were placed in center of the Y-maze and allowed to explore the maze for 8 minutes. The experimenter recorded the sequence and number of arms the rats entered during the 8 min. period. The maze was cleaned with 70% ethanol after each rat. The number of arms each rat entered was used as a measure of activity. A percent alternation score was computed for all rats that entered at least 10 arms. An alternation was defined as entering three different arms consecutively. The percent alternation score was computed by dividing the number of alternations each rat made by the number of arms entered minus two (i.e., the number of alternations possible) and then multiplying that resulting quotient by 100.

### **High performance liquid chromatography (HPLC)**

The amount of GABA in each sample of perfusate was assayed using reverse-phase HPLC (Waters 2695 Separations Module; Waters Corporation) and fluorescence detection (Waters 474; Waters Corporation) with Empower software. The amino acids were derivatized with o-phthalaldehyde (OPA, Pierce) and the products of the reaction, 1-alkyl-2-alkylthio-substituted isoindoles, exhibit optimal excitation at 260 nm and maximal emission at 455 nm. A 5.0 µl p-fluoraldehyde reagent was added to a 5.0 µl portion of standard, blank, or sample via an autosampler (Waters Corporation). The sample was held in the injection loop of the solvent management system (Alliance 2690XE; Waters Corporation) for 1.5 min prior to being injected.

A solvent delivery system (Alliance 2690IX; Waters Corporation) moved the solvent, which consisted of a mixture of 70% mobile phase A (900 ml 0.08 M NaH<sub>2</sub>PO<sub>4</sub>, 240 ml MeOH, 20 ml acetonitrile, and 10 ml tetrahydrofuran [THF]; pH = 6.2; vacuum filtered [0.2 µM]): 30% mobile phase B (670 ml 0.04 M NaH<sub>2</sub>PO<sub>4</sub>, 555 ml MeOH, and 30 ml THF; pH = 6.2; pH = 6.2; vacuum filtered [0.2 µM]). The precolumn (Waters µBondapak C18; Waters Corporation) and the column (Waters Spherisorb ODS2 C18 [4.6 x 250 mm, 5 µm]) were used at a constant temperature of 30 °C. The initial gradient was maintained for 20 min at a flow rate of 0.5 ml/min. Then the gradient was changed to 100% mobile phase B, and after 23 min, the flow rate was increased to 1.0 ml/min for 20 min. Fresh stock solutions were used daily and stock solutions were sealed under N<sub>2</sub> and stored at 4 °C. A calibration curve consisting of a series of concentrations of GABA (.0078- 1 ng/5µL) was constructed with each assay run.

## **Histology**

After behavioral testing, the rats were euthanized with an overdose of sodium pentobarbital (400 mg/kg, ip) and perfused intracardially with 0.9% saline followed by 10% formalin. Their brains were stored in a 10% formalin solution for at least 2 days before sectioning. All brains were sectioned on a cryostat (Leica CM 30510 S) and 45-60 µm sections were taken through the septal cannulae tracts. The brain sections were stained with thionin and an unbiased observer determined the cannulae placement using a light microscope (Olympus BX41). Acceptable medial septal cannulae placement was defined as septal injection sites located within the medial septum and dorsal portions of the ventral diagonal band of Broca, but not within the lateral septum. Moreover, the cannula must not have penetrated the fimbria.

## Statistical Analysis

The neurochemical data were expressed as a percentage of the mean of the baseline samples and were analyzed with a mixed ANOVA. The SA data were expressed as means and standard errors of the mean (SEM) and analyzed using a one-way analysis of variance (ANOVA) and Tukey post hoc tests. An alpha level of 0.05 was used as the criterion for statistical significance.

## Results

Figure 3.1 shows the approximate location of septal microdialysis probes. The 2 mm probes sampled primarily from the medial septum and ventral diagonal band of Broca. Drug infusions into the septum and hippocampus [ $F(3,23) = 11.60$ ;  $p < .05$ ] significantly affected SA performance (see Figure 3.2). Septal infusions of muscimol or glucose alone did not affect SA performance. The scores of rats given septal infusions of muscimol or glucose were not significantly different from the scores of rats given infusions of PBS ( $p > .05$ ). As in previous research, co-infusions of muscimol with glucose into the septum impaired SA performance. Specifically, the percent alternation scores of rats given muscimol combined with glucose in the septum were significantly lower than those of rats given PBS ( $p < .05$ ). In contrast, the drug infusions into the septum [ $F(3,23) = 1.59$ ;  $p > .05$ ] did not significantly affect the number of arms the rat entered in the maze (see Figure 3.3), although there was a tendency for rats given muscimol in the septum to enter more arms than rats given PBS ( $p = .052$ ). More importantly, the drug infusions into the septum did not significantly affect septal ECF GABA levels [ $F(27, 117) = .856$ ,  $p > .05$ ; see Figure 3.4].

## Discussion

These results demonstrate that septal co-infusions of the GABA receptor agonist muscimol with glucose, at doses that have no effect alone, impair spatial working memory. These data are consistent with previous research indicating that co-infusions of muscimol with glucose into the septum impair memory (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). The present findings also show that septal infusions of glucose or septal co-infusions of glucose with muscimol did not increase septal ECF GABA concentrations. These findings are inconsistent with previous findings showing that acute increases in glucose or experimentally-induced diabetes elevate GABA levels in the cortex and substantia nigra (Amoroso, Schmid-Antomarchi, Fosset, & Lazdunski, 1990; During, Leone, Davis, Kerr, & Sherwin, 1995; Fink & Gothert, 1993; Fink, Zentner, & Gothert, 1994; Ohtani, Ohta, & Sugano, 1997; Schmid-Antomarchi, Amoroso, Fosset, & Lazdunski, 1990). These findings suggest that acute elevations in septal ECF GABA levels are not necessary for the memory-impairing effects of septal glucose infusions.

## Experiment 2

Acute drug infusions were used in Experiment 1 to mimic the procedures used in our previous experiments showing that septal infusions of glucose with muscimol impair memory. It is possible, however, that the microdialysis and assay procedures could not detect changes in ECF GABA levels produced by the acute infusions of glucose. As a result, the goal of Experiment 2 was to determine whether elevating glucose in the septum, over multiple sample periods, would increase ECF GABA levels. Also, as a positive control, we determined whether a manipulation known to increase GABA levels, including GABA levels in the septum (Parent et al., 2001), would increase septal ECF GABA levels using our procedures. The concentration of

glucose was selected based on previous research showing that it increased hippocampal ECF ACh levels in rats performing in the SA task (Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Stefani & Gold, 2001b). The concentration of potassium was chosen based on previous data showing it significantly increased ECF GABA levels in the septum and other brain regions (Campbell et al., 1993; Herbison, Heavens, & Dyer, 1990; Hu, Watson, Kennedy, & Becker, 2006; Parent et al., 2001; Takeda, Hirate, Tamano, & Oku, 2003; Tossman & Ungerstedt, 1986). The same procedures were used as in Experiment 1, with the following exceptions: After baseline samples were collected, the aCSF was changed to one that contained higher concentrations of glucose (6.6 mM) or potassium (50 mM K<sup>+</sup>) for two sample periods. The Na concentration in the aCSF containing elevated K<sup>+</sup> was decreased to correct for the increase in hyperosmolarity. To increase statistical power, the data for different sampling periods were pooled. Specifically, the ECF GABA values for the baseline samples (i.e., samples 1-3) and the ECF GABA values for the two post-baseline samples in which glucose or K<sup>+</sup> were elevated (i.e., samples 4-5) were averaged. To correct for non-normality and non-heterogeneity of variance, the data were transformed to the log(base10) of the percent of baseline. Paired t-tests were used to detect differences between the average baseline and post-baseline values for glucose or K<sup>+</sup>. Six (6.6 mM glucose) and 12 (50 mM K<sup>+</sup>) male Sprague-Dawley-derived rats were used.

## Results

Increasing the concentration of glucose in the septal perfusate did not affect septal ECF GABA levels [ $t(1,5) = .45$ ,  $p > .05$ ; see Figure 3.5]. In contrast, increasing the concentration of K<sup>+</sup> did significantly increase septal ECF GABA levels [ $t(1,11) = 6.98$ ,  $p < .05$ ]. Specifically, compared to baseline levels the mean ECF GABA levels of rats were significantly higher during the sample periods when K<sup>+</sup> was elevated ( $p < .05$ ).

## **General Discussion**

The findings from the present experiment demonstrated that septal infusions of muscimol with glucose combined in the same solution impaired spatial working memory. These findings are consistent with previous research showing that septal co-infusions of glucose with muscimol impair memory (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). More importantly, the present experiment also showed that the same manipulations did not increase septal ECF GABA levels. Furthermore, these data showed that perfusing the septal area with glucose, at a concentration that increases hippocampal ECF ACh levels (Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Stefani & Gold, 2001b), did not increase septal ECF GABA levels. In contrast, septal ECF GABA levels were increased by septal perfusion of aCSF containing elevated K<sup>+</sup>. These data are in agreement with previous results showing that elevating K<sup>+</sup> within the septum increases ECF GABA levels (Parent et al., 2001). In contrast, these findings are inconsistent with previous research showing that elevating glucose levels increases GABA release in the cortex and substantia nigra (Amoroso, Schmid-Antomarchi, Fosset, & Lazdunski, 1990; During, Leone, Davis, Kerr, & Sherwin, 1995; Fink & Gothert, 1993; Fink, Zentner, & Gothert, 1994; Ohtani, Ohta, & Sugano, 1997; Schmid-Antomarchi, Amoroso, Fosset, & Lazdunski, 1990).

The absence of a glucose-induced increase in ECF GABA levels in Experiment 1 could be due to insufficient drug delivery. For example, the microdialysis probe that was adjacent to the injection needle may have collected some of drugs being delivered into the septum. This is not likely, though, because septal co-infusions of glucose with muscimol effectively produced memory deficits. Another possibility is that any glucose-induced increases in GABA were cleared from the synapse more quickly than the temporal resolution of the dialysis procedure



(Timmerman & Westerink, 1997). This is also not likely given the present finding that K<sup>+</sup> did increase septal ECF GABA levels.

Collectively, these results suggest that glucose-induced increases in ECF GABA levels are not necessary for the impairing effects of glucose on memory. Glucose may instead influence memory by influencing the binding properties of the GABA receptor. For instance, glucose may modulate memory by directly influencing the binding properties of the GABA receptor to either promote or attenuate binding of GABA agonists to the receptor, although there is no evidence to date showing that glucose can do so. There is evidence, though, showing that glucose does reduce the binding of morphine to opioid receptors (Brase, Han, & Dewey, 1987). Another possibility is that adenosine tri-phosphate (ATP)-dependent potassium (K<sup>+</sup>) channels (K<sup>+</sup>-ATP) are involved in the negative effects of glucose on memory. K-ATP channels, which can affect neurotransmitter release (Amoroso, Schmid-Antomarchi, Fosset, & Lazdunski, 1990; During, Leone, Davis, Kerr, & Sherwin, 1995), are closed by glucose (Larsson, Kindmark, Branstrom, & Berggren, 1997; Straub, James, Dunne, & Sharp, 1998; Valdeolmillos, Nadal, Contreras, & Soria, 1992). It is not clear how K-ATP channels could participate in for the memory-impairing effects of glucose; however, because the opening of the septal K-ATP channels impairs memory (Rashidy-Pour, 2001; Stefani & Gold, 1998, 2001a; Stefani, Nicholson, & Gold, 1999) and blocking septal K-ATP channels with glucose enhances memory and reverses memory deficits (Rashidy-Pour, 2001; Stefani & Gold, 1998, 2001a; Stefani, Nicholson, & Gold, 1999). Alternatively, the impairing effects of glucose on memory may involve a product of glycolytic metabolism. Glucose metabolism yields two molecules of pyruvate and two molecules of ATP (Hertz & Dienel, 2002). Pyruvate metabolism, in turn, yields by-products necessary for glutamate, ACh, and GABA synthesis (Hertz & Dienel, 2002).

Previous research has demonstrated that infusions of the glycolytic metabolite pyruvate mimics the memory-impairing effects of glucose (Shah & Parent, 2003, 2004), suggesting that a product of glycolytic metabolism could mediate the impairing effects of glucose on memory.

Another possibility is that glucose impairs memory by increasing ACh release in the septum as it does in the hippocampus. Although increasing brain ACh activity, including the septum (Decker, Majchrzak, & Anderson, 1992; Givens & Olton, 1995; Givens & Olton, 1990; Olton et al., 1991), typically has positive consequences for learning and memory (Chang & Gold, 2003a; Degroot, Kornecook, Quirion, DeBow, & Parent, 2003; Ragozzino, Unick, & Gold, 1996), some studies have shown that septal administration of the cholinergic agonist oxotremorine can produce memory deficits (Elvander et al., 2004; Pang, 1999). Furthermore, oxotremorine infusions into the striatum impair striatal long-term-potential (Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998), which would be expected to produce memory deficits. Alternatively, elevations in glucose in the septum impair memory via increases in septal glutamate activity. Glucose administration also elevates glutamate levels in the brain (Burke & Nadler, 1989; Gruetter, 2002; Hamberger, Chiang, Nylen, Scheff, & Cotman, 1979). Furthermore, there is some limited evidence that suggesting that septal infusions of glutamate may have negative consequences for memory. For example, septal infusions of low concentrations of glutamate tend to impair SA (Parent, Laurey, Wilkniss, & Gold, 1997). In addition, septal infusions of glutamate also decrease hippocampal sodium-dependent high affinity choline uptake (SDHACU; Marighetto, Micheau, & Jaffard, 1994), which would be expected to impair memory (Chang & Gold, 2003a; Gold, 2003a; Parent & Baxter, 2004). Furthermore, septal infusions of glutamate antagonists increase SDHACU (Marighetto, Micheau, & Jaffard, 1994). Septal elevations in ACh or glutamate may enhance the activity of GABA

interneurons in the septum and thus decrease the function of the cholinergic, GABAergic, and/or glutamatergic septo-hippocampal projections, which would be expected to impair memory (Durkin, 1992b; Parent & Baxter, 2004).

In summary, septal infusions of glucose with muscimol impair spatial working memory. More importantly, septal infusions of glucose did not increase septal ECF GABA levels. Elevations in K<sup>+</sup> did increase septal ECF GABA levels, indicating that the procedures were able to detect changes in septal ECF GABA levels. Collectively, these findings suggest that GABA release is not necessary for the memory-impairing effects of septal infusions of glucose.

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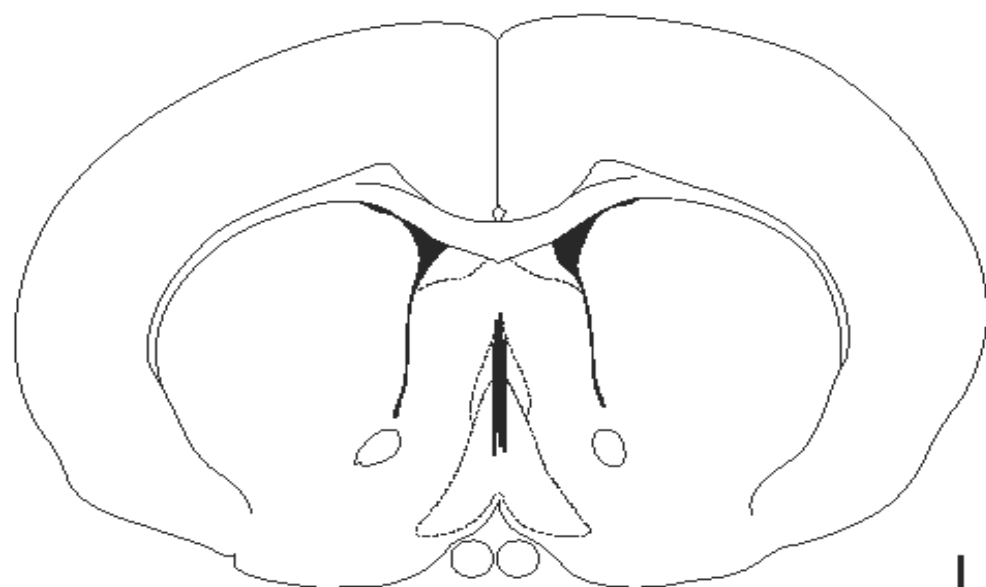
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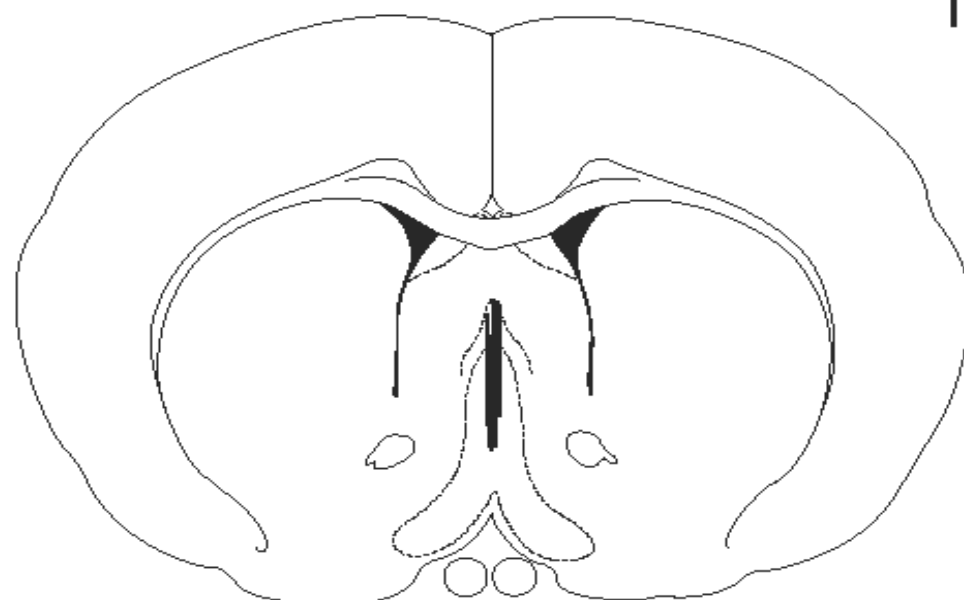


Figure 3.1. Schematic illustration of coronal sections of the rat brain showing the approximate locations of medial septal dialysis probe and infusion sites. Atlas plates were adapted from Paxinos and Watson (1998).

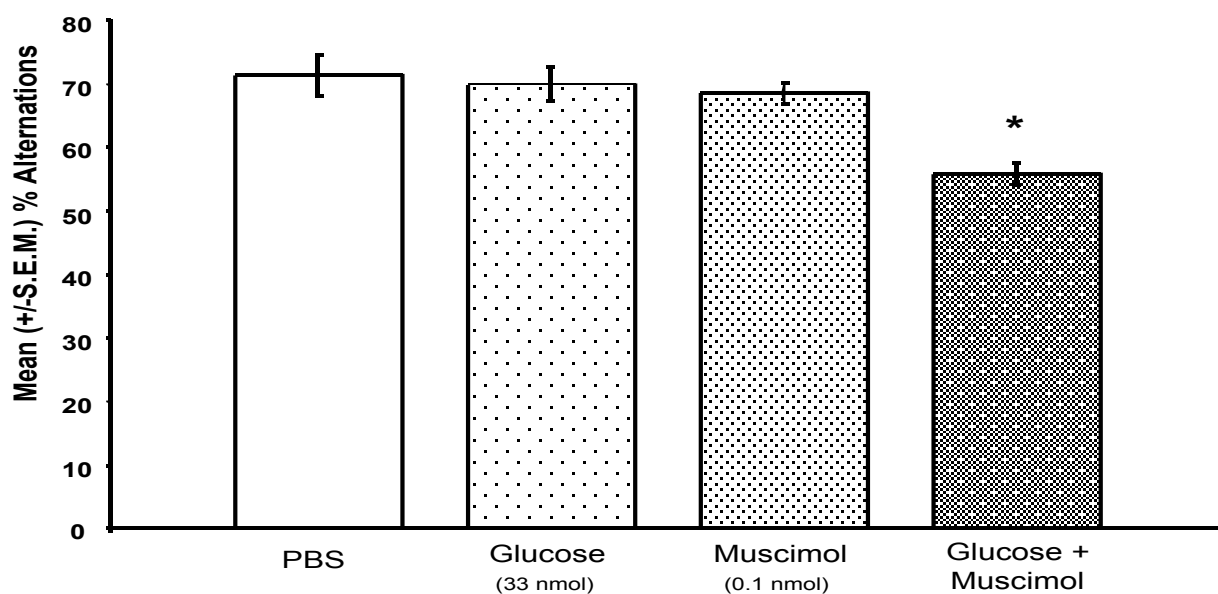


Figure 3.2. Septal infusions of muscimol or glucose alone did not significantly decrease mean (+/- S.E.M.) percent alternation scores ( $p > .05$  vs. control). Septal infusions of glucose with muscimol significantly decreased percent alternation scores ( $*p < .05$  vs. control).



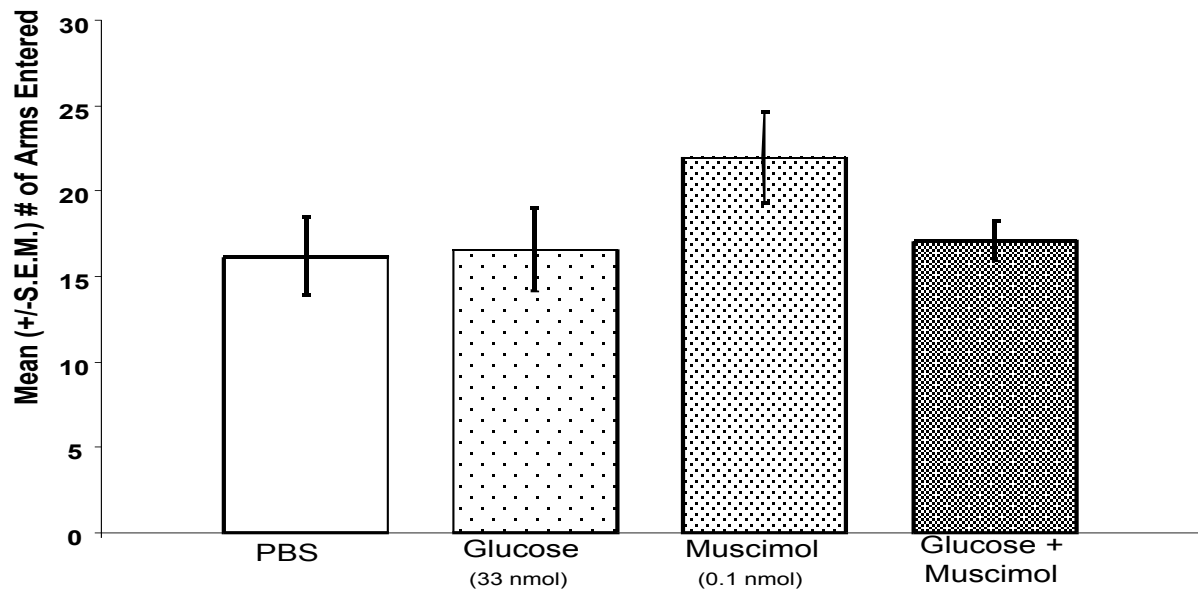


Figure 3.3. Septal drug treatments did not significantly affect the number of arms entered in the maze ( $p > .05$ ).

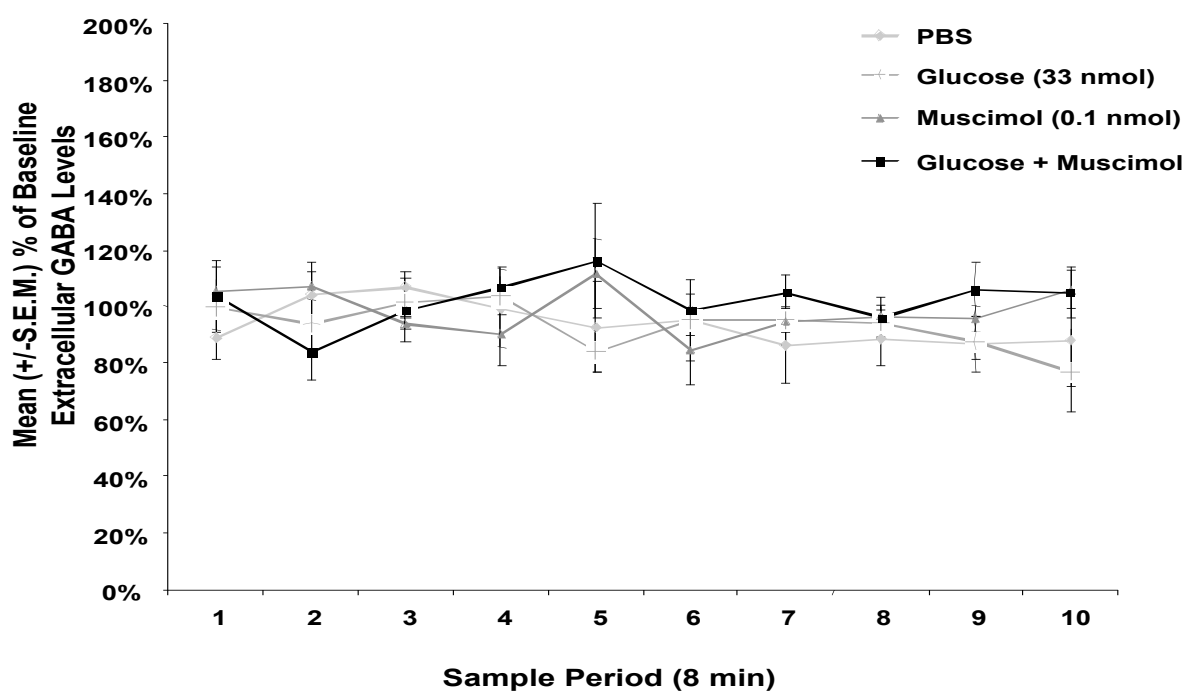


Figure 3.4. Septal drug infusions did not significantly affect mean ( $\pm$  S.E.M.) ECF GABA levels ( $p > .05$ ).

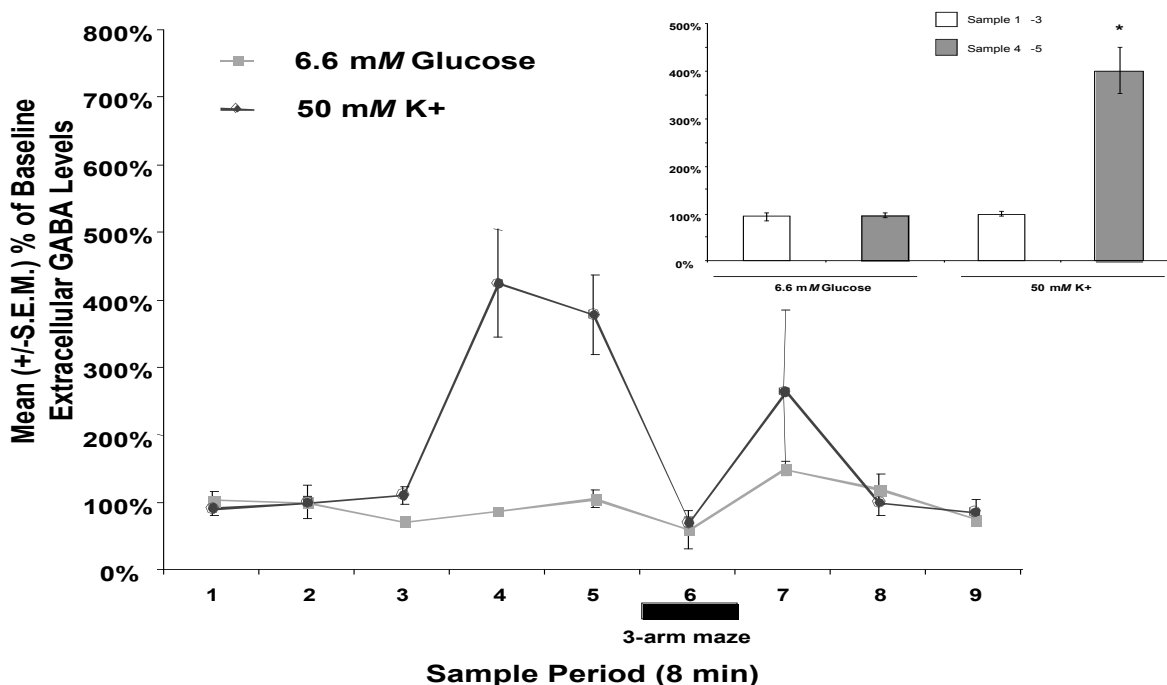


Figure 3.5. Septal perfusions of 6.6 mM glucose did not significantly affect mean (+/-S.E.M.) ECF GABA levels ( $p > .05$ ); whereas, septal perfusions of 50 mM potassium did ( $p < .05$ ). Inset shows that pooled mean (+/-S.E.M.) ECF GABA values for the baseline samples (i.e., samples 1-3) and the ECF GABA values for the two post-baseline samples in which glucose or K<sup>+</sup> were elevated (i.e., samples 4-5). Septal perfusion of glucose (6.6 mM) did not significantly affect the mean septal ECF GABA levels ( $p > .05$  vs. baseline). The mean septal ECF GABA levels in the samples in which K<sup>+</sup> was elevated were significantly higher than the ECF GABA values of the baseline samples ( $p < .05$ ).

## **CHAPTER 4**

### **THE MEMORY-IMPAIRING EFFECTS OF MS GABA RECEPTOR ACTIVATION INVOLVE THE GABAERGIC SEPTO-HIPPOCAMPAL PROJECTION**

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### Abstract

Medial septal (MS) infusions of the  $\gamma$ -aminobutyric acid (GABA) agonist muscimol impair memory, and the effect likely involves the hippocampus. GABA<sub>A</sub> receptors are present on the cell bodies of both cholinergic and GABAergic septo-hippocampal (SH) projections. The current experiments determined whether the memory-impairing effects of MS GABA receptor activation involve the GABAergic SH projection. Experiment 1 tested whether hippocampal infusions of a GABA<sub>A</sub> receptor antagonist would block the effects of MS muscimol infusions. Experiment 2 tested whether combining MS co-infusions of sub-effective doses of muscimol with scopolamine, a drug that selectively influences the GABA projection, would summate to produce deficits. Fifteen minutes prior to assessing spontaneous alternation (SA) or training in a continuous multiple trial inhibitory avoidance (CMIA) task, male Sprague-Dawley-derived rats were given MS infusions of vehicle or muscimol combined with unilateral hippocampal infusions of vehicle or bicuculline (Experiment 1) or MS infusions of vehicle, muscimol, scopolamine, or co-infusions of muscimol with scopolamine (Experiments 2). Hippocampal infusions of bicuculline, at a dose that produced no effect alone, blocked deficits produced by MS muscimol infusions in SA and attenuated retention deficits produced in CMIA. MS co-infusions of muscimol with scopolamine, at doses that had no effect on memory alone, significantly impaired SA and CMIA. Combined, these findings suggest that the memory-impairing effects of MS GABA receptor activation involve the GABAergic SH projection.

Key Words: GABA, Muscimol, Bicuculline, Septum, Hippocampus, Scopolamine, Spontaneous Alternation, Inhibitory Avoidance

## Introduction

Extensive evidence indicates that activation of GABA receptors in the medial septum (MS) impairs memory in a variety of learning and memory tasks (Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990; Chrobak, Stackman, & Walsh, 1989; Durkin, 1992b; Nagahara & McGaugh, 1992; Pang & Nocera, 1999). These impairing effects of MS GABA receptor activation likely involve the hippocampus. For instance, MS infusions of the GABA agonist muscimol impair hippocampal theta rhythm (Allen & Crawford, 1984; Smythe, Colom, & Bland, 1992; Sotty et al., 2003), a rhythmic oscillation important for memory (Hasselmo, 2005; Hasselmo, Hay, Ilyn, & Gorchetchnikov, 2002; O'Keefe, 1993; Vertes & Kocsis, 1997). Also, the memory-impairing effects of MS infusions of muscimol are reversed by hippocampal infusions of glucose, pyruvate, or acetylcholinesterase (AChE) inhibitors (Degroot & Parent, 2000, 2001; Krebs & Parent, 2005a, 2005c; Parent, Laurey, Wilkniss, & Gold, 1997).

The MS is connected to the hippocampus via the fimbria-fornix, which is composed primarily of cholinergic and GABAergic projection neurons (Kohler, Chan-Palay, & Wu, 1984; Lewis, Shute, & Silver, 1967; Rye, Wainer, Mesulam, Mufson, & Saper, 1984; see Figure 4.1A). Although a putative glutamatergic projection has been recently shown, very little is known about the connectivity or receptor make-up of this septo-hippocampal (SH) projection (Bland, Oddie, & Colom, 1999; Castaneda et al., 2005; Colom, Castaneda, Reyna, Hernandez, & Garrido-Sanabria, 2005). The GABAergic SH projection synapses onto GABA interneurons in the hippocampus (Freund & Antal, 1988), which then synapse onto glutamatergic pyramidal cells (Toth, Freund, & Miles, 1997). The GABAergic afferents to the hippocampus, therefore, produce a net disinhibition of pyramidal cells. Cholinergic SH projections terminate broadly in the hippocampus, synapsing onto pyramidal cells, dentate granule cells, and inhibitory interneurons

(Frotscher & Leranth, 1985). Activation of the cholinergic SH projection excites pyramidal cells, which would be expected to have positive consequences for learning and memory (Chabot, Massicotte, Milot, Trudeau, & Gagne, 1997; Izquierdo & Medina, 1993; Teyler, 1987; see Figure 4.1A).

GABA<sub>A</sub> receptors are present on the cell bodies of both the GABAergic and the cholinergic SH neurons (Gao, Hornung, & Fritschy, 1995; Rouse & Levey, 1996; Van der Zee & Luiten, 1994); therefore, the memory-impairing effects of MS GABA receptor activation may involve either or both SH projections. Extensive evidence indicates that the cholinergic SH projection is involved in the memory-impairing effect of muscimol. For example, MS infusions of muscimol, at doses that impair spatial working memory, decrease hippocampal acetylcholine (ACh; Degroot, Kornecook, Quirion, DeBow, & Parent, 2003; Durkin, 1992a; Gorman, Pang, Frink, Givens, & Olton, 1994). Furthermore, only those doses of muscimol that decrease hippocampal ACh impair memory (Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990). Also, manipulations that increase hippocampal ACh levels reverse the memory deficits produced by MS GABA receptor activation (Degroot & Parent, 2000, 2001; Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997). Furthermore, MS GABA receptor activation increases the dose of ACh receptor agonists needed in the hippocampus to reverse memory deficits (Farr, Uezu, Flood, & Morley, 1999).

More recent evidence suggests the GABA SH projection may also be involved in memory. Ibotenic acid lesions, which primarily affect GABAergic SH neurons, impair place learning (Cahill & Baxter, 2001). Furthermore, the GABAergic SH projection is likely involved in the memory-impairing effects of MS GABA receptor activation because selective lesions of the cholinergic SH projection do not prevent the memory-impairing effects of muscimol (Pang,

Nocera, Secor, & Yoder, 2001a; Pang & Nocera, 1999). Moreover, electrophysiological studies show that MS administration of muscarine, a cholinergic receptor agonist that is expected to have memory-enhancing actions when infused into the MS (Givens & Olton, 1995; Givens & Olton, 1990), selectively excites the GABAergic SH projection, but not the cholinergic SH projection (Alreja et al., 2000; Wu, Shanabrough, Leranath, & Alreja, 2000). Similarly, the muscarinic receptor antagonist scopolamine, a drug that impairs memory when infused into the MS (Pang & Nocera, 1999), also selectively influences the GABAergic SH projection (Alreja et al., 2000).

The present set of experiments determined whether the memory-impairing effects of MS GABA receptor activation involve the GABAergic SH projection. Experiment 1 determined whether the memory-impairing effects of MS infusions of the GABA<sub>A</sub> receptor agonist muscimol would be prevented by concurrent hippocampal infusions of the GABA<sub>A</sub> receptor antagonist bicuculline. Experiment 2 determined whether combining infusions of a subeffective dose of the muscarinic antagonist scopolamine, a drug that selectively influences the GABAergic SH projection (Alreja et al., 2000) with muscimol would summate to produce memory deficits (see Figure 4.1B). Such a finding would suggest that muscimol and scopolamine are acting on a common mechanism (i.e., the GABAergic SH projection) to impair memory (Seeley & Moran, 2002). For both experiments, rats participated in two behavioral tasks that assess different types of memory that vary in motivational, temporal, and cognitive demands. This would allow us to assess whether the manipulations affect a process that influences memory rather than some process that influences performance on a memory task.



## Experiment 1

### Materials and Methods

This experiment tested whether the memory-impairing effects of MS GABA receptor activation would be prevented by blocking the activity of the GABAergic SH projection. Specifically, we determined whether hippocampal infusions of bicuculline, a GABA<sub>A</sub> receptor antagonist, would prevent memory deficits produced by concurrent MS infusions of muscimol. Hippocampal infusions of bicuculline are expected to inhibit the GABA<sub>A</sub> receptors on both the inhibitory interneurons and pyramidal cells. Consequently, combining hippocampal infusions of bicuculline with MS infusions of muscimol should inhibit the activity of the GABAergic SH projection (see Figure 4.1A).

### Subjects

Forty-three male Sprague-Dawley derived rats (n = 9-12 per group) weighing 200-250 g upon arrival (Charles River, Wilmington, MA) were used for the SA task. Thirty-six rats (n = 5-11 per group) were used for the continuous multiple trial inhibitory avoidance (CMIA) task. The rats were housed individually in polycarbonate cages (20x40x20 cm) with corncob bedding on a 12 hour light-dark cycle (lights on at 7:00 a.m.) in a temperature-controlled colony room (70-74°F). Food and water was available *ad libitum*. The Georgia State University Institutional Animal Care and Use Committee (IACUC) approved all procedures involving rats.

### Surgery

At least 1 week after their arrival, the rats were given injections of atropine sulfate (0.4 mg/kg, ip, Baxter, Deerfield, IL), anesthetized with sodium pentobarbital (Nembutal; 50 mg/kg, ip, Abbott Laboratories, Deerfield, IL), and then given an injection of penicillin (1500 units, im,

Crystiben). The incision site was shaved with a #50 electric clipper blade (Oster) and betadine solution was applied to the surgical area. The incision site was numbed with a 2% lidocaine/.001% epinephrine cocktail (0.5-2.0 cc, sc, Abbott Labs, Chicago, IL). After the incision, the 2% lidocaine/.001 % epinephrine cocktail solution (.05- 1.0 cc) was applied topically to the skull to facilitate seeing lambda and bregma. Stereotaxic surgical procedures (David Kopf Instruments, Tujunga, CA) were used to implant one 22-gauge stainless-steel guide cannula (Plastics One, Inc., Roanoke, VA) aimed at the MS (0.5 mm anterior [AP] to bregma, 4.9 mm ventral to dura [DV]) and one guide cannula aimed at the dorsal hippocampus (4.5 mm AP, 1.6 mm DV, and 4.0 mm from the interaural line; Paxinos & Watson, 1998). The hemisphere in which the unilateral hippocampal cannulae were implanted was counterbalanced across rats. The cannulae were secured to the skull with three jeweler's screws and cranioplastic cement (DuraLay, Worth IL) and a dummy cannula (Plastics One, Inc., Roanoke, VA) was inserted to keep the cannulae free of debris. Immediately after surgery, the rats were given an injection of 0.9 % sterile saline (3.0 cc, sc) and then wrapped with a paper towel and kept under a warm lamp until recovery from anesthesia. Two days following surgery, the patency of each cannula was checked and betadine was applied to the surgical wound. If signs of infection were evident, the rats were anesthetized with isoflurane gas (5 %; Baxter, Deerfield, IL) delivered in 1000 ml/min of oxygen and given an additional injection of penicillin (1500 units im, Crystiben).

### **Drug Preparation and Drug Infusions**

Two days prior to behavioral testing, the experimenter handled each rat for two minutes. Behavioral testing occurred at least 1 week after surgery and was conducted between 7:00 a.m and 7:00 p.m. Drug treatments were counterbalanced over the course of the day. Before and

after all handling and behavioral testing, the rats were allowed a minimum of 30 minutes to habituate to the laboratory environment. Fifteen minutes prior to behavioral testing, different groups of rats were given unilateral hippocampal infusions of vehicle (1  $\mu$ l, 0.5  $\mu$ l/min; phosphate-buffered saline [PBS], pH = 7.4) or bicuculline methiodide (0.3 nmol; Sigma). The dose of bicuculline was selected based on pilot experiments showing that it was the highest dose that, when infused alone, did not affect memory or cause apparent seizure activity. One minute after the hippocampal infusion was initiated; the rats were given a MS injection of vehicle (PBS; 0.5 $\mu$ l, 0.5 $\mu$ l/min) or muscimol (0.15 nmol: SA or 5 nmol: CMIA; Sigma). The doses of muscimol were selected based on findings showing that these doses decreased percent alternation scores and CMIA retention latencies without affecting activity measures or acquisition (Krebs & Parent, 2005a, 2005c). Bicuculline was prepared on the day of testing. Muscimol was prepared in 5 and 1 nmol/0.5  $\mu$ L concentrations and aliquoted (200  $\mu$ l) into microcentrifuge tubes and frozen (2-8  $^{\circ}$ C). On the day of the experiment, the muscimol was thawed and diluted with PBS (pH = 7.4) to generate the desired concentration. The drugs were infused through a 28-gauge injection needle that extended 1.2 mm (hippocampus) or 1.0 mm (MS) beyond the guide cannulae. The needle was connected to a 25  $\mu$ l Hamilton syringe by polyethylene tubing (PE-50), and the infusions were delivered using an infusion pump (Harvard Apparatus 11). Following the completion of both injections, the needle was left in place for one minute to facilitate drug diffusion.

### **Spontaneous Alternation (SA)**

Spontaneous alternation (SA) is assumed to be a hippocampal-dependent measure of spatial working memory (Deacon, Bannerman, Kirby, Croucher, & Rawlins, 2002; Dember, 1989; Lalonde, 2002; Richman, Dember, & Kim, 1987; Stevens & Cowey, 1973). The underlying assumption is that in order to alternate successfully between locations the rat must remember its visits to previous places. This assumption is supported by the finding that SA is impaired by removing directional cues or by increasing the interval between arm choices (Dember, 1989; Lalonde, 2002; Richman, Dember, & Kim, 1987). Fifteen minutes after the drug injections, SA performance was assessed by placing each rat in a Y-maze composed of three equally spaced arms (60°; 60 cm long x 17.5 cm high). The floor of each arm was composed of stainless steel (3.5 cm wide) and the top (14 cm wide) was covered with a colorless, transparent plexiglass lid. All rats were placed in the same starting arm of the Y-maze and allowed to explore the maze for 8 minutes. The experimenter, who was blind to drug treatment, recorded the sequence and number of arms the rats entered during the 8 min. period. The maze was cleaned with 70% ethanol after each rat. The number of arms each rat entered was used as a measure of activity. A percent alternation score was computed for all rats that entered at least 10 arms. An alternation was defined as entering three different arms consecutively. The percent alternation score was computed by dividing the number of alternations each rat made by the number of arms entered minus two (i.e., the number of alternations possible) and then multiplying that resulting quotient by 100.

### **Continuous Multiple Trial Inhibitory Avoidance (CMIA)**

A minimum of 3 days after performing in the SA task, rats were trained on the shock avoidance task. The drug infusions were counterbalanced across the two behavioral tasks. The avoidance apparatus consisted of a trough-shaped alley (91 cm long, 15 cm high, 20 cm wide at the top, and 6.4 cm wide at the bottom) that was divided into a lighted (31 cm long) and a dark (60 cm long) compartment by a retractable guillotine door. The dark compartment had a metal floor through which shock could be delivered. A 15-watt lamp was placed over the lighted compartment and was the only source of illumination in the room. The table underneath the avoidance apparatus was lined with bench paper and the apparatus was cleaned with 70% ethanol after each rat was trained or tested.

For the training, each rat was placed in the lighted compartment with its head facing away from the door. Once the rat turned around to face the door or after 12 s passed, the retractable door was opened and the rat was allowed to cross over to the dark (shock) compartment. After the rat crossed with all four paws, the rat was given a footshock (1.2 mA) until it returned to the lighted compartment (maximum 4 s). This sequence constituted one training trial. Training continued until the rat remained in the lighted compartment for 100 consecutive s or for a maximum of 5 trials. The rat was not removed from the avoidance apparatus between trials. The number of trials needed to reach the criterion was recorded and used as a measure of acquisition.

Retention of the training was tested 48 hrs (+/- 2 hrs) later. Each rat was placed in the lighted compartment of the avoidance chamber with its head facing away from the closed door. After the rat turned to face the door or 12 s passed, the door was opened and the latency (s) to cross over to the dark (shock) compartment was recorded and used as a measure of retention.

Each rat was given a maximum of 600 s to enter the dark compartment and foot-shock was not delivered.

## **Histology**

After behavioral testing, the rats were euthanized with an overdose of sodium pentobarbital (Sleepaway; 400 mg/kg, ip, Fort Dodge, IA) and perfused intracardially with 0.9% saline followed by 10% formalin. Their brains were stored in a 10% formalin solution for at least 2 days before sectioning. All brains were sectioned on a cryostat (Leica CM 30510 S) and 45-60  $\mu$ m sections were taken through the MS and hippocampal cannulae tracts. The brain sections were stained with thionin and an unbiased observer determined the cannulae placement using a light microscope (Olympus BX41). Acceptable medial MS cannulae placement was defined as MS injection sites located within the MS, but not within the lateral septum or the ventral diagonal band of Broca. Moreover, the cannula must not have penetrated the fimbria. Acceptable placement for hippocampal cannulae was defined as injection sites located within hippocampal fields CA1, CA2, CA3, or dentate gyrus. Only rats with acceptable cannulae placements in both brain regions were included in the statistical analyses.

## **Statistical Analysis**

The data were expressed as means and standard errors of the mean (S.E.M.) and analyzed using 2 (MS drug treatment) x 2 (hippocampal drug treatment) univariate analysis of variance (ANOVA) and Tukey post hoc tests where appropriate. The acquisition and retention latency data were not normally distributed due to the fact that several of the rats reached the maximum trials to criterion and 600 s retention latency cut-off. Consequently, these avoidance data were

expressed as medians and inter-quartile ranges (I.Q.) and the non-parametric Kruskal-Wallis and Mann-Whitney U tests were used to detect differences between treatment groups. An alpha level of 0.05 was used as the criterion for statistical significance.

## Results

Figure 4.2 illustrates the approximate locations of MS and hippocampal infusions. Figure 4.3A illustrates that drug infusions into the MS [ $F(1,42) = 5.611, p < .05$ ] and the hippocampus [ $F(1,42) = 9.900, p < .05$ ] significantly affected percent alternation scores, and the effects of drug infusions into both regions interacted significantly [ $F(1,42) = 4.542, p < .05$ ].

Hippocampal infusions of bicuculline alone did not affect percent alternation scores. Compared to the percent alternation scores of rats that were given infusions of vehicle in both brain regions (V-V), the percent alternation scores of rats given MS infusions of vehicle and hippocampal infusions of bicuculline (V-B) were not significantly different ( $p > .05$  vs. V-V). MS infusions of muscimol impaired memory. Specifically, compared to the percent alternation scores of rats that were given infusions of vehicle in both brain regions (V-V), the percent alternation scores of rats given MS infusions of muscimol and hippocampal infusions of vehicle (M-V) were significantly decreased ( $p < .05$  vs. V-V). More importantly, hippocampal infusions of bicuculline prevented the alternation deficits in rats given MS infusions of muscimol (M-B). Specifically M-B rats had percent alternation scores that were not significantly different from V-V rats ( $p > .05$  vs. V-V), but were significantly higher than M-V rats ( $p < .05$  vs. M-V). Drug infusions into the MS [ $F(1,42) = .542, p > .05$ ] and the hippocampus [ $F(1,42) = 1.152, p > .05$ ] did not significantly affect the number of arms entered during SA, and the effect of drug

infusions into both regions on arm entries did not interact significantly ( $[F(1,42) = .113, p > .05]$ ; see Figure 4.3B).

Drug infusions into the MS and hippocampus [ $\chi^2(3,36) = 6.45, p > .05$ ] did not significantly affect trials to criterion during CMIA training (see Figure 4.4A). Figure 4.4B illustrates that the pre-training drug infusions into the MS and hippocampus [ $\chi^2(3,36) = 11.74, p < .01$ ] significantly affected subsequent CMIA retention performance. Consistent with previous research, MS infusions of muscimol impaired CMIA retention. Specifically, M-V rats had significantly shorter retention latencies than did V-V rats [ $U(1,21) = 8; p < .01$ ]. Hippocampal infusions of bicuculline alone did not affect CMIA retention. The retention latencies of V-B rats were not significantly different from those of V-V rats [ $U(1,16) = 26, p > .05$ ]. More importantly, hippocampal infusions of bicuculline attenuated the CMIA retention deficits produced by muscimol. M-B rats did not have significantly shorter retention latencies than did V-V rats [ $U(1,21) = 36.50; p > .05$ ], but did not have significantly longer retention latencies than did M-V rats [ $U(1,20) = 39.50; p > .05$ ].

## Discussion

These results replicate the finding that MS infusions of the GABA receptor agonist muscimol impair performance in both SA and CMIA tasks (Chrobak, Stackman, & Walsh, 1989; Durkin, 1992a; Krebs & Parent, 2005a, 2005c; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997). More importantly, the findings show that these memory deficits are reversed (SA) or attenuated (CMIA) by hippocampal infusions of the GABA antagonist bicuculline. These findings indicate that the memory-impairing effects of MS GABA receptor activation involve, at least in part, the GABAergic SH projection.



## Experiment 2

The goal of this experiment was to provide converging evidence to support the hypothesis that GABA receptor activation impairs memory, at least in part, via the GABAergic SH projection. MS infusions of the muscarinic receptor antagonist scopolamine impair memory (Elvander et al., 2004; Givens & Olton, 1995; Gorman, Pang, Frink, Givens, & Olton, 1994; Pang & Nocera, 1999). Recent findings show that scopolamine in the MS influences the activity of the GABAergic, but not the cholinergic SH projection (Alreja et al., 2000). When two drugs act via a common mechanism such as the GABAergic SH projection to impair memory, then the effects of those two drugs should summate (Seeley & Moran, 2002). We reasoned, therefore, that if memory-impairing effects of MS GABA receptor activation involve an influence on the GABAergic SH projection, then co-infusions of muscimol and scopolamine, at doses that have no effect on memory alone, should produce memory deficits. The goal of Experiment 2 was to test this hypothesis.

The same procedures were used as in Experiment 1, with the following exceptions. Rats were given MS infusions of vehicle (PBS; 0.5  $\mu$ L, 0.5  $\mu$ L/min), muscimol (0.10 nmol: SA or 1 nmol: CMIA), scopolamine (2.5  $\mu$ g: SA or 40  $\mu$ g: CMIA) or muscimol with scopolamine combined in the same solution 15 min prior to assessing SA or training in CMIA. The drugs that were combined in the same solution were prepared at double the desired concentration and then combined reducing the concentration of each by half. The doses of muscimol and scopolamine were selected based on our pilot data showing that these were the maximum doses that did not significantly impair memory when infused alone (Krebs & Parent, unpublished findings). Sixty-three rats (14-16 per group) were used for the SA task and 55 rats (12-19 per group) were used for the CMIA task.

## Results

The locations of the MS infusions were similar to those presented in Figure 4.2 from Experiment 1. Drug infusions into the MS [ $F(3, 62) = 4.26$ ;  $p < .01$ ] significantly affected SA performance (see Figure 4.5A). MS infusions of muscimol or scopolamine alone did not impair SA performance. Specifically, the percent alternation scores of rats given MS infusions of muscimol or scopolamine alone were not significantly different from those of rats given infusions of vehicle ( $p > .05$ ). Interestingly, the findings showed that MS co-infusions of muscimol with scopolamine significantly impaired SA performance. Specifically, the percent alternation scores of rats given muscimol and scopolamine in the same solution were significantly lower than of those of rats given MS infusions of vehicle ( $p < .05$ ). Drug infusions into the MS [ $F(3, 62) = 2.95$ ;  $p < .05$ ] significantly affected the number of arms that the rat entered in the maze (see Figure 4.5B). The data showed that the MS infusions of scopolamine alone decreased the number of arms the rats entered in the maze. Specifically, the rats given MS infusions of scopolamine alone entered fewer arms than did rats given infusions of vehicle ( $p < .05$ ).

MS drug infusions [ $\chi^2(3, 57) = 2.04$ ;  $p > .05$ ] did not significantly affect trials to criterion during CMIA training (see Figure 4.6A). Figure 4.6B illustrates that the pre-training drug infusions into the MS significantly affected subsequent CMIA retention performance tested 48 hrs later [ $\chi^2(3, 57) = 7.92$ ;  $p < .05$ ]. MS infusions of muscimol or scopolamine did not significantly impair CMIA retention. Specifically, the retention latency scores of rats that were given MS infusions of muscimol [ $U(1, 30) = 98.5$ ;  $p > .05$ ] or scopolamine [ $U(1, 33) = 97.5$ ;  $p > .05$ ] alone were not different from those of rats that were given MS infusions of vehicle. Importantly, MS co-infusions of muscimol with scopolamine impaired avoidance retention. The

retention latencies of rats that were given MS co-infusions of muscimol with scopolamine were significantly lower from those of rats that were given MS infusions of vehicle [ $U(1,30) = 51$ ;  $p < .01$ ].

## Discussion

The present findings showed that MS infusions of scopolamine with muscimol, at doses that did not impair memory when infused alone, produced significant memory deficits when infused together. These findings are consistent with previous research showing that MS infusions of GABA agonists or ACh antagonists alone produce memory deficits (Chrobak, Stackman, & Walsh, 1989; Durkin, 1992b; Krebs & Parent, 2005a, 2005c; Pang & Nocera, 1999). These data also showed that MS infusions of scopolamine decreased the activity of rats while they were performing on the memory task. This finding is consistent with previous research showing that systemic infusions of scopolamine decrease locomotor activity in the radial arm maze (Masuoka, Fujii, & Kamei, 2006). The scopolamine-induced decrease in the number of arms entered doesn't likely impact the interpretation of the alternation data, because rats that were given MS infusions of scopolamine did not have impaired alternation scores. Moreover, MS co-infusions of scopolamine with muscimol affected alternation scores without affecting the mean number of arm entries. The additive interaction between scopolamine and muscimol supports the hypothesis that MS GABA receptor activation impairs memory via an influence on the GABAergic SH projection.

## General Discussion

The present results show that unilateral hippocampal infusions of bicuculline prevent the memory-impairing effects of MS GABA receptor activation in two memory tasks. These

findings are consistent with previous research suggesting that MS GABA receptor activation impairs memory via an influence on the hippocampus (Allen & Crawford, 1984; Degroot, Kornecook, Quirion, DeBow, & Parent, 2003; Degroot & Parent, 2000, 2001; Durkin, 1992a; Krebs & Parent, 2005a, 2005c; Parent, Laurey, Wilkniss, & Gold, 1997). These data are also congruent with previous findings showing that unilateral hippocampal infusions are sufficient to reverse the memory-impairing effects of MS GABA receptor activation (Degroot & Parent, 2000, 2001; Krebs & Parent, 2005a, 2005c; Parent, Laurey, Wilkniss, & Gold, 1997). These data extend these previous findings by showing that MS infusions of muscimol impair memory through a process that involves, at least in part, the GABAergic SH projection. The present findings also show that MS co-infusions of sub-effective doses of scopolamine, a drug that selectively influences the GABAergic SH projection (Alreja et al., 2000), with muscimol impair SA and avoidance retention. These latter findings provide converging evidence to support the hypothesis that the memory-impairing effects of MS infusions of muscimol involve the GABAergic SH projection. This interpretation is consistent with evidence showing that selective lesions of the cholinergic SH projection do not prevent the memory-impairing effects of MS GABA receptor activation (Pang & Nocera, 1999). Rather, these lesions reduce the dose of muscimol needed to produce a memory deficit when infused into the medial MS (Pang & Nocera, 1999), suggesting MS GABA receptor activation impairs memory via a process that involves the GABA SH projection. Combined with previous electrophysiological findings (Alreja et al., 2000; Wu, Shanabrough, Leranth, & Alreja, 2000), the present findings show that MS manipulations that enhance or impair memory likely do so via a process that involves the GABAergic SH projection.

The possibility remains that the memory-impairing effects of MS infusions of muscimol also involve the cholinergic SH projection. MS infusions of muscimol decrease a variety of markers of hippocampal ACh (Allen & Crawford, 1984; Moor, Schirm, Jasco, & Westerink, 1998; Walsh, Stackman, Emerich, & Taylor, 1993). More importantly, memory-impairing doses of muscimol decrease hippocampal ACh (Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990; Degroot, Kornecook, Quirion, DeBow, & Parent, 2003; Durkin, 1992a) and only those doses of muscimol that impair memory decrease hippocampal ACh function (Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990). Furthermore, previous results suggest that hippocampal infusions of AChE inhibitors or drugs that enhance hippocampal ACh levels (Ragozzino, Pal, Unick, Stefani, & Gold, 1998b) reverse memory deficits produced by MS GABA receptor activation (Degroot & Parent, 2000, 2001). Thus, collectively the evidence suggests that both projections are involved the deficits produced by MS GABA receptor activation.

The hippocampal infusions of bicuculline may not have completely reversed the deficits in the CMIA task because a larger dose of muscimol was infused for the CMIA task than for the SA task. The dose of bicuculline infused into the hippocampus for the CMIA task was not increased to account for the higher dose of muscimol because infusing a larger dose would increase the probability of memory-impairing seizure activity (Hu, Karnup, Zhou, & Stelzer, 2005). The finding that bicuculline prevented the memory deficits in the SA but not the CMIA task raises the possibility that the cholinergic SH projection is involved in the CMIA task because hippocampal infusions of bicuculline would not be expected to reverse deficits produced by the cholinergic SH projection. This possibility is unlikely because previous research has shown that the same selective lesions of the cholinergic projection that impair spatial working

memory do not impair shock avoidance (Freo, Pizzolato, Dam, Ori, & Battistin, 2002; LeBlanc et al., 1999). Furthermore, previous findings show that lesions of the cholinergic projection do not impair shock avoidance (Bannon, Curzon, Gunther, & Decker, 1996; Ragozzino, Wenk, & Gold, 1994).

To more definitively determine whether the GABAergic SH projection is required for the memory-impairing effects of MS GABA receptor activation, it would be necessary to directly lesion the GABAergic SH projections and then administer muscimol into the MS. Recent findings show that MS administration of kainic acid may be one way to lesion the GABAergic SH projection. MS kainic acid lesions decrease the number of MS glutamate decarboxylase and parvalbumin immunoreactive cells (Pang, Nocera, Secor, & Yoder, 2001b). Parvalbumin immunoreactive cells in the MS are assumed to reflect GABA SH projection neurons (Gao, Hornung, & Fritschy, 1995; Pang, Nocera, Secor, & Yoder, 2001b; Toth, Borhegyi, & Freund, 1993). It is not clear, though, if the kainic acid lesions also destroy MS GABA interneurons. MS kainic acid-induced lesions do not impair spatial working memory, and 192 IgG-induced lesions of the cholinergic SH projections only produce mild deficits (Pang, Nocera, Secor, & Yoder, 2001a; Pang, Nocera, Secor, & Yoder, 2001b). In contrast, combined lesions of the cholinergic and GABAergic SH projections severely impair spatial working memory (Pang, Nocera, Secor, & Yoder, 2001a; Pang, Nocera, Secor, & Yoder, 2001b). This suggests that the activity of either projection alone is sufficient but not necessary to influence memory. It is important to note that the interpretation of lesion studies is always limited by the possibility that the lesions are incomplete and can induce compensatory changes. For example, recent evidence suggests that 192 IgG-induced lesions of the cholinergic SH projection do not completely deplete

hippocampal extracellular ACh levels, moreover, there is an upregulation of the residual ACh functioning (Chang & Gold, 2004).

The motivational, temporal, and cognitive differences between the SA and CMIA tasks support the hypothesis that the drug infusions are influencing memory rather than some other process such as attention or motivation that could influence performance in the memory task. The finding that MS GABA receptor activation impairs memory in both SA and CMIA indicate that the SH system is involved in several mnemonic processes. Specifically, the findings from the SA task suggest that the SH system is involved in on-line spatial associations, and the findings from the CMIA task reveal that the SH system also affects emotional and long-term memory. The fact that the pretraining infusions of muscimol did not affect acquisition in the CMIA task suggests further that MS GABA receptor activation influences consolidation of newly formed emotional memories.

The present findings also show that higher doses of MS infusions of muscimol are needed to impair avoidance retention than SA. This is consistent with previous evidence investigating the effects of MS infusions of muscimol on different behavioral measures of memory (Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990; Krebs & Parent, 2005a, 2005c; Nagahara & McGaugh, 1992; Parent, Laury, Wilkniss, & Gold, 1997; Parent & Gold, 1997). Collectively, this research shows that higher doses of muscimol are needed in the MS to impair shock avoidance than are needed to produce deficits in a rewarded alternation, SA, or spatial water maze task. It is not clear why higher doses are needed to impair shock avoidance. It is possible that the higher doses of muscimol may influence avoidance via non-GABAergic mechanisms or through diffusion to other brain regions. The finding that lower doses of muscimol are effective in impairing avoidance retention when they are co-infused with glucose (Parent, Laury,

Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004) or scopolamine (present findings) suggests that this possibility is unlikely. The task-dependent effects of muscimol do not appear to be related to the stress level of the task, because both water maze and shock avoidance produce stress responses (De Boer, Van der Gugten, & Slangen, 1990; Mabry, Gold, & McCarty, 1995; Van der Borght, Meerlo, Luiten, Eggen, & Van der Zee, 2005). The differences also do not appear to be related to the presence of shock, because MS infusions of low doses of muscimol impair shock avoidance in a shock-probe burying test (Degroot & Treit, 2003). Thus, these findings suggest that more MS GABA receptors or more prolonged MS receptor activation is required to impair avoidance memory than other types of memory. Additional research is needed to determine the factors that contribute to these task-dependent, dose-response differences.

In summary, intra-hippocampal infusions of a GABA antagonist prevent the memory-impairing effects of MS GABA receptor activation. Furthermore, MS co-infusions of scopolamine with muscimol act synergistically to impair memory. These effects were observed in two different memory tasks. Collectively, these findings support the hypothesis that the effects of MS GABA receptor activation on memory are mediated through a process involving the GABAergic SH projection. These studies are important because they constitute the first behavioral evidence indicating the GABAergic SH projection is important for both spatial working memory and emotional, long-term memory.

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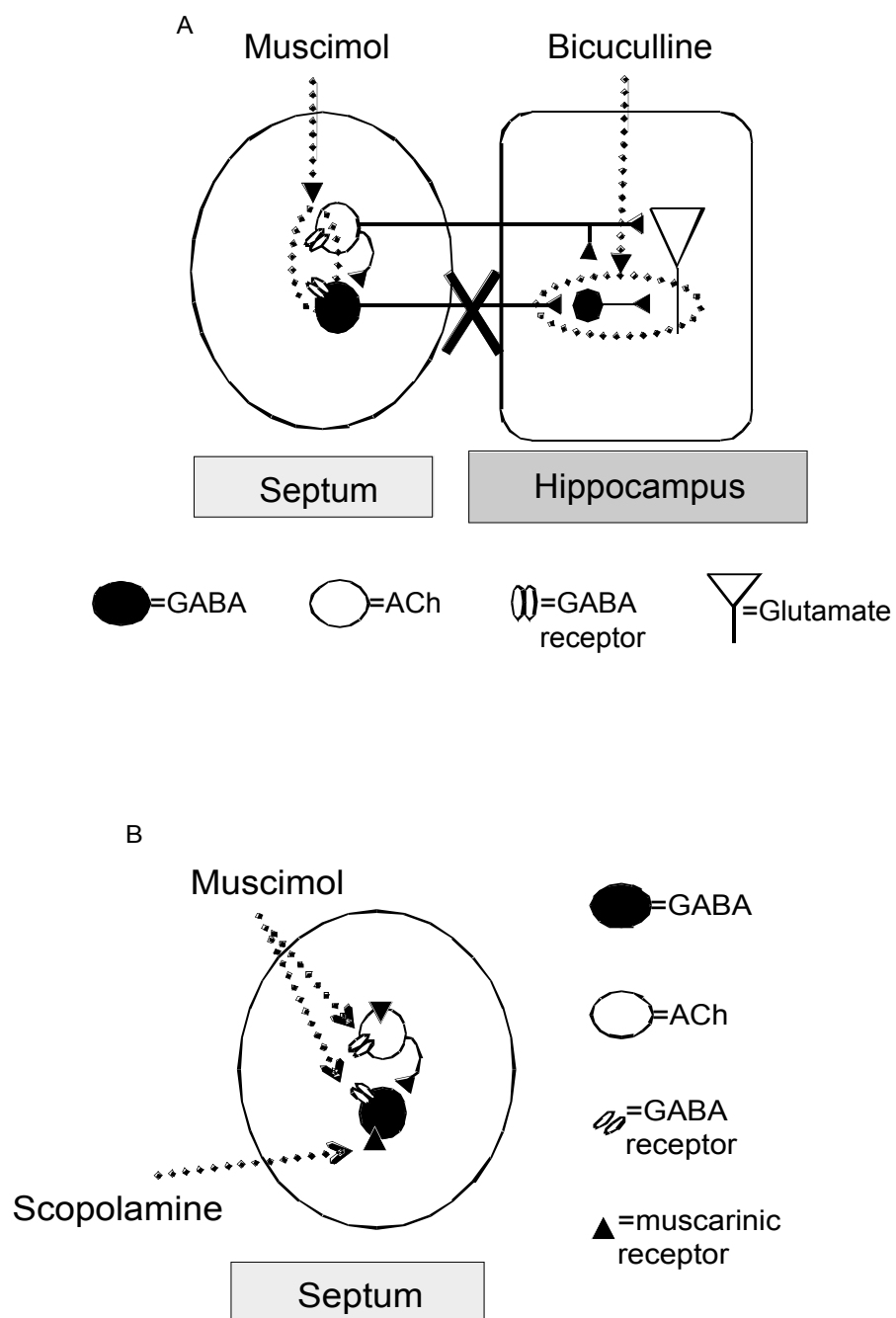


Figure 4.1. (A). Schematic illustration of the septo-hippocampal system. Muscimol infused into the medial MS binds to receptors present on both cholinergic and GABAergic SH projections (Gao, Hornung, & Fritschy, 1995) and inhibits the activity of both projections (Alreja, personal communication). Importantly, in this concurrent infusion paradigm, the hippocampal infusions of the GABA antagonist are blocking the contributions of the GABAergic SH projection. We assume that the hippocampal bicuculline infusions are not preventing the effects of the ACh collaterals onto the GABAergic SH projections, because presumably these ACh collaterals are already inhibited by the MS GABA receptor activation. As a result, if hippocampal infusions of bicuculline reverse the deficits produced by MS infusions of muscimol then this will suggest that the impairing effects of muscimol involve the GABAergic SH projection. (B). Schematic illustration of the MS. Muscarinic receptors are present on the cell bodies of both ACh and GABA projections (Van der Zee & Luiten, 1994). MS administration of scopolamine only inhibits the activity of the GABAergic SH projection (Alreja et al., 2000). If MS co-infusions of muscimol with scopolamine impair memory, then these results would also suggest that the impairing affects of muscimol involve the GABAergic SH projection.

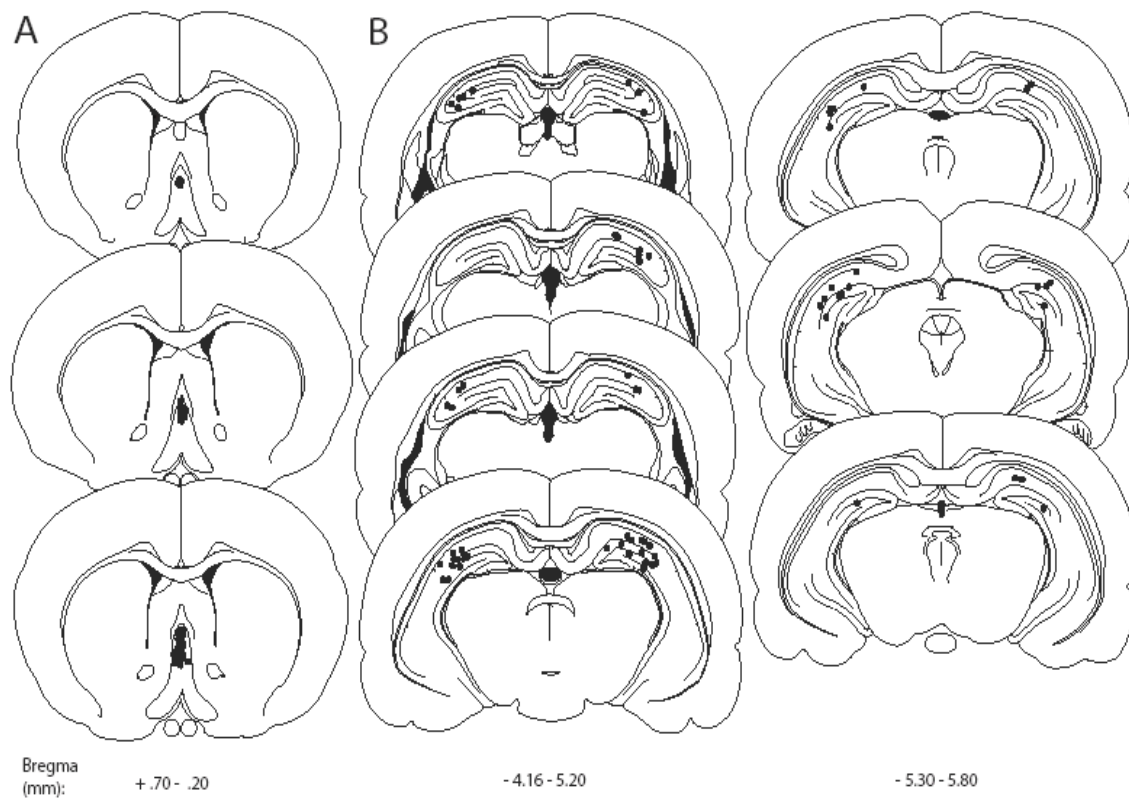


Figure 4.2. Schematic illustration of coronal sections of the rat brain showing the approximate location of (A) medial MS and (B) hippocampal infusion sites in Experiment 1. Atlas plates were adapted from Paxinos and Watson (1986).

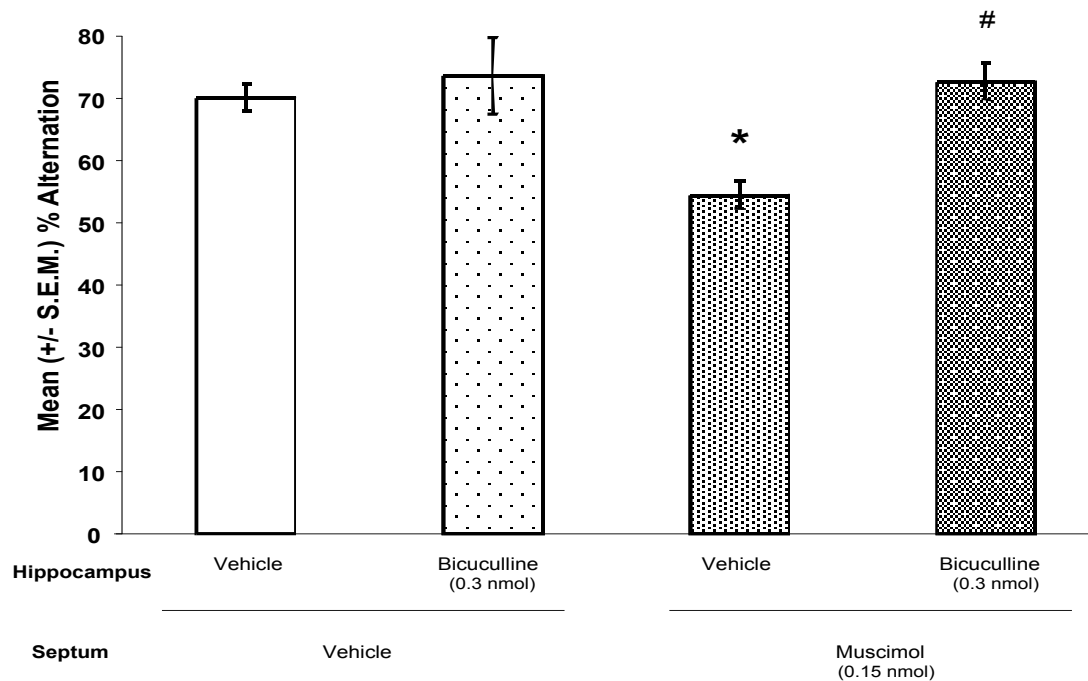


Figure 4.3A. MS infusions of muscimol decreased mean ( $\pm$  S.E.M.) percent alternation scores ( $*p < .05$  vs. V-V). Hippocampal infusions of bicuculline, at a dose that did not affect percent alternation scores when infused alone, reversed the deficits produced by muscimol ( $\#p < .05$  vs. M-V,  $p > .05$  vs. V-V).

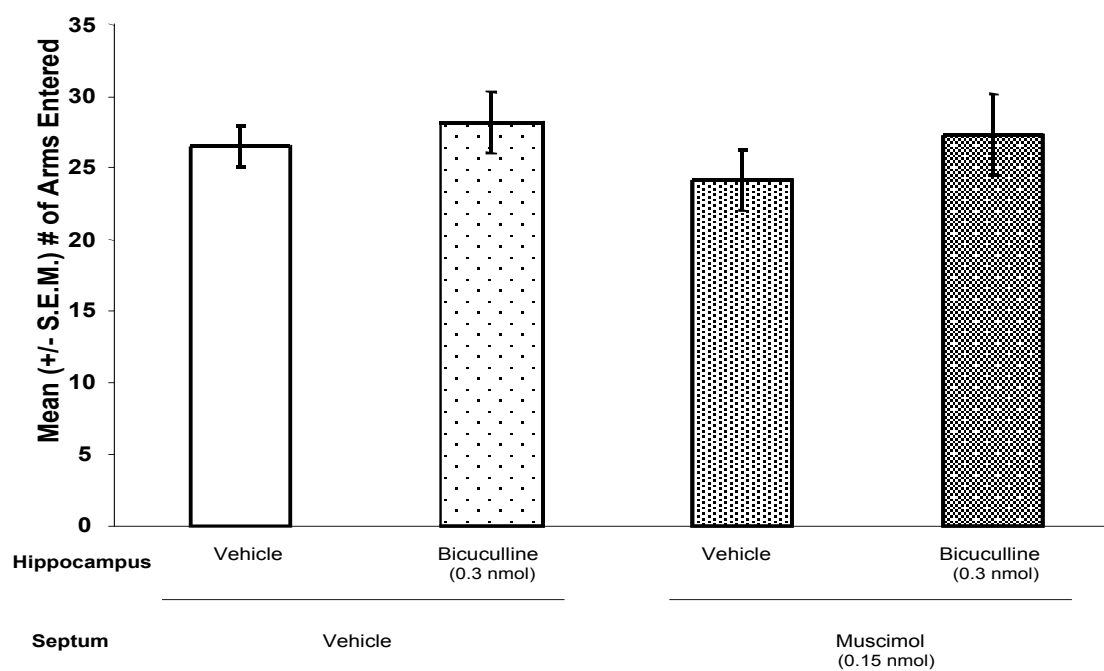


Figure 4.3B. There were no significant effects of any of the manipulations on the mean ( $\pm$  S.E.M.) number of arm entries.

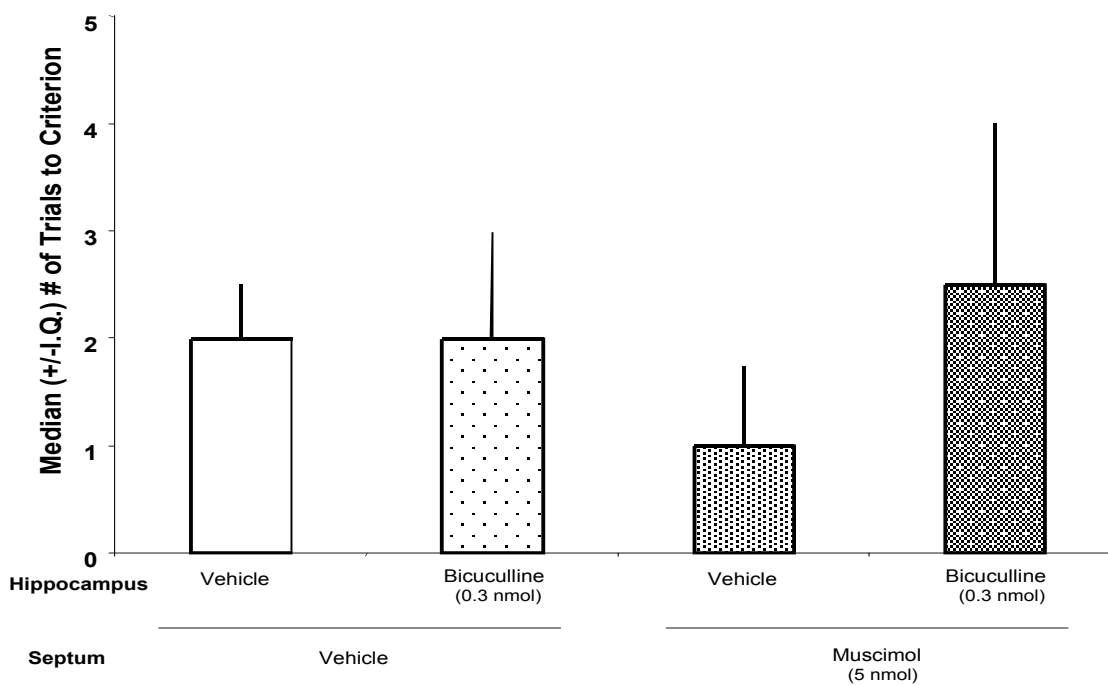


Figure 4.4A. There were no significant effects of any of the manipulations on the median (+/- I.Q.) trials to criterion during CMIA training ( $p > .05$  vs. V-V).



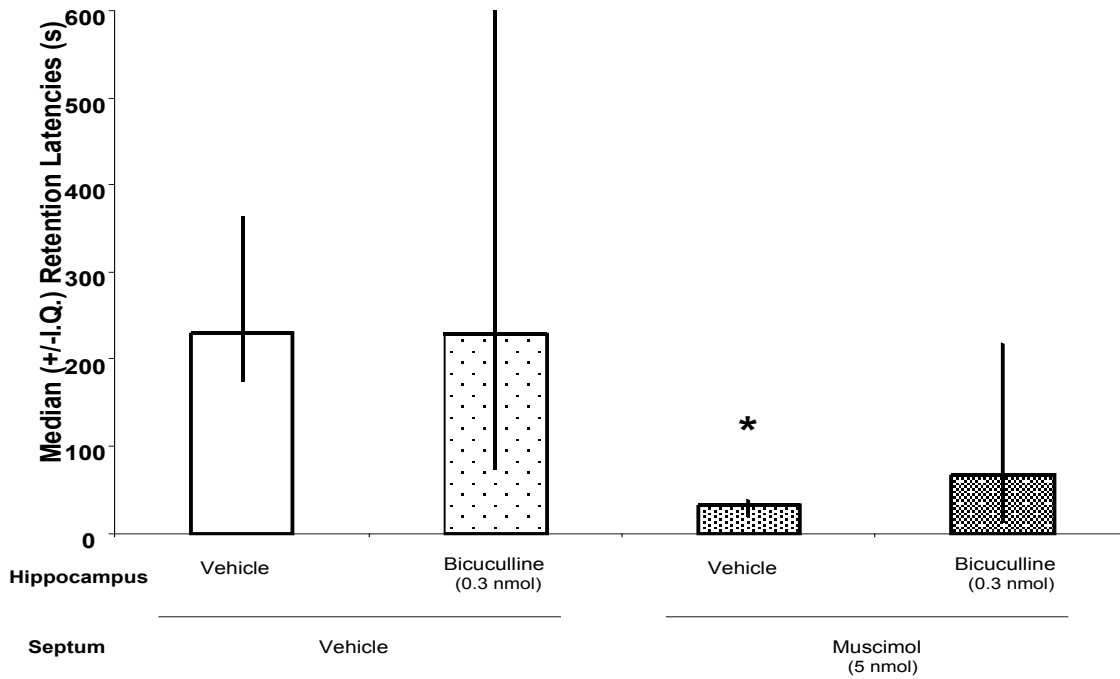


Figure 4.4B. Pretraining MS infusions of muscimol decreased the median (+/- I.Q.) retention latencies tested 48 hr later (\* $p < .05$  vs. V-V). Hippocampal infusions of bicuculline, at a dose that did not affect retention latencies when infused alone, attenuated the deficits produced by muscimol ( $p > .05$  vs. M-V or V-V).

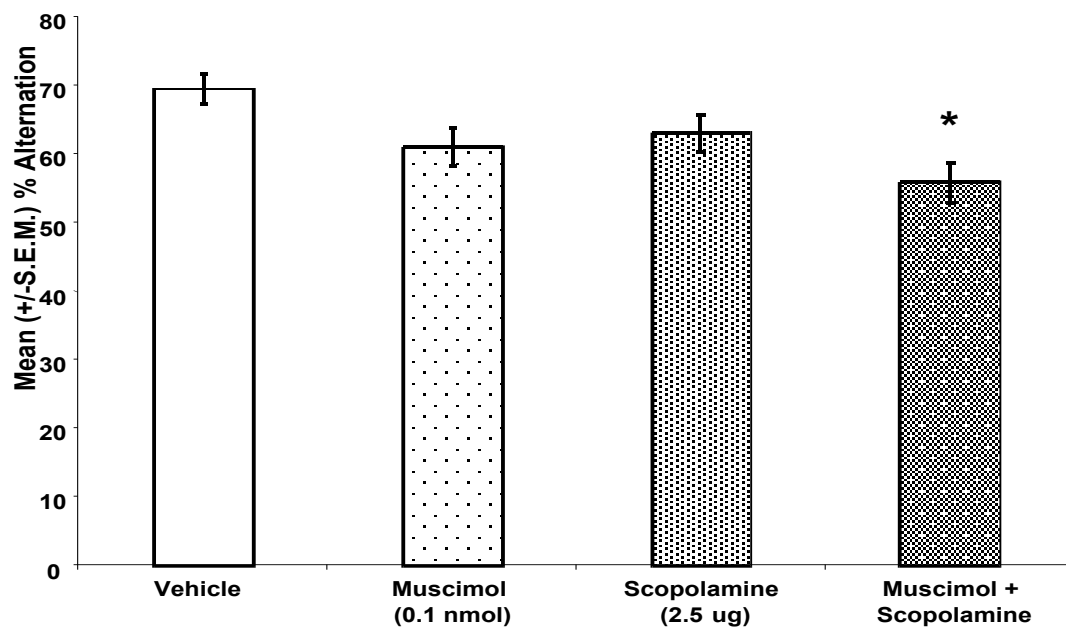


Figure 4.5A. MS co-infusions of muscimol with scopolamine, at doses that had no effect on memory alone, significantly decreased mean ( $\pm$  S.E.M.) percent alternation scores (\*  $p < .05$  vs. vehicle rats).

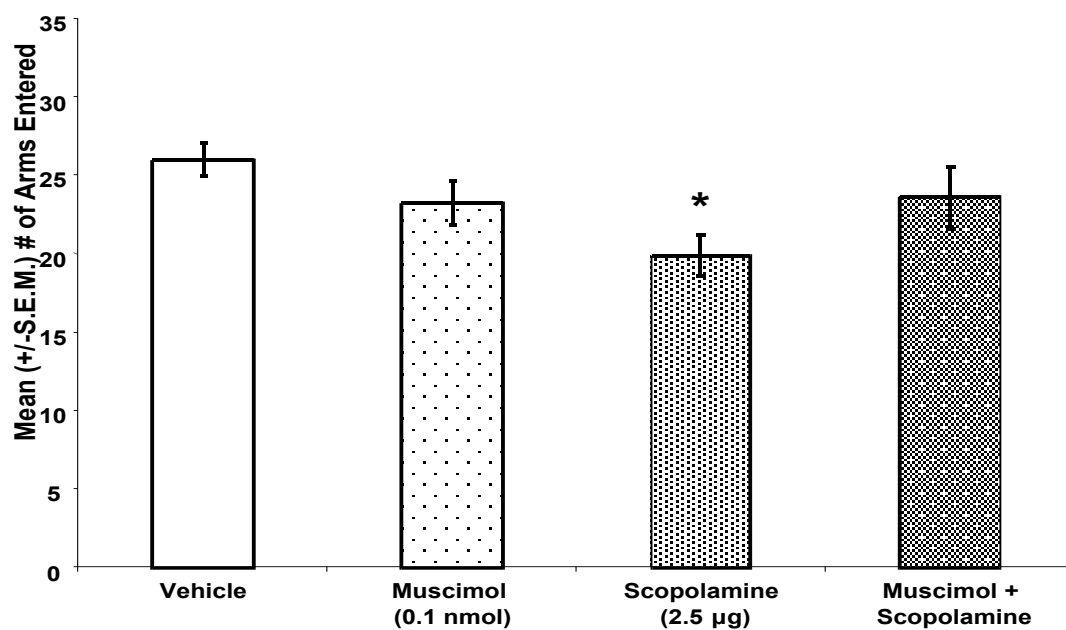


Figure 4.5B. MS infusions of scopolamine significantly decreased the mean (+/- S.E.M.) number of arms rats entered in the maze ( $p < .05$  vs. vehicle rats).

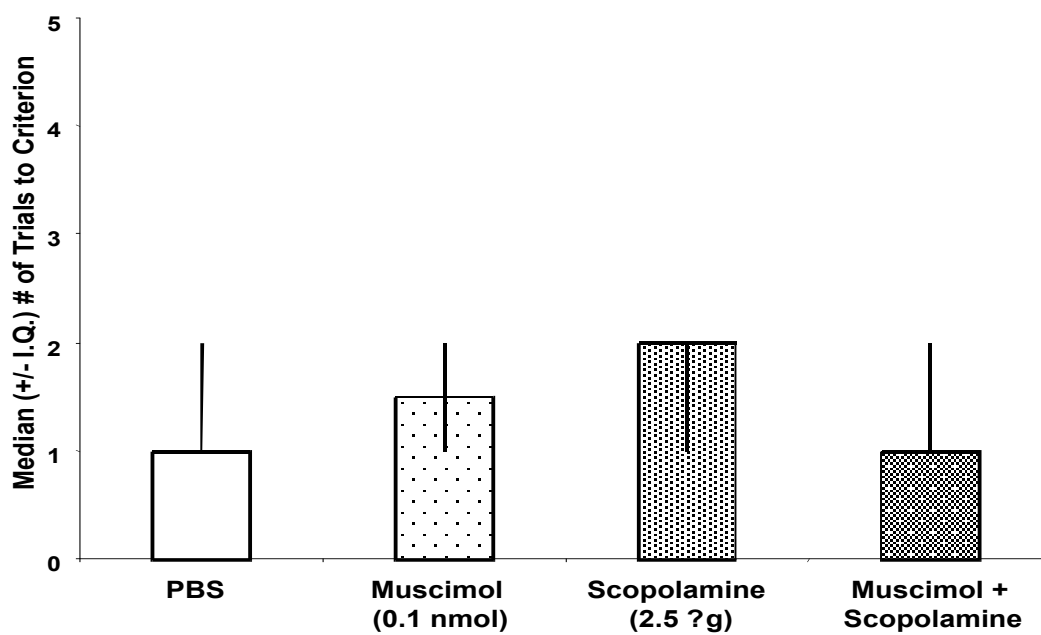


Figure 4.6A. There were no significant effects of MS infusions on median (+/- I.Q.) number of trials to criterion (\* $p > .05$  vs. vehicle rats).

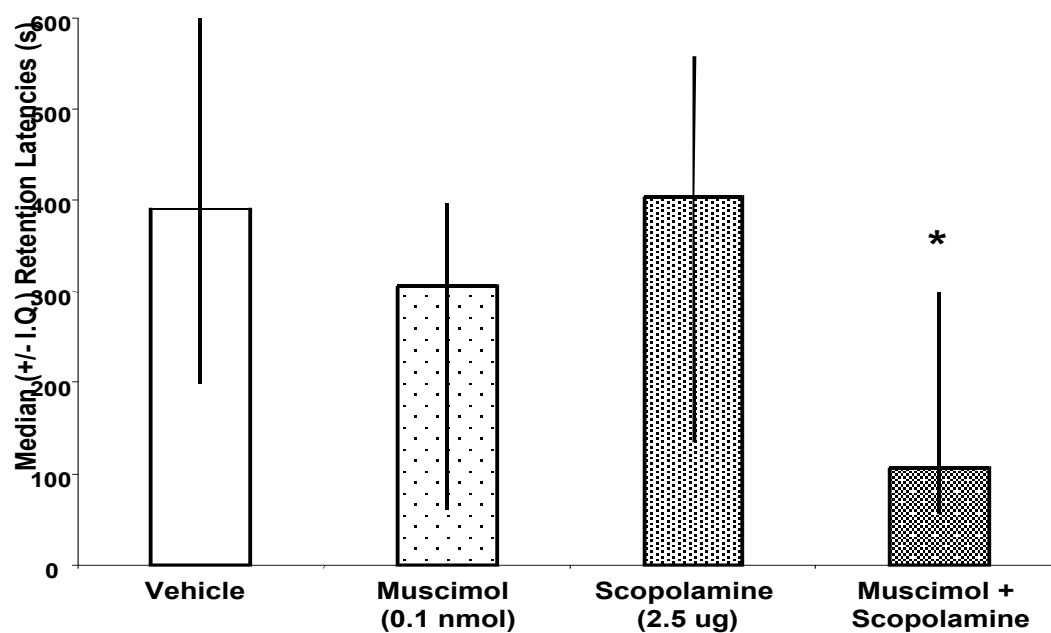


Figure 4.6B. MS co-infusions of muscimol with scopolamine, at doses that had no effect on memory when infused alone, significantly decreased median (+/- I.Q.) retention latencies (\*  $p < .05$  vs vehicle rats).

**CHAPTER 5**

**THE MEMORY-ENHANCING EFFECTS OF HIPPOCAMPAL INFUSIONS OF  
GLUCOSE OVERRIDE THE MEMORY-IMPAIRING EFFECTS OF SEPTAL  
GLUCOSE INFUSIONS**

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### Abstract

Although glucose typically has positive effects on memory, there are instances where it is deleterious. For instance, septal co-infusions of glucose with  $\gamma$ -aminobutyric acid (GABA) receptor agonists, such as muscimol impair memory. We hypothesize that the memory-impairing effects of septal co-infusions of glucose with muscimol involve the hippocampus. If this is the case, then infusions of glucose into the hippocampus, which enhance hippocampal function, should prevent the memory-impairing effects of septal infusions of glucose. As a result, the present experiment determined whether hippocampal infusions of glucose, which enhance hippocampal function, would reverse the deficits produced by septal infusions of muscimol with glucose. Fifteen min prior to assessing spontaneous alternation (SA) or training in a continuous multiple trial inhibitory avoidance (CMIA) task, male Sprague-Dawley-derived rats were given septal infusions of vehicle (phosphate-buffered saline [PBS]), glucose (33 nmol), muscimol (0.10 nmol; SA or 3 nmol; CMIA), or a cocktail composed of muscimol with glucose, combined with concurrent hippocampal infusions of vehicle (PBS) or glucose (50nmol). The results showed that the septal co-infusions of muscimol with glucose, at doses that had no effect on their own, decreased SA scores and CMIA retention latencies. More importantly, hippocampal infusions of glucose reversed these deficits. These findings show for the first time that the memory-enhancing effects of hippocampal glucose override the memory-impairing effects of MS glucose, suggesting that the memory-impairing effects of septal glucose involve an influence on the hippocampus. Moreover, these findings indicate that the mnemonic effects of glucose are brain region-dependent.

**Key Words:** Muscimol, GABA, Glucose, Spontaneous Alternation, Shock Avoidance, Hippocampus, Septum, Memory



## Introduction

Brain glucose levels increase in response to meals, drugs, stress, exercise and most commonly diabetes (Bequet et al., 2000; Canal, McNay, & Gold, 2005; Fellows, Boutelle, & Fillenz, 1993; Jacob, Fan, Evans, Dziura, & Sherwin, 2002; Silver & Erecinska, 1994). Extensive evidence suggests that glucose is involved in the regulation of memory processes (Benton & Nabb, 2003; Corey-Bloom, 2002; Korol & Gold, 1998; Messier, 2004; Messier & Gagnon, 2000). More specifically, glucose has both positive and negative effects on memory. For example, glucose enhances memory (Lee, Graham, & Gold, 1988; Stefani, Nicholson, & Gold, 1999; Sunram-Lea, Foster, Durlach, & Perez, 2002) and reverses the memory deficits and cognitive dysfunction that are associated with Alzheimer's disease, aging, Down's syndrome, and schizophrenia (Craft, Murphy, & Wemstrom, 1994; Gold, 1995; Korol, 2002; Korol & Gold, 1998; Manning, Ragozzino, & Gold, 1993; Newcomer et al., 1999; Petit, 1988). In contrast, acute and chronic hyperglycemia are associated with memory dysfunction (Biessels et al., 1996; Convit, 2005; Elias et al., 1997; Flood, Mooradian, & Morley, 1990; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; Hagemann, Sartory, Hader, & Kobberling, 2005; McCall, 2002; Popovic, Biessels, Isaacson, & Gispen, 2001; Winocur et al., 2005). The mechanisms underlying the memory-modulating effects of glucose, particularly the conditions that differentiate the enhancing and impairing effects of glucose, are poorly understood.

The effects of glucose on memory are mediated, at least in part, by the brain. Glucose readily crosses the blood-brain barrier via glucose transporters (Pardridge, Boado, & Farrell, 1990; Rahner-Welsch, Vogel, & Kuschinsky, 1995). Interestingly, extracellular levels of hippocampal glucose decrease with cognitive demand, and systemic infusions of glucose both enhance memory and replenish these decreases in hippocampal glucose (McNay & Gold, 1999).

These data suggest that during tasks that engage the hippocampus, peripheral glucose is preferentially directed to the hippocampus and enhances memory formation. Moreover, direct brain infusions of glucose affect memory. For example, infusions of glucose into the cerebral ventricular system enhance retention performance on a one-trial inhibitory avoidance task in rodents (Lee, Graham, & Gold, 1988). Direct injections of glucose into specific brain regions, including the medial septum (MS) or hippocampus, enhance spontaneous alternation (SA) performance (Ragozzino & Gold, 1995b; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Stefani & Gold, 1998; Stefani, Nicholson, & Gold, 1999), a measure of spatial working memory (Lalonde, 2002; Richman, Dember, & Kim, 1987). MS or hippocampal infusions of glucose also reverse drug-induced deficits in SA (Ragozzino & Gold, 1995b). MS infusions of glucose potentiate memory deficits produced by MS  $\gamma$ -aminobutyric acid (GABA) receptor activation (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997), suggesting that the memory-impairing effects of glucose are also mediated, at least in part, in the brain.

Both the memory-enhancing and memory-impairing effects of glucose appear to involve the hippocampus. For instance, glucose enhances memory and reverses hippocampal-dependent memory deficits (Craft, Murphy, & Wemstrom, 1994; Gold, 1995; Korol, 2002; Korol & Gold, 1998; Krebs & Parent, 2005a; Manning, Ragozzino, & Gold, 1993; McNay, Fries, & Gold, 2000; Messier, 2004; Newcomer et al., 1999; Parent, Laurey, Wilkniss, & Gold, 1997; Stefani & Gold, 2001b; Winocur & Gagnon, 1998). Furthermore, direct hippocampal infusions of glucose reverse memory deficits (Convit, Wolf, C, & de Leon, 2003; Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Stefani & Gold, 2001b). In contrast, the elevated glucose levels associated with diabetes are correlated with hippocampal-dependent memory deficits (Mooradian, 1988; Ryan & Geckle, 2000) and

increased rates of brain atrophy (Perros, Deary, Sellar, Best, & Frier, 1997). Diabetes impairs the function of the hippocampus (Stewart & Liolitsa, 1999; Weir, Murray, Dyker, & Lees, 1997). For example, experimentally-induced diabetes impairs memory and hippocampal synaptic plasticity (Biessels, Bravenboer, & Gispen, 2004; Biessels et al., 1996; Gispen & Biessels, 2000; Kamal, Biessels, Urban, & Gispen, 1999). The memory deficits associated with diabetes are more commonly found in older adults, and several levels of evidence suggest that this involves an interaction with the impairing effects of age on hippocampal function (Ryan & Geckle, 2000). Finally, impaired glucose tolerance is associated with both memory deficits and hippocampal atrophy (Convit, Wolf, C, & de Leon, 2003).

Under certain conditions, MS infusions of glucose impair memory. Specifically, MS infusions of glucose potentiate the memory-impairing effects of GABA<sub>A</sub> agonists (Parent & Gold, 1997). In addition, MS co-infusions of glucose with the GABA agonist muscimol, the GABA<sub>A</sub> modulator chlordiazepoxide, or the GABA<sub>B</sub> agonist baclofen, at doses that individually do not affect memory performance, produce memory deficits (Erickson, Watts, & Parent, 2006; Krebs & Parent, 2004; Parent, Laurey, Wilkniss, & Gold, 1997; Shah & Parent, 2003, 2004). The fact that the effects of MS infusions of muscimol with glucose interact synergistically to produce memory deficits, suggests that muscimol and glucose act via a common mechanism (Seeley & Moran, 2002). It is well established that muscimol impairs memory through an influence on the hippocampus (Allen & Crawford, 1984; Degroot, Kornecook, Quirion, DeBow, & Parent, 2003; Degroot & Parent, 2000, 2001; Krebs & Parent, 2005a, 2005c; Parent, Laurey, Wilkniss, & Gold, 1997; Smythe, Colom, & Bland, 1992); therefore, it is likely that the memory-impairing effects of MS infusions of glucose with muscimol involve, at least in part, an effect on the hippocampal function. We hypothesize that MS infusions of glucose do not impair memory

unless hippocampal function is compromised, by concurrent MS GABA receptor activation. As a result, we predict that augmenting hippocampal function by elevating hippocampal glucose levels will prevent the memory-impairing effects of MS infusions of glucose with muscimol.

## **Materials and Methods**

### **Subjects**

One hundred fourteen [(n=10-19 per group); SA] or 84 [(n= 9-13 per group); continuous multiple trial inhibitory avoidance (CMIA)] male Sprague-Dawley-derived rats weighing 200-250 g upon arrival (Charles River, Wilmington, MA) were used. The rats were housed individually in polycarbonate cages (20x40x20 cm) with corncob bedding on a 12 hour light-dark cycle (lights on at 7:00 a.m.) in a temperature-controlled colony room (70-74 °F). Animals had free access to food and water. The rats were acclimated to lab conditions for approximately 1 week prior to surgery. The Georgia State University Institutional Animal Care and Use Committee (IACUC) approved all procedures involving rats.

### **Surgery**

At least 1 week after arrival, the rats were given atropine sulfate (0.4 mg/kg, i.p.), anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and then given an injection of penicillin (1500 units, i.m., Crystiben). Stereotaxic surgical procedures (David Kopf Instruments, Tujunga, CA) were used to implant one 22-gauge stainless-steel guide cannula (Plastics One, Inc., Roanoke, VA) aimed at the MS (0.5 mm anterior [AP] to bregma, 4.9 mm ventral to dura [DV]) and one guide cannula aimed at the dorsal hippocampus (4.5 mm AP, 1.6 mm DV, and 4.0 mm from the interaural line; Paxinos & Watson, 1998). The hemisphere in which the unilateral hippocampal cannulae were implanted was counterbalanced. The cannulae were secured to the

skull with three jeweler's screws (Plastics One, Inc., Roanoke, VA) and a mixture of dental cement and acrylic (DuraLay, Worth, IL). A dummy cannula was inserted to keep the cannulae free of debris. Immediately after surgery, the rats were given an injection of 0.9 % sterile saline (3.0 cc, s.c.) and then wrapped with a paper towel and kept under a warm lamp until recovery from anesthesia. Two days following surgery, the patency of each cannula was checked and betadine was applied to the surgical wound. If signs of infection were evident, the rats were anesthetized with isoflurane gas (5 %; Baxter, Deerfield, IL) delivered in 1000 ml/min of medical grade oxygen and given an additional injection of penicillin (1500 units i.m., Crystiben).

### **Drug Preparation and Drug Infusions**

Two days prior to behavioral testing, the experimenter handled each rat for 2 min. Behavioral testing occurred at least 1 week after surgery and was conducted between 8:00 a.m. and 6:00 p.m. Before and after all handling and behavioral testing, the rats were allowed a minimum of 30 min to habituate to the laboratory environment. Fifteen min prior to behavioral testing, different groups of rats were given unilateral hippocampal infusions of vehicle (1  $\mu$ l, 0.5  $\mu$ l/ min; phosphate-buffered saline [PBS], pH=7.4) or glucose (50 nmol). The dose of glucose was selected based on prior experiments showing that this dose of glucose reverses memory deficits in SA and CMIA produced by septal manipulations (Krebs & Parent, 2005a; Parent, Laury, Wilkniss, & Gold, 1997). One minute after the hippocampal infusion was initiated, the rats were given a MS injection of vehicle (PBS; 0.5 $\mu$ l, 0.5 $\mu$ l/ min), glucose (33 nmol), muscimol (0.10 nmol: SA or 3 nmol: CMIA), or glucose combined with muscimol in the same solution. The hippocampal and MS injections overlapped for the last minute and ended simultaneously.

The drugs that were combined in the same solution were prepared at double the desired concentration and then combined reducing the concentration of each by half. Drug treatments were counterbalanced over the course of the day. The dose of glucose infused into the MS was selected on the basis of previous findings showing that it significantly impaired memory when co-infused with muscimol, although it has no effects on its own (Krebs & Parent, 2004; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). The doses of muscimol were selected based on pilot experiments (SA) or previous research (CMIA; Parent & Gold, 1997) showing that these doses were the maximum subeffective doses (ie. do not significantly impair memory when given alone).

All drugs except for muscimol were prepared on the day of testing. Muscimol was prepared in 5 or 1 nmol /0.5  $\mu$ L concentrations and aliquoted (200  $\mu$ l) into microcentrifuge tubes and frozen (2-8  $^{\circ}$ C). On the day of the experiment, the muscimol was thawed and diluted, if necessary, with PBS (pH = 7.4) to generate the desired concentration. The drugs were infused through a 28-gauge injection needle that extended 1.2 mm (hippocampus) or 1.0 mm (MS) beyond the guide cannulae. The needle was connected to a 25  $\mu$ l Hamilton syringe by polyethylene tubing (PE-50), and the infusions were delivered using an infusion pump (Harvard Apparatus 11). Following the completion of both injections, the needle was left in place for 1 min to facilitate drug diffusion. The experimenter was blind to the identity of the solutions that were administered.

### **Spontaneous Alternation**

SA is a hippocampal-dependent task (Johnson, Olton, Gage, & Jenko, 1977; Stevens & Cowey, 1973) that is assumed to be a measure of spatial working memory (Lalonde, 2002;

Richman, Dember, & Kim, 1987). The underlying assumption is that in order to alternate successfully between locations the rat must remember its visits to previous locations. This assumption is supported, in part, by the finding that SA is impaired by removing directional cues or by increasing the interval between arm choices (Lalonde, 2002). Fifteen min after the drug injections, SA performance was assessed by placing each rat in a Y-maze composed of three equally spaced arms (60°; 60 cm long x 17.5 cm high). The floor of each arm was composed of stainless steel (3.5 cm wide) and the top (14 cm wide) was covered with a colorless, transparent plexiglass lid. The rats were placed in the same starting arm of the Y-maze and allowed to explore the maze for 8 min. The experimenter, who was blind to drug treatment, recorded the sequence and number of arms the rats entered. The maze was cleaned with 70% ethanol after each rat was tested. The number of arms each rat entered was used as a measure of activity. A percent alternation score was computed for all rats that entered at least 10 arms. An alternation was defined as entering three different arms consecutively. The percent alternation score was computed by dividing the number of alternations each rat made by the number of arms entered minus two (i.e., the number of alternations possible) and then multiplying that resulting quotient by 100.

### **Continuous Multiple Trial Inhibitory Avoidance (CMIA)**

A minimum of 3 days after SA testing, rats performed in a CMIA task. The avoidance apparatus consisted of a trough-shaped alley (91 cm long, 15 cm high, 20 cm wide at the top, and 6.4 cm wide at the bottom) that was divided into a lighted (31 cm long) and a dark (60 cm long) compartment by a retractable door. The dark compartment had a metal floor through which shock could be delivered. A 15-watt lamp was placed over the lighted compartment and was the

only source of illumination in the room. The table underneath the avoidance apparatus was lined with bench paper and the apparatus was cleaned with 70% ethanol after each rat was trained.

For the training the rat was placed in the lighted compartment with its head facing away from the door. Once the rat turned around to face the door or after 12 s passed, the retractable door was opened and the rat was allowed to cross over to the dark (shock) compartment. After the rat crossed with all four paws, it was given a footshock (1.2 mA) until it returned to the lighted compartment (maximum 4 s). This sequence constituted one training trial. Subsequent trials started immediately after the rat moved back into the lighted compartment; the rat was never removed from the apparatus between trials. Rats that did not escape the dark compartment within 4 s on each trial were excluded from data analysis. Training continued until the rat remained in the lighted compartment for 100 consecutive s or for a maximum of 5 trials. The number of trials needed to reach the criterion was recorded and used as a measure of acquisition.

Retention of the training was tested 48 hrs (+/- 2 hr) later. For the retention test, each rat was placed in the lighted compartment of the avoidance chamber with its head facing away from the closed door. After the rat turned to face the door or 12 s passed, the door was opened and the latency (s) to cross over to the dark (shock) compartment was recorded and used as a measure of retention. Each rat was given a maximum of 600 s to enter the dark compartment during the retention test. Footshock was not delivered on the retention test.

## **Histology**

After behavioral testing, the rats were euthanized with an overdose of sodium pentobarbital (400 mg/kg, ip) and perfused intracardially with 0.9% saline followed by 10% formalin. Their brains were stored in a 10% formalin solution for at least 2 days before



sectioning. All brains were sectioned on a cryostat (Leica CM 30510 S) and 45-60  $\mu\text{m}$  sections were taken through the MS and hippocampal cannulae tracts. The brain sections were stained with thionin and an unbiased observer determined the cannulae placement using a light microscope (Olympus BX41). Acceptable MS cannulae placement was defined as injection tips located within the MS, but not within the lateral septum or the ventral diagonal band of Broca. Moreover, the cannula must not have penetrated the fimbria. Acceptable placement for hippocampal cannulae was defined as injection sites located within hippocampal fields CA1, CA2, CA3, or dentate gyrus. Only rats with acceptable cannulae placements in both brain regions were included in the statistical analyses.

### **Statistical Analysis**

The SA data were expressed as means and standard errors of the mean (S.E.M.) and analyzed using 4 (MS drug treatment) X 2 (hippocampal drug treatment) univariate analysis of variance (ANOVA) and Tukey HSD post hoc tests where appropriate. The acquisition and retention latency data were not normally distributed. This was because several rats required the maximum number of trials to reach the acquisition criterion or reached the maximum retention latency cut-off of 600 s. Consequently, these data were expressed as medians and interquartile ranges (I.Q.) and the non-parametric Kruskal-Wallis and Mann-Whitney U tests were used to detect differences between treatment groups. An alpha level of 0.05 was used as the criterion for statistical significance. Bonferonni corrections were used for the Mann-Whitney U tests based on the number of planned comparisons.

### **Results**

The approximate locations of the MS and hippocampal infusions are shown in Figure 5.1. Infusions of vehicle or glucose into the MS [ $F(3,41) = .32, p > .05$ ], hippocampus [ $F(3,41) = 1.18, p > .05$ ] or both the MS and hippocampus [ $F(3,41) = .42, p > .05$ ] did not significantly affect percent alternation scores (data not shown). Therefore, the scores of these rats were collapsed into one control group (C-C) in order to increase statistical power. The results of this analysis indicated that drug infusions into the MS and hippocampus [ $F(4,83) = 3.48; p < .05$ ] significantly affected SA performance (see Figure 5.2A). MS infusions of muscimol alone did not affect SA performance. The scores of rats given MS infusions of muscimol and hippocampal infusions of vehicle (M-PBS) were not significantly different from the scores of rats given control infusions ( $p > .05$  vs. C-C). As in previous research, co-infusions of muscimol with glucose into the MS produced memory deficits. Specifically, the percent alternation scores of rats given muscimol combined with glucose in the MS and vehicle in the hippocampus (M+G-PBS) were significantly lower than C-C rats ( $p < .01$ ). Importantly, the findings indicated that hippocampal infusions of glucose reversed alternation deficits produced by the combination of muscimol with glucose into the MS. Specifically, the percent alternation scores of rats given MS infusions of muscimol with glucose combined with hippocampal infusions of glucose (M+G-G) were not significantly different than those of C-C rats ( $p > .05$ ), and were significantly higher than those of M+G -PBS rats ( $p < .05$ ). Drug infusions of vehicle or glucose into the MS [ $F(3,41) = 2.27, p > .05$ ], hippocampus [ $F(3,41) = .40, p > .05$ ], or the MS and hippocampus [ $F(3,41) = .25, p > .05$ ] did not significantly affect the number of arms rats entered in the maze (data not shown). Therefore, the scores of these rats were collapsed into one control group in order to increase statistical power. The findings of this experiment indicated that drug infusions

into the MS and hippocampus [ $F(4,83) = 2.43$ ;  $p > .05$ ] did not significantly affect the number of arms the rats entered in the maze (see Figure 5.2B).

Drug infusions of vehicle or glucose into the MS and hippocampus [ $\chi^2(3,60) = 11.49$ ,  $p < .05$ ] significantly affected the number of trials to criterion during CMIA training (see Figure 5.3A). MS infusions of glucose combined with hippocampal infusions of vehicle facilitated shock avoidance acquisition. Specifically, rats that were given MS infusions of glucose with hippocampal infusions of vehicle (G-PBS) required significantly fewer trials to reach criterion than did rats given MS and hippocampal infusions of vehicle ( $p < .05$  vs. PBS-PBS rats). As a result of this difference, the scores of these rats were not collapsed into one control group. Figure 5.3A demonstrates that the drug infusions significantly affected the trials to criterion during CMIA training [ $\chi^2(7,113) = 17.95$ ,  $p < .05$ ]. After Bonferonni correction ( $p < .006$ ), the post-hoc tests revealed no significant differences between any of the groups on the number of trial to criterion during CMIA training. There was a strong trend, however, for MS infusions of muscimol combined with hippocampal infusions of glucose to also facilitate acquisition of shock avoidance. Specifically, rats that were given MS infusions of muscimol with hippocampal infusions of glucose tended to take fewer trials to reach criterion than did rats given PBS-PBS ( $p = .008$ ).

Pre-training infusions of vehicle or glucose into the MS and hippocampus [ $\chi^2(3,60) = 1.37$ ,  $p > .05$ ] did not significantly affect retention latencies (data not shown). Therefore, the scores of these rats were collapsed into one control group. Figure 5.3B illustrates that the pre-training drug infusions into the MS and hippocampus significantly affected subsequent CMIA retention performance [ $\chi^2(4,113) = 30.51$ ,  $p < .01$ ]. After Bonferonni correction ( $p < .013$ ), MS

infusions of muscimol alone impaired avoidance retention. The retention latencies of rats given MS infusions of muscimol with hippocampal infusions of vehicle (M-PBS) were significantly shorter than those of rats given control infusions [ $U(1,75) = 176.0$ ;  $p < .01$ ]. MS infusions of muscimol with glucose also impaired CMIA retention. Specifically, M+G-PBS rats had significantly shorter retention latencies than did C-C rats [ $U(1,75) = 34$ ;  $p < .01$ ]. MS infusions of muscimol with glucose produced a bigger deficit than did MS infusions of muscimol alone. Specifically, the M+G-PBS has significantly shorter retention latencies than did M-PBS rats ( $p < .01$ ). More importantly, hippocampal infusions of glucose reversed CMIA retention deficits produced by MS infusions of muscimol alone and muscimol combined with glucose. Rats given MS infusions of muscimol and hippocampal infusions of glucose (M-G rats) had significantly longer retention latencies than did M-V rats [ $U(1,31) = 70$ ;  $p < .01$ ], and their latencies did not significantly differ from those of C-C rats [ $U(1,77) = 501.0$ ;  $p > .05$ ]. Similarly, rats given MS infusions of muscimol with glucose and hippocampal infusions of glucose (M+G-G rats) had significantly longer retention latencies than did M+G-V rats [ $U(1,20) = 20$ ;  $p < .01$ ], and their latencies did not significantly differ from those of C-C rats [ $U(1,70) = 286.0$ ;  $p > .05$ ].

### **General Discussion**

These results demonstrate that MS co-infusions of the GABA receptor agonist muscimol with glucose, at doses that have no effect alone, impair spatial working memory. These effects were also observed in an emotional, long-term memory task. More importantly, the findings showed that these deficits were reversed by concurrent hippocampal infusions of glucose. These data are consistent with previous research indicating that co-infusions of muscimol with glucose into the MS impair memory (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997;

Shah & Parent, 2003, 2004) and that hippocampal infusions of glucose reverse memory deficits produced by MS manipulations (Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997). The present findings extend these previous results by showing that, when both phenomena happen simultaneously, the memory-enhancing effects of hippocampal infusions of glucose override the memory-impairing effects of MS glucose infusions.

The fact that hippocampal infusions prevented the deficits produced in the MS suggests that MS infusions of glucose impair memory by impairing hippocampal function. This interpretation is consistent with the finding that MS manipulations influence hippocampal activity and hippocampal-dependent memory (Allen & Crawford, 1984; Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990; Dragoi, Carpi, Recce, Csicsvari, & Buzsaki, 1999; Leutgeb & Mizumori, 1999; Parent, Laurey, Wilkniss, & Gold, 1997; Smythe, Colom, & Bland, 1992). For example, septal manipulations influence hippocampal theta, a rhythmic 4-8 Hz waveform associated with hippocampal excitation (Bland, Oddie, & Colom, 1999), synaptic plasticity (Huerta & Lisman, 1993; Larson, Wong, & Lynch, 1986), and memory (Kahana, Seelig, & Madsen, 2001; Kahana, Sekuler, Caplan, Kirschen, & Madsen, 1999; Raghavachari et al., 2001). Furthermore, extensive evidence shows that MS GABA receptor activation impairs memory (Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990; Chrobak, Stackman, & Walsh, 1989; Durkin, 1992b; Izquierdo & Medina, 1997; Krebs & Parent, 2005a, 2005c; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004) and decreases hippocampal acetylcholine (ACh) levels (Allen & Crawford, 1984; Moor, DeBoer, & Westerink, 1998; Walsh, Stackman, Emerich, & Taylor, 1993), a neurotransmitter important for memory (Gold, 2003a). Collectively, these results may account for the finding that the impairing effects

of diabetes are most robustly observed in populations with compromised hippocampal function (Ryan & Geckle, 2000).

Elevations in blood glucose levels associated with diabetes are accompanied by generalized increases in brain extracellular glucose levels (Biessels, Bravenboer, & Gispen, 2004; Hofer & Lanier, 1991) and memory deficits (Biessels et al., 1996; Flood, Mooradian, & Morley, 1990; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; Messier & Gagnon, 1996; Ryan & Geckle, 2000). This would lead to the expectation that elevating glucose in multiple brain areas should produce memory deficits. These present findings showed, though, that elevating glucose simultaneously in the MS and hippocampus did not impair memory. The absence of a memory deficit is not likely due to insufficient increases in glucose, because these doses of glucose ( $33 \text{ nmol}/0.5 \text{ }\mu\text{L} = 66 \text{ mM}$  or  $50 \text{ nmol}/1.0 \text{ }\mu\text{L} = 50 \text{ mM}$ ) produce extracellular levels that are higher than those that would be produced by hyperglycemia (Jacob, Fan, Evans, Dziura, & Sherwin, 2002; Silver & Erecinska, 1994). This suggests that additional brain regions must experience elevated glucose levels or that hippocampal function must be impaired in order for glucose to produce memory deficits.

The finding that glucose infusions in the hippocampus have positive effects on memory; whereas, infusions in MS can have negative effects shows that the effect of glucose on memory are brain region-dependent. This hypothesis is supported by previous evidence showing that the effects of glucose on electrochemical signaling varies by neuronal phenotype, brain area, and glucose concentration (Fioramonti, Lorsignol, Taupignon, & Penicaud, 2004; Song, Levin, McArdle, Bakhos, & Routh, 2001; Wang et al., 2004). For instance, within the hypothalamus, glucose increases the electrical activity of some cells and decreases the activity of others via an effect on different channels (Song, Levin, McArdle, Bakhos, & Routh, 2001). Moreover, basal

extracellular glucose concentrations, glucose transporter distribution, activation, and expression are brain region-dependent (Barros, Porras, & Bittner, 2005; Choeiri, Staines, Miki, Seino, & Messier, 2005; Khandelwal et al., 2004; McNay, McCarty, & Gold, 2001). Importantly, changes in brain extracellular glucose levels associated with performance in a memory task are brain region-specific. Specifically, performance in a spatial working memory task decreases brain extracellular glucose levels in the hippocampus, without changing extracellular glucose levels in the striatum (McNay, McCarty, & Gold, 2001). Similarly, performance in an operant conditioning task produces selective increases in glucose transporter expression in the hippocampus (Choeiri, Staines, Miki, Seino, & Messier, 2005). More importantly, recent findings from our lab show that the memory-impairing effects of glucose are brain-region dependent. For example, the memory-impairing interaction between glucose and GABA receptor activation that is observed in the MS is not observed in the hippocampus (Parent & Krebs, 2004). Specifically, hippocampal co-infusions of glucose with muscimol reverse muscimol-induced memory deficits, rather than producing deficits as they do in the MS.

The motivational, temporal, and cognitive differences between the SA and continuous multiple trial inhibitory avoidance task support the hypothesis that glucose influences memory rather than some other process that influences performance in a memory task. The finding that septo-hippocampal manipulations affect memory in both SA and CMIA indicate that the septo-hippocampal system is involved in several mnemonic processes. Specifically, the findings from the SA task suggest that the hippocampus is involved in on-line spatial associations, and the findings from the CMIA task indicate that the hippocampus also affects emotional and long-term memory. The fact that the pretraining infusions of glucose did not affect acquisition in the

continuous multiple trial inhibitory avoidance task suggests further that elevating glucose in the hippocampus influences consolidation of newly formed emotional memories.

In summary, hippocampal infusions of glucose reverse the memory deficits produced by MS infusions of glucose. The enhancing and impairing effects of glucose were observed in a septo-hippocampal-dependent task that involves short-term, spatial working memory and one that is more dependent on long-term, emotional memory. These results suggest that the memory-impairing effects of MS infusions of glucose involve an influence on the hippocampus, and that the impairing effects of glucose may only be observed when hippocampal function is impaired. These results also show that the mnemonic effects of glucose are brain region-dependent.

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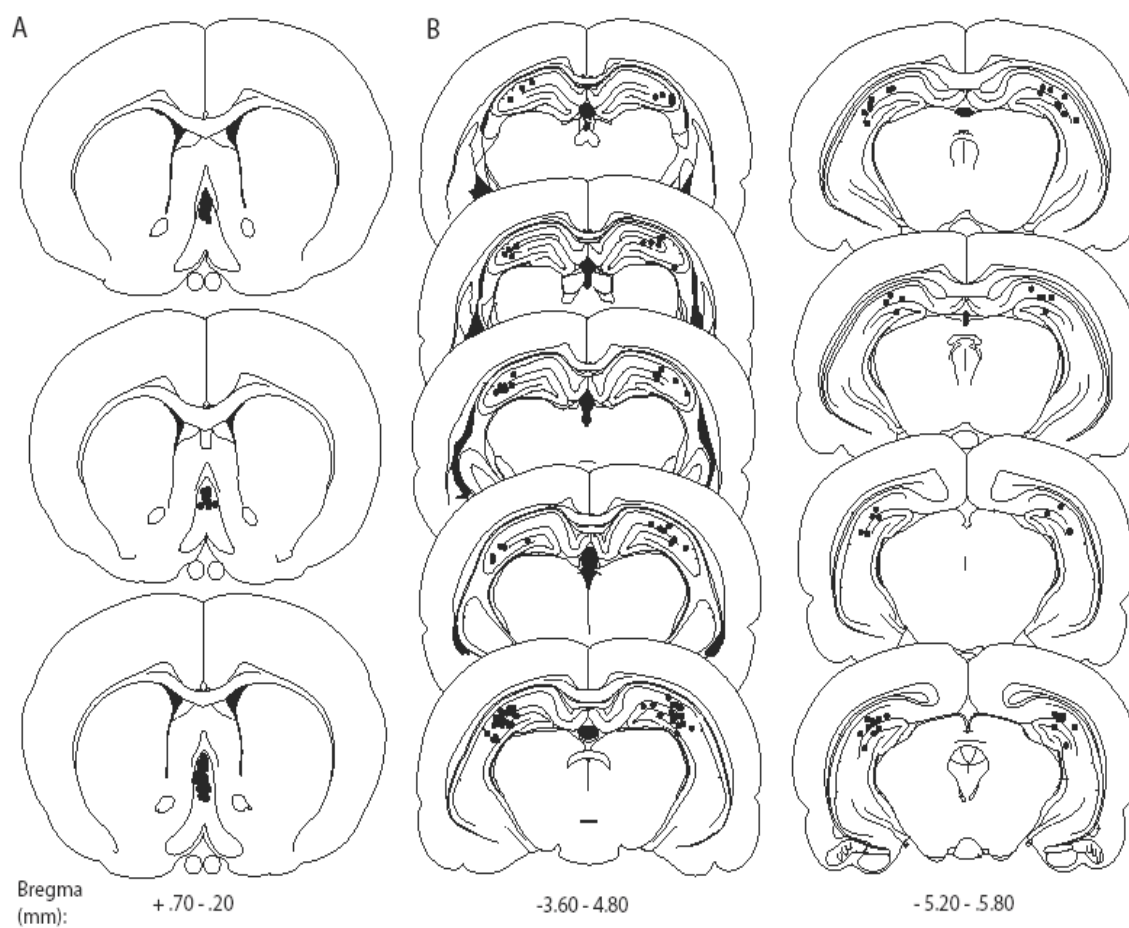


Figure 5.1. Schematic illustration of coronal sections of the rat brain showing the approximate locations of (A) MS and (B) hippocampal infusion sites in Experiment 1. Atlas plates were adapted from Paxinos and Watson (1998).

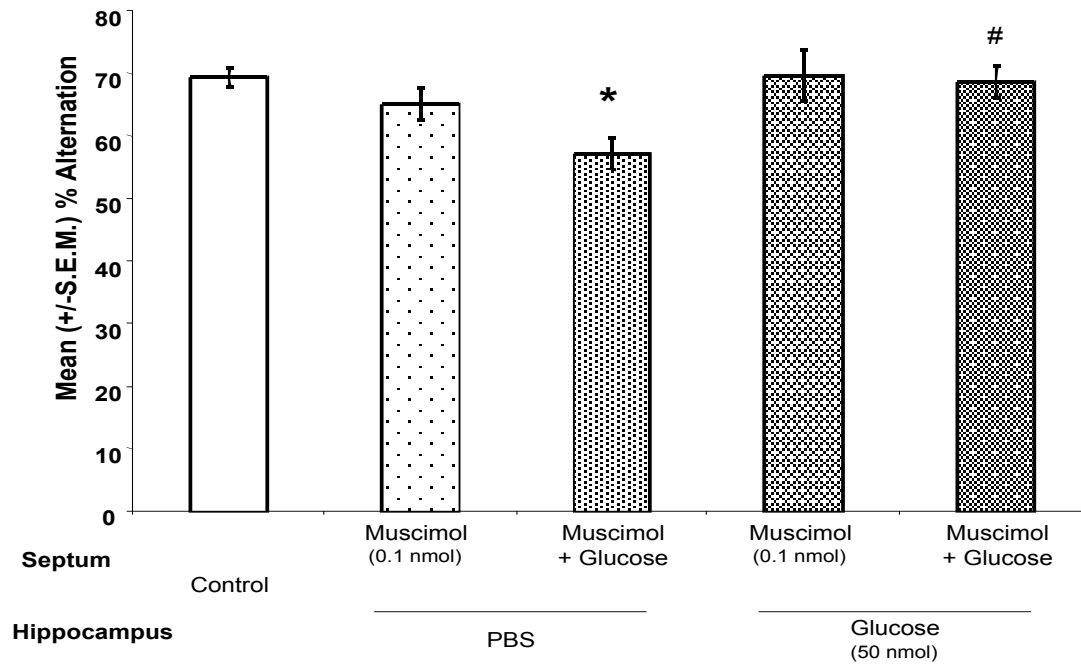


Figure 5.2A. MS infusions of muscimol alone did not significantly decrease mean ( $\pm$  S.E.M.) percent alternation scores ( $p > .05$  vs. control), but combined MS infusions of glucose with muscimol did ( $*p < .05$  vs. control). Hippocampal infusions of glucose reversed the deficits produced by MS co-infusions of glucose with muscimol ( $\#p < .05$  vs. muscimol + glucose,  $p > .05$  vs. control).

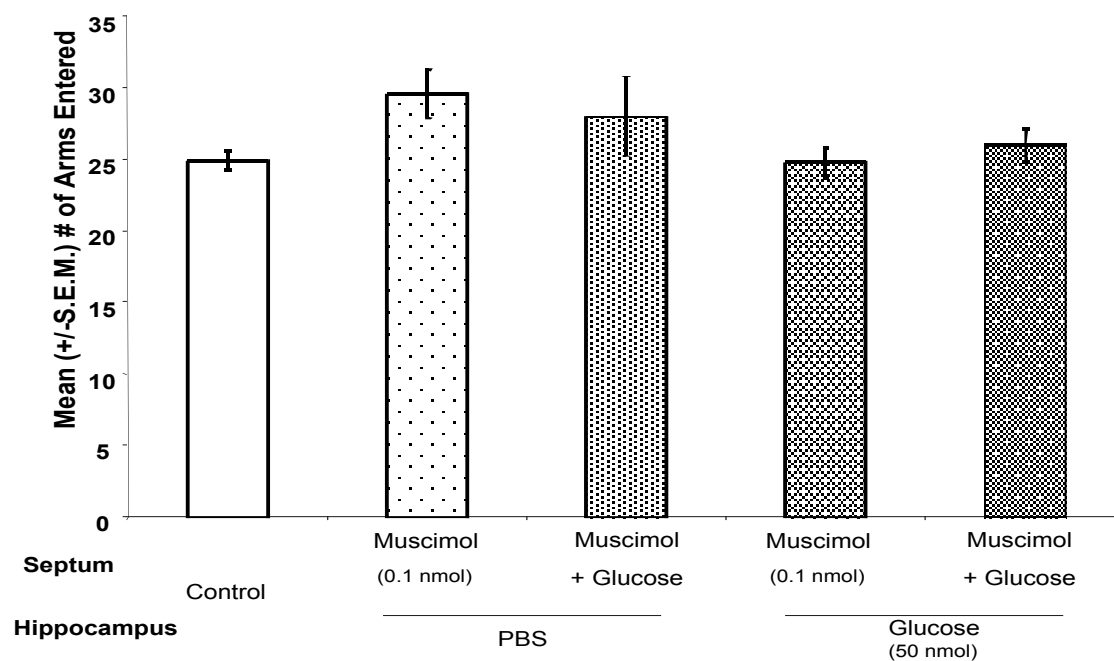


Figure 5.2B. MS and hippocampal drug treatments did not significantly affect the number of arms entered in the maze ( $p > .05$ ).

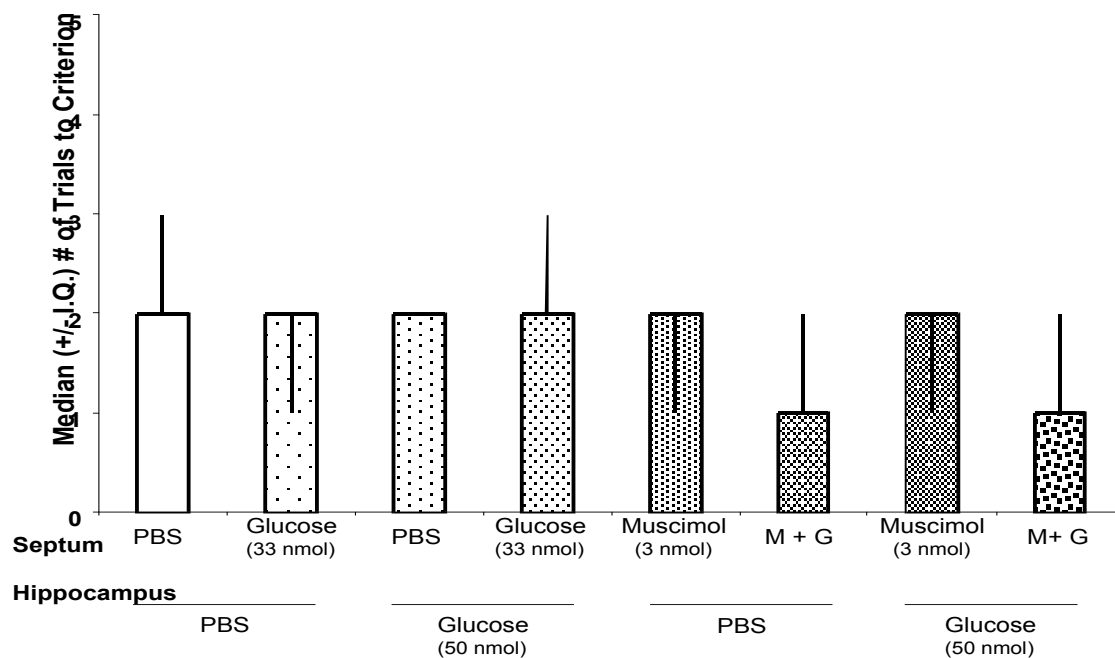


Figure 5.3A. MS infusions significantly affected the median (+/-I.Q.) number of trials to criterion ( $p < .05$ ). After Bonferonni correction ( $p < .006$ ), the post-hoc tests revealed a tendency for MS infusions of muscimol with hippocampal infusions of glucose to decrease the number of trials to reach criterion ( $p = .008$  vs. PBS-PBS).

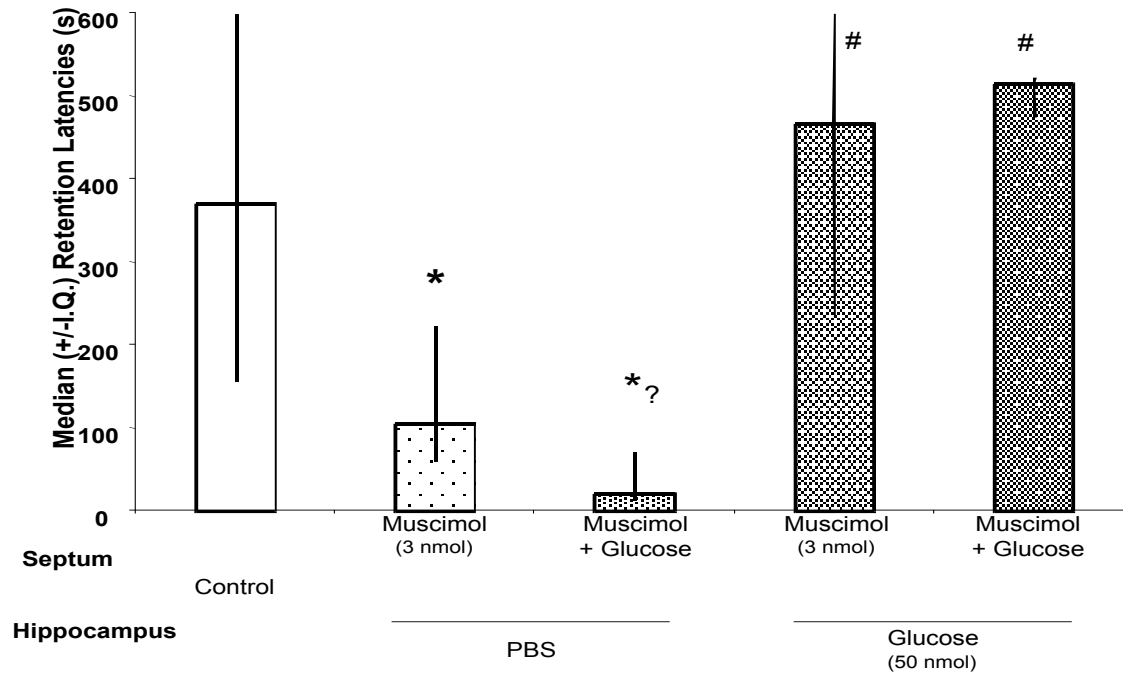


Figure 5.3B. MS infusions of muscimol alone or combined with glucose significantly decreased median (+/-I.Q.) retention latencies (\* $p < .05$  vs. control; ? $p < .05$  vs. muscimol). Hippocampal infusions of glucose reversed the deficits (# $p < .05$  vs. muscimol or muscimol + glucose,  $p > .05$  vs. control).



## **CHAPTER 6**

### **THE EFFECTS OF GLUCOSE ON MEMORY ARE BRAIN REGION-DEPENDENT**

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### **List of Abbreviations**

GABA:  $\gamma$ -aminobutyric acid

SA: spontaneous alternation

CMIA: continuous multiple trial inhibitory avoidance

PBS: phosphate buffered saline

S.E.M.: standard error of the mean

I.Q.: interquartile range

s: second(s)

ACh: acetylcholine

### Abstract

Elevations in brain glucose can have positive or negative effects on memory. One of the variables that appears to dissociate the impairing and enhancing effects of glucose are the neurochemical systems that are engaged at the time that glucose levels are elevated. For instance, septal infusions of glucose reverse memory deficits produced by morphine and galanin, but exacerbate deficits produced by co-infusions of  $\gamma$ -aminobutyric acid (GABA) receptor agonists. In addition to being neurotransmitter-dependent, emerging evidence suggests that the effects of glucose may also be brain region-dependent. As a result, the present experiments tested whether the negative interaction between glucose and GABA in the septum would be observed in the hippocampus, a brain region where glucose typically has positive effects on memory. Specifically, we determined whether hippocampal infusions of glucose would exacerbate or reverse memory deficits produced by hippocampal co-infusions of the GABA receptor agonist muscimol. Fifteen minutes prior to assessing either spontaneous alternation (SA) or continuous multiple trial inhibitory avoidance (CMIA) training, male Sprague-Dawley-derived rats were given bilateral hippocampal infusions of vehicle (phosphate-buffered saline [PBS], 1  $\mu$ l/ 2 min), glucose (33 or 50 nmol), muscimol (0.3  $\mu$ g or 0.4  $\mu$ g, SA or 3  $\mu$ g, CMIA) or muscimol and glucose combined in one solution. The results indicated that hippocampal infusions of muscimol alone decreased SA scores and CMIA retention latencies. More importantly, hippocampal infusions of glucose, at doses that had no effect when infused alone, attenuated (33 nmol) or reversed (50 nmol) the muscimol-induced memory deficits. Thus, although co-infusions of glucose with muscimol into the septum impair memory, the present findings show that an opposite effect is observed in the hippocampus. Collectively, these

findings show that the memory-impairing interaction between glucose and GABA in the septum is not a general property of the brain, but rather is brain region-dependent.

**Key Words:** Muscimol, GABA, Hippocampus, Spontaneous Alternation, Inhibitory Avoidance

## Introduction

One key variable that influences memory is the level of glucose in the brain. Increases in brain glucose levels can occur in response to meals, drugs, stress, exercise, and diabetes (Bequet et al., 2000; Canal, McNay, & Gold, 2005; Fellows, Boutelle, & Fillenz, 1993; Jacob, Fan, Evans, Dziura, & Sherwin, 2002; Silver & Erecinska, 1994). Although glucose typically enhances memory (Lee, Graham, & Gold, 1988; Stefani, Nicholson, & Gold, 1999; Sunram-Lea, Foster, Durlach, & Perez, 2002) and reverses memory deficits (Craft, Murphy, & Wemstrom, 1994; Korol, 2002; Manning, Ragozzino, & Gold, 1993; Petit, 1988), elevated glucose levels are also associated with memory dysfunction (Biessels et al., 1996; Convit, 2005; Elias et al., 1997; Flood, Mooradian, & Morley, 1990; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; Hagemann, Sartory, Hader, & Kobberling, 2005; McCall, 2002; Popovic, Biessels, Isaacson, & Gispen, 2001; Winocur et al., 2005). The mechanisms underlying the memory-modulating effects of glucose, particularly the conditions that differentiate the enhancing and impairing effects of glucose, are poorly understood.

The effects of glucose on memory are mediated, at least in part, by the brain. Glucose readily crosses the blood-brain barrier via glucose transporters (Pardridge, Boado, & Farrell, 1990; Rahner-Welsch, Vogel, & Kuschinsky, 1995; Takata, Hirano, & Kasahara, 1997). Interestingly, extracellular levels of hippocampal glucose decrease with cognitive demand, and systemic infusions of glucose both enhance memory and replenish these decreases in hippocampal glucose (McNay & Gold, 1999). These data suggest that during tasks that engage the hippocampus, peripheral glucose is preferentially directed to the hippocampus and enhances memory formation. Moreover, direct brain infusions of glucose affect memory. For example, infusions of glucose into the cerebral ventricular system enhance retention performance on a

one-trial inhibitory avoidance task in rodents (Lee, Graham, & Gold, 1988). In addition, direct injections of glucose into specific brain regions, including the medial septum (MS) or hippocampus, enhance spontaneous alternation (SA) performance (Ragozzino & Gold, 1995b; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Stefani & Gold, 1998; Stefani, Nicholson, & Gold, 1999), a measure of spatial working memory (Lalonde, 2002; Richman, Dember, & Kim, 1987). MS or hippocampal infusions of glucose also reverse drug-induced deficits in SA (Ragozzino & Gold, 1995b). MS co-infusions of glucose with muscimol potentiate memory deficits produced by MS  $\gamma$ -aminobutyric acid (GABA) receptor activation (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997), suggesting that the memory-impairing effects of glucose are also mediated, at least in part, in the brain.

In addition to its general role in providing energy to neurons, glucose affects memory through more specific mechanisms. For instance, the effects of glucose on memory depend on the chemical systems that are engaged at time that glucose levels are elevated. Specifically, glucose administration into the MS reverses deficits produced by the opiate agonist morphine and the peptide galanin (Ragozzino & Gold, 1995b; Ragozzino, Parker, & Gold, 1992; Stefani & Gold, 1998). Likewise, MS co-infusions of glucose do not reverse the memory-impairing effects of GABA agonists (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997). Rather, MS infusions of glucose potentiate these memory-impairing effects of GABA<sub>A</sub> agonists (Parent, Laurey, Wilkniss, & Gold, 1997). Furthermore, MS co-infusions of glucose with the GABA agonist muscimol, at doses that individually do not affect memory performance, produce memory deficits (Parent & Gold, 1997; Shah & Parent, 2003, 2004). Collectively, this evidence indicates that the effects of glucose on memory are neurotransmitter-dependent.

Several lines of evidence indicate that the effects of glucose on brain function vary by brain area. Previously, glucose was assumed to be contained within one universal brain compartment and thought to have the same fate and effect in all neuronal cells (Lund-Anderson, 1979; Sokoloff et al., 1977). More recent evidence, however, suggests that the distribution and effects of glucose are not uniform throughout the brain. For instance, there are brain region differences in glucose utilization (Gage, Kelly, & Bjorklund, 1984; Gerber, Choki, Brunswick, Reivich, & Frazer, 1983; Kimbrell et al., 2002a; Kimbrell et al., 2002b; McNay, McCarty, & Gold, 2001). In addition, basal extracellular glucose concentrations and glucose transporter distribution, activation, and expression are brain region-dependent (Barros, Porras, & Bittner, 2005; Choeiri, Staines, Miki, Seino, & Messier, 2005; Khandelwal et al., 2004; McNay, McCarty, & Gold, 2001). Acute increases in blood glucose either increase or decrease cerebral blood flow depending on the brain region (Duckrow, 1988). Moreover, the effects of glucose on electrochemical signaling also vary by neuronal phenotype, brain area, and glucose concentration (Fioramonti, Lorsignol, Taupignon, & Penicaud, 2004; Wang et al., 2004). For instance, within the hypothalamus, glucose increases the electrical activity of some cells and decreases the activity of others via an effect on different channels (Song, Levin, McArdle, Bakhos, & Routh, 2001). Importantly, changes in brain extracellular glucose levels associated with performance in a memory task are brain region-specific. Specifically, performance in the SA task decreases brain extracellular glucose levels in the hippocampus, but does not affect striatal extracellular glucose levels (McNay, McCarty, & Gold, 2001). Similarly, performance in an operant conditioning task produces selective increases in glucose transporter expression in the hippocampus (Choeiri, Staines, Miki, Seino, & Messier, 2005).

Collectively, the evidence reviewed above raises the possibility that, in addition to being neurotransmitter-dependent, the memory-modulating effects of glucose are brain-region dependent. The goal of the present set of experiments was to test whether the memory-impairing interaction between glucose and muscimol that is observed in the MS would be observed in the hippocampus, a brain region where glucose typically has positive effects on memory (Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Ragozzino, Unick, & Gold, 1996). To test this, we determined whether hippocampal infusions of glucose would exacerbate or reverse memory deficits produced by hippocampal co-infusions of the GABA receptor agonist muscimol. If the memory-impairing effects of glucose are brain-region dependent, then hippocampal co-infusions of glucose with muscimol will reverse the muscimol-induced memory deficits. If, however, the memory-impairing interaction between glucose and GABA receptor activation is a general property of the brain, then hippocampal co-infusions of glucose with muscimol will produce memory deficits as they do in the MS.

## **Experimental Procedures**

### **Experiment 1**

The goal of Experiment 1 was to determine whether hippocampal co-infusions of glucose with muscimol, at doses that do not significantly impair memory, would produce SA deficits as they do in the medial MS.

### **Subjects**

Seventy-three male Sprague-Dawley-derived rats ( $n = 9-19$  per group) weighing 200-250 g upon arrival (Charles River, Wilmington, MA) were used. The rats were housed individually



in polycarbonate cages (20x40x20 cm) with corncob bedding on a 12 hour light-dark cycle (lights on at 7:00 a.m.) in a temperature-controlled colony room (70-74 °F). Animals had free access to food and water. The rats were acclimated to lab conditions for approximately 1 week prior to surgery. The Georgia State University Institutional Animal Care and Use Committee approved all procedures involving rats.

### **Surgery**

Prior to surgery, rats were placed in a clear plastic gas induction chamber and anesthetized with 5% isoflurane gas (Baxter, Deerfield, IL) delivered in 1000 ml/min medical grade oxygen. After the rat was no longer ambulatory, it was removed from the chamber and placed on a face-mask that delivered 3% isoflurane gas in 1000 ml/min of oxygen. Rats were then given injections of atropine sulfate (0.4 mg/kg, ip, Baxter, Deerfield, IL) and penicillin (1500 units, im, Hanfords US Vet, Syracuse, NY). The incision site was shaved with a #50 electric clipper blade (Oster) and betadine solution was applied to the surgical area. Anesthesia was maintained by delivering 1-3% isoflurane gas in 500 ml/min oxygen to the face-mask. A stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) that was equipped with an anesthesia mask was used to implant two 22-gauge stainless-steel injection guide cannulae (Plastics One, Inc., Roanoke, VA) aimed at the dorsal hippocampus (4.5 mm anterior to bregma, 1.6 mm ventral to dura, and +/- 4.0 mm from the interaural line; Paxinos & Watson, 1998). The incision site was injected with a 2% lidocaine/.001% epinephrine cocktail (0.5-2.0 cc, sc, Abbott Labs, Chicago, IL). After the incision, the 2% lidocaine/.001 % epinephrine cocktail solution (.05- 1.0 cc) was applied topically to the skull to facilitate seeing lambda and bregma. The cannulae were secured to the skull with three jeweler's screws and cranioplastic cement (Duralay, Worth, IL) and a dummy cannula (Plastics One, Inc., Roanoke, VA) was inserted to

keep the cannulae free of debris. Immediately after surgery, the rats were given an injection of 0.9 % sterile saline (3.0 cc, sc) and the non-steroidal anti-inflammatory analgesic flunixin meglumine (2.5 mg/kg, ip, Fort Dodge Animal Health, Fort Dodge, IA), and then wrapped with a paper towel and kept under a warm lamp until recovery from anesthesia. Two days following surgery, the patency of each cannula was checked and betadine was applied to the surgical wound. If signs of infection were evident at this time, the rats were anesthetized with isoflurane gas (5%) delivered in 1000 ml/min of oxygen and given an additional injection of penicillin (1500 units im).

### **Drug Preparation and Intracranial Infusions**

Two days prior to behavioral testing, the experimenter handled each rat for 2 minutes. Before and after all handling and behavioral testing, the rats were allowed a minimum of 30 minutes to habituate to the laboratory environment. Behavioral testing was conducted between 7:00 a.m. and 7:00 p.m. Drug treatments were counterbalanced over the course of the day. Fifteen minutes prior to behavioral testing, different groups of rats were given bilateral hippocampal infusions of vehicle (1  $\mu$ l, 0.5  $\mu$ l/ min; phosphate-buffered saline [PBS], pH = 7.4), glucose (33 or 50 nmol), muscimol (0.3  $\mu$ g, Sigma) or muscimol combined with glucose in one solution. The drugs that were combined in the same solution were prepared at double the desired concentration and then combined to reduce the concentration of each by half. The effects of two doses of glucose were tested to ensure that any negative effects were not due to brain region-dependent differences in the dose-response properties of glucose. The 33 nmol dose of glucose was selected because it produces memory deficits when infused with GABA receptor agonists in the MS (Parent & Krebs, 2004; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997;

Shah & Parent, 2003, 2004). The 50 nmol dose of glucose was selected because it reverses memory deficits when infused into the hippocampus (Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997). These doses produce increases in extracellular glucose (33 nmol/1.0  $\mu$ l = 33 mM) that are higher than levels that would be produced by a meal, stress, or acute or chronic hyperglycemia (8.7 - 10.5 mM; Jacob, Fan, Evans, Dziura, & Sherwin, 2002; Silver & Erecinska, 1994). We have elected to use these doses because 1) they are the same doses that can have positive effects on memory, 2) these doses do not affect memory in our tasks when infused alone, 3) there are dose-response differences in this dose range, indicating that the effects of glucose are not saturated, 4) extracellular glucose concentrations must be high in order to provide a driving force for uptake into neurons, 5) there is no apparent negative effect of these doses, and 6) high amounts are sometimes necessary for probing subtle effects. The 0.3  $\mu$ g dose of muscimol was used based on our pilot data indicating that this is the highest dose that does not significantly impair SA. Co-infusions of glucose with this dose of muscimol were used to test whether hippocampal infusions of glucose would mimic the effects of MS infusions of glucose and produce memory deficits.

With the exception of muscimol, drug solutions were prepared on the day of testing. Muscimol was prepared in 6  $\mu$ g /1.0  $\mu$ L concentrations and aliquoted (200  $\mu$ l) into microcentrifuge tubes and frozen (2-8  $^{\circ}$ C). On the day of the experiment, the muscimol was thawed and diluted with PBS (pH = 7.4) to generate the desired concentration. The drugs were infused through a 28-gauge injection needle that extended 1.2 mm beyond the guide cannulae. The needle was connected to a 25  $\mu$ l Hamilton syringe by polyethylene tubing (PE-50), and the infusions were delivered using an infusion pump (Harvard Apparatus 11). Following the

completion of the injections, the needle was left in place for one minute to facilitate drug diffusion.

### **Spontaneous Alternation**

SA is assumed to be a hippocampal-dependent measure of spatial working memory (Deacon, Bannerman, Kirby, Croucher, & Rawlins, 2002; Dember, 1989; Lalonde, 2002; Pacteau, Einon, & Sinden, 1989; Richman, Dember, & Kim, 1987; Stevens & Cowey, 1973). The underlying assumption is that in order to alternate successfully between locations the rats must remember its visits to previous arms. This interpretation is supported, in part, by the finding that SA is impaired by removing directional cues or by increasing the interval between arm choices (Dember, 1989; Lalonde, 2002; Richman, Dember, & Kim, 1987). Fifteen minutes after the drug injections, SA performance was assessed by placing each rat in a Y-maze composed of three equally spaced arms (60°; 60 cm long x 17.5 cm high). The floor of each arm was composed of stainless steel (3.5 cm wide) and the top (14 cm wide) was covered with a translucent plexiglass lid. All rats were placed in the same starting arm of the Y-maze and allowed to explore the maze for 8 minutes. The experimenter, who was blind to drug treatment, recorded the sequence and number of arms the rats entered during the 8 min. period. The maze was cleaned with 70% ethanol after each rat. The number of arms each rat entered was used as a measure of activity. A percent alternation score was computed for all rats that entered at least 10 arms. An alternation was defined as entering three different arms consecutively. The percent alternation score was computed by dividing the number of alternations each rat made by the number of arms entered minus two (i.e., the number of alternations possible) and then multiplying that resulting quotient by 100.

## **Histology**

After behavioral testing, the rats were euthanized with an overdose of sodium pentobarbital (400 mg/kg, ip) and perfused intracardially with 0.9% saline followed by 10% formalin. Their brains were stored in a 10% formalin solution for at least 2 days before sectioning. All brains were sectioned on a cryostat (Leica CM 30510 S) and 45-60  $\mu$ m sections were taken through the hippocampal cannulae tracts. The brain sections were stained with thionin and an unbiased observer determined the cannulae placement using a light microscope (Olympus BX41). Acceptable placement for hippocampal cannulae was defined as injection sites located within hippocampal fields CA1, CA2, CA3, or the dentate gyrus. Only rats with acceptable cannulae placements in both hemispheres of the hippocampus were included in the statistical analyses.

## **Statistical Analysis**

The data were expressed as means and standard errors of the mean (S.E.M.) and analyzed using one-way univariate analysis of variance and Tukey post hoc tests where appropriate. An alpha level of 0.05 was used as the criterion for statistical significance.

## **Experiment 2**

The goal of Experiment 2 was to test whether hippocampal infusions of glucose would reverse the memory-impairing effects of co-infusions of muscimol. The same procedures were used as in Experiment 1, with the exception that a higher dose of muscimol (0.4  $\mu$ g, Sigma) was used. This dose was selected based on our pilot data indicating that this dose significantly

impaired SA. Eighty-three male Sprague-Dawley-derived rats ( $n = 9-18$  per group) were used for Experiment 2.

### Experiment 3

The goal of Experiment 3 was to determine whether hippocampal co-infusions of glucose with muscimol would reverse or exacerbate memory retention deficits produced by hippocampal co-infusions of the GABA receptor agonist muscimol in another memory task. Specifically, we determined whether hippocampal co-infusions of glucose with a mildly impairing dose of muscimol would potentiate or reverse muscimol-induced footshock avoidance retention deficits. This experiment would thus show whether the brain region-dependent effects of glucose would generalize to another behavioral task that is dependent on the hippocampus. Given that SA and CMIA vary in motivational, temporal, and cognitive demands, the use of two behavioral tasks would also allow us to determine whether the manipulations were affecting a process that influences memory rather than some process that influences performance on the memory task. The same procedures were used as in Experiment 1 with the following exceptions: A minimum of three days after performing a SA task, different groups of rats were given bilateral hippocampal infusions of vehicle ( $1\ \mu\text{l}$ ,  $0.5\ \mu\text{l}/\text{min}$ ; PBS,  $\text{pH} = 7.4$ ), glucose (33 or 50 nmol), muscimol ( $3\ \mu\text{g}$ , Sigma) or muscimol combined with glucose in one solution 15 minutes prior to CMIA training. The  $3.0\ \mu\text{g}$  dose of muscimol was used in CMIA because our pilot data indicated that it produced a mild deficit in shock-avoidance retention that could be either potentiated or reversed. Sixty-three rats ( $n = 9-12$  per group) were used for Experiment 3.

### **Continuous Multiple Trial Inhibitory Avoidance**

The avoidance apparatus consisted of a trough-shaped alley (91 cm long, 15 cm high, 20 cm wide at the top, and 6.4 cm wide at the bottom) that was divided into a lighted (31 cm long) and a dark (60 cm long) compartment by a retractable guillotine door. The dark compartment had a metal floor through which shock could be delivered. A 15-watt lamp was placed over the lighted compartment and was the only source of illumination in the room. The table underneath the avoidance apparatus was lined with bench paper and the apparatus was cleaned with 70% ethanol after each rat was trained or tested.

For the training the rat was placed in the lighted compartment with its head facing away from the door. Once the rat turned around to face the door or after 12 seconds (s) passed, the retractable door was opened and the rat was allowed to cross over to the dark (shock) compartment. After the rat crossed with all four paws, the rat was given a footshock (1.2 mA) until it returned to the lighted compartment (maximum 4 s). This sequence constituted one training trial. Training continued until the rat remained in the lighted compartment for 100 consecutive s or for a maximum of 5 trials. The rat was not removed from the avoidance apparatus between trials. The number of trials needed to reach the criterion was recorded and used as a measure of acquisition.

Retention of the training was tested 48 hours (+/- 2 hrs) later. For the retention test, the rats were placed in the lighted compartment of the avoidance chamber with their heads facing away from the closed door. After each rat turned to face the door or 12 s passed, the door was opened and the latency (s) to cross over to the dark (shock) compartment was recorded and used as a measure of retention. Each rat was given a maximum of 600 s to enter the dark compartment during the retention test. Footshock was not delivered on the retention test.

## Statistical Analysis

The retention latency data were not normally distributed due to the fact that several of the rats reached the maximum number of trials to criterion during training and the 600 s latency cut-off on the retention test. Consequently, the data were expressed as medians and inter-quartile (I.Q.) ranges and the non-parametric Kruskal-Wallis and Mann-Whitney U tests were used to detect differences between treatment groups.

## Results

### Experiment 1

Figure 6.1 illustrates the approximate locations of the bilateral hippocampal infusions. Drug infusions into the hippocampus did not significantly affect SA performance [ $F(5, 72) = 1.99$ ;  $p > .05$ ; see Figure 6.2A]. Specifically, these data showed that hippocampal infusions of glucose did not interact with co-infusions of the subeffective dose of muscimol to produce a memory deficit. Drug infusions into the hippocampus also did not significantly affect the number of arms that the rats entered in the maze [ $F(5, 72) = 2.30$ ;  $p > .05$ ; see Figure 6.2B].

### Experiment 2

Figure 6.3A shows that drug infusions into the hippocampus using the higher dose of muscimol significantly affected SA performance [ $F(5, 72) = 2.98$ ;  $p < .05$ ]. Hippocampal infusions of muscimol (0.4  $\mu$ g) significantly impaired SA performance. Specifically, the scores of rats given hippocampal infusions of muscimol (0.4  $\mu$ g) were significantly lower than those of rats given hippocampal infusions of vehicle ( $p < .05$ ). Hippocampal infusions of glucose alone did not significantly affect percent alternation scores. That is, the scores of rats given hippocampal infusions of glucose (33 or 50 nmol) were not different from those of rats given



hippocampal infusions of vehicle ( $p > .05$ ). More importantly, hippocampal co-infusions of glucose attenuated (33 nmol) or reversed (50 nmol) the memory deficits produced by muscimol. Specifically, the scores of rats given hippocampal co-infusions of 33 nmol of glucose with muscimol were not significantly different from those of rats given hippocampal infusions of vehicle or muscimol ( $p > .05$ ). The scores of rats given hippocampal co-infusions of glucose (50 nmol) with muscimol (0.4  $\mu$ g) were not significantly different from those of rats given hippocampal infusions of vehicle ( $p > .05$ ), but were significantly higher than those rats given hippocampal infusions of muscimol ( $p < .05$ ). Drug infusions into the hippocampus did not significantly affect the number of arms that the rats entered in the maze [ $F(5,72) = 1.68$ ;  $p > .05$ ; see Figure 6.3B].

### Experiment 3

Hippocampal drug infusions did not significantly affect trials to criterion during CMIA training [ $\chi^2(5,62) = 9.05$ ;  $p > .05$ ; see Figure 6.4A]. Figure 6.4B illustrates that the pre-training drug infusions into the hippocampus significantly affected subsequent CMIA retention performance [ $\chi^2(5,62) = 14.45$ ;  $p < .05$ ]. Hippocampal infusions of glucose (33 or 50 nmol) did not significantly affect CMIA retention. Specifically, the retention latencies of rats that were given hippocampal infusions of glucose (33 or 50 nmol) were not significantly different from those given hippocampal infusions of vehicle [ $U(1,21) = 52.0$ ;  $p > .05$ ]. Hippocampal infusions of muscimol (3  $\mu$ g) significantly impaired shock avoidance performance. The retention latencies of rats given hippocampal infusions of muscimol were significantly lower than those of rats given MS infusions of vehicle [ $U(1,23) = 19.0$ ;  $p < .05$ ]. Hippocampal infusions of glucose attenuated (33 nmol) or reversed (50 nmol) the avoidance deficits produced by hippocampal muscimol infusions. The retention latencies of rats that were given hippocampal infusions of

muscimol with 33 nmol of glucose did not significantly differ from those of rats given hippocampal infusions of vehicle [ $U(1,20) = 28.5$ ;  $p > .05$ ] or muscimol [ $U(1,21) = 39$ ;  $p > .05$ ]. Furthermore, the retention latencies of rats that were given hippocampal infusions of muscimol with 50 nmol of glucose were not significantly different from those of rats given hippocampal infusions of vehicle [ $U(1,22) = 51.5$ ;  $p > .05$ ], but were significantly higher than those of rats given hippocampal infusions of muscimol alone [ $U(1,23) = 20.0$ ;  $p < .05$ ].

### Discussion

Collectively, the present findings show that hippocampal co-infusions of muscimol and glucose, at doses that have no effect alone, do not produce memory deficits. Rather, these present findings demonstrate that the memory deficits produced by hippocampal GABA receptor activation are *reversed* by hippocampal co-infusions of glucose in two memory tasks. The present findings are consistent with previous research indicating that hippocampal infusions of glucose enhance memory (McNay, Fries, & Gold, 2000; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Stefani & Gold, 2001b) and reverse the memory-impairing effects of MS GABA receptor activation (Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997). These findings are also consistent with data showing that hippocampal infusions of glucose affect both spatial working memory and emotional, long-term memory tasks (Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b). These findings, however, are in direct contrast with previous research showing that MS glucose administration exacerbates the impairing effects of muscimol or interacts with subeffective doses to produce memory deficits (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). Combined with previous data showing that the effects of glucose are

neurotransmitter-dependent, the present data show that the effects of glucose on memory are also brain region-dependent.

The motivational, temporal, and cognitive differences between the SA and continuous multiple trial inhibitory avoidance task support the hypothesis that glucose influences memory rather than some other process that influences performance in a memory task. The finding that the hippocampal infusions affected memory in both tasks is consistent with extensive data showing that the hippocampus is involved in these two types of memory (Chang & Gold, 2004; Deacon, Bannerman, Kirby, Croucher, & Rawlins, 2002; Johnson, Olton, Gage, & Jenko, 1977; Krebs & Parent, 2005a; Lovely, Grossen, Moot, Bauer, & Peterson, 1971; Parent, Laurey, Wilkniss, & Gold, 1997). Specifically, the findings from the SA task suggest that the hippocampus is involved in on-line spatial associations, and the findings from the continuous multiple trial inhibitory avoidance task reveal that the hippocampus also affects emotional and long-term memory. The fact that the pretraining infusions of glucose did not affect acquisition in the CMIA task suggests further that elevating glucose in the hippocampus influences consolidation of newly formed emotional memories.

To the best of our knowledge, these are the first data showing that the effects of glucose on memory are brain region-dependent. These data add to the growing evidence showing that the effects of glucose vary by brain area. For instance, variations in brain glucose transport and brain extracellular glucose levels associated with performance on a memory task are brain region-specific (Choeiri, Staines, Miki, Seino, & Messier, 2005; Fellows & Boutelle, 1993; Fellows, Boutelle, & Fillenz, 1992; McNay, Fries, & Gold, 2000; McNay, McCarty, & Gold, 2001). Specifically, SA performance decreases hippocampal extracellular glucose levels, without changing striatal extracellular glucose levels (McNay, McCarty, & Gold, 2001). These

differences could contribute to the differential effects of glucose on memory in the MS versus the hippocampus by affecting the dose-response properties of glucose in these two brain areas. For example, if extracellular glucose levels are higher in the MS than the hippocampus, then smaller increases in glucose may be required to produce memory deficits in the MS than in the hippocampus.

There are several other possible mechanisms that may contribute to the brain region-dependent effects of glucose. One possibility is that glucose affects the functioning of different neurotransmitters in different brain areas. Extensive evidence indicates that glucose has positive effects on memory via increases in acetylcholine (ACh) particularly in the hippocampus (Kopf, Buchholzer, Hilgert, Loffelholz, & Klein, 2001; Messier, Durkin, Mrabet, & Destrade, 1990; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b). In contrast, the fact the glucose produces memory deficits when combined with GABA agonists in the MS (Parent & Krebs, 2004; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004) suggests that glucose may have negative effects on memory in the MS by increasing GABA synthesis or release. This possibility is supported by the finding that acute administration of large amounts of glucose and experimentally induced chronic hyperglycemia increase GABA levels in the brain (Amoroso, Schmid-Antomarchi, Fosset, & Lazdunski, 1990; During, Leone, Davis, Kerr, & Sherwin, 1995; Fink & Gothert, 1993; Fink, Zentner, & Gothert, 1994; Ohtani, Ohta, & Sugano, 1997). Glucose metabolism yields two molecules of pyruvate and two molecules of adenosine-tri-phosphate (Hertz & Dienel, 2002). Previous research has demonstrated that infusions of the glycolytic metabolite pyruvate mimic both the memory-impairing and memory-enhancing effects of glucose (Krebs & Parent, 2005c; Shah & Parent, 2003, 2004). Pyruvate metabolism yields by-products necessary for GABA and ACh synthesis (Dolezal & Tucek, 1981; Gibson, Jope, &

Blass, 1975; Hertz & Dienel, 2002; Lefresne, Guyenet, & Glowinski, 1973). Collectively, these findings raise the possibility that different products of glycolytic metabolism could mediate the positive versus negative effects of glucose on memory. These different products could vary as a function of brain region.

Another possibility is that glucose both enhances and impairs memory via an influence on the same process in different brain areas. For instance, glucose may increase ACh in the MS as it does in the hippocampus. Although increasing ACh function in the brain, including the MS, typically has positive consequences for learning and memory (Gold, 2003a, 2003b), there are cases where MS administration of the cholinergic agonist oxotremorine can produce memory deficits (Elvander et al., 2004; Pang & Nocera, 1999) and decrease hippocampal ACh levels (Gorman, Pang, Frink, Givens, & Olton, 1994). Furthermore, oxotremorine has negative consequences for long-term potentiation in the striatum (Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998), supporting the possibility that ACh can have negative effects on memory.

In summary, hippocampal co-infusions of glucose reverse memory deficits produced by hippocampal GABA receptor activation. The effects of glucose were observed in a hippocampal-dependent task that involves short-term, spatial working memory and one that is more dependent on long-term, emotional memory. Combined with previous results showing that similar manipulations in the MS produce memory deficits, these findings show that the memory-impairing interaction between glucose and GABA in the MS is not a general property of the brain, but rather is brain region-dependent.

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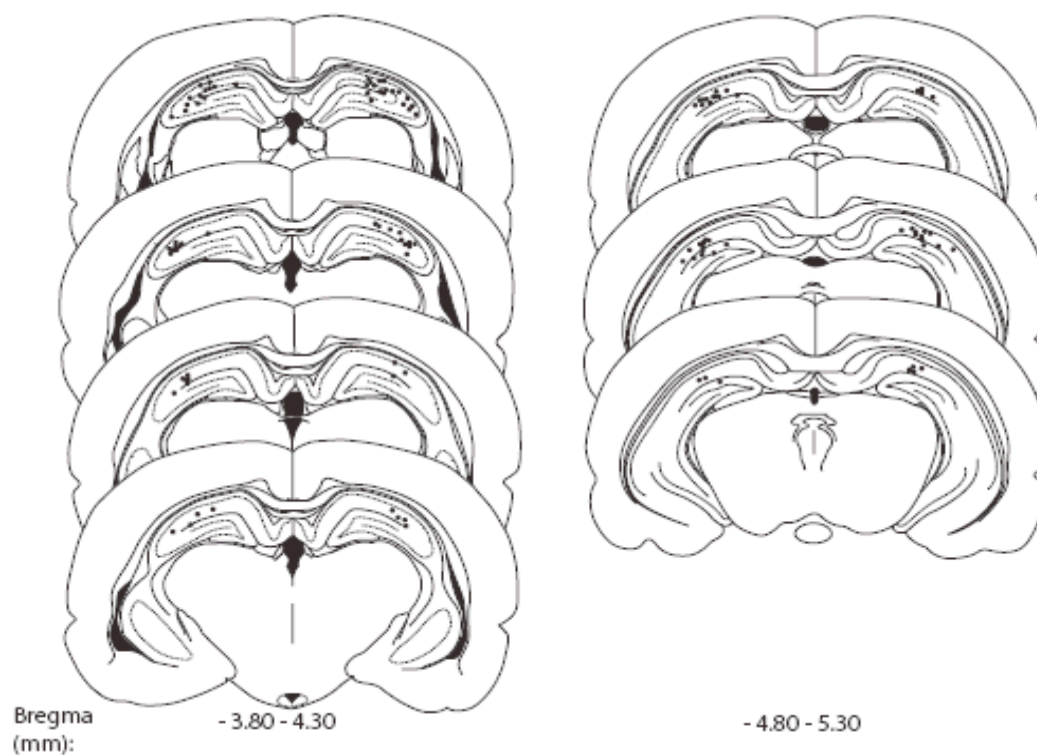


Figure 6.1. Schematic illustration of coronal sections of the rat brain showing the approximate location of bilateral hippocampal infusion sites in Experiment 1. Atlas plates were adapted from Paxinos and Watson (1998).

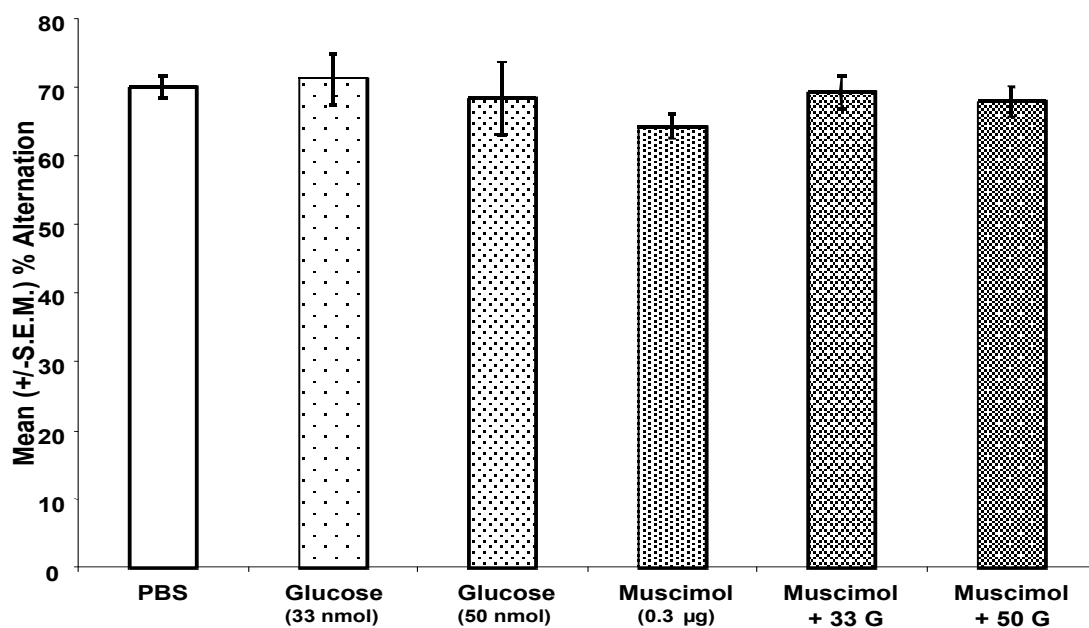


Figure 6.2A. Hippocampal co-infusions of glucose with muscimol did not significantly affect the mean (+/-S.E.M.) percent alternation scores ( $p > .05$  vs. PBS).

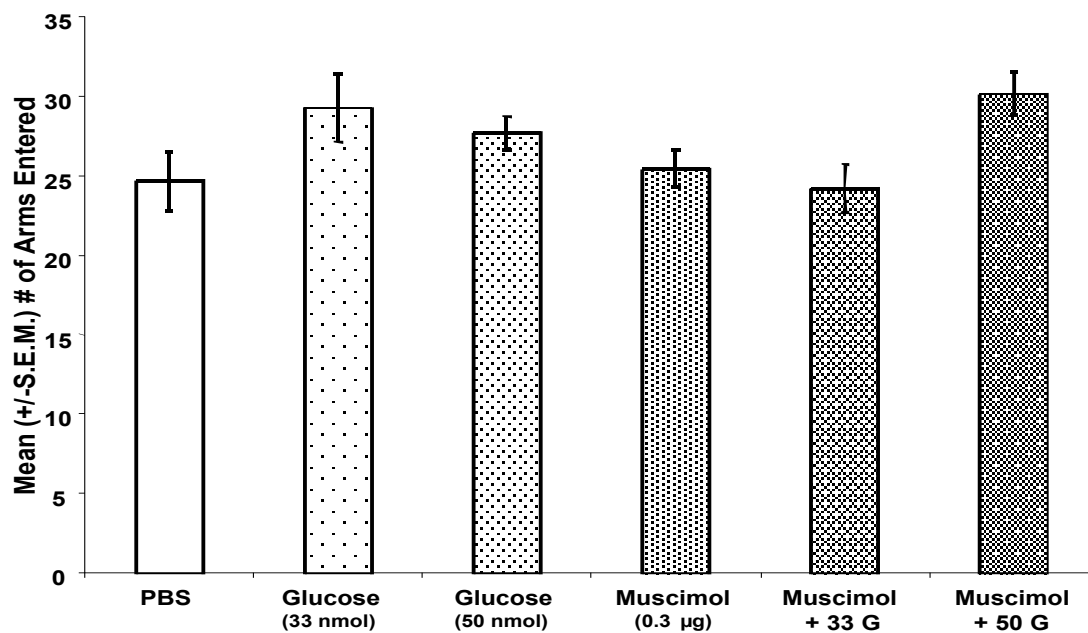


Figure 6.2B. Hippocampal co-infusions of glucose with a low dose of muscimol did not affect the mean (+/-S.E.M.) number of arm entries ( $p > .05$  vs. PBS).

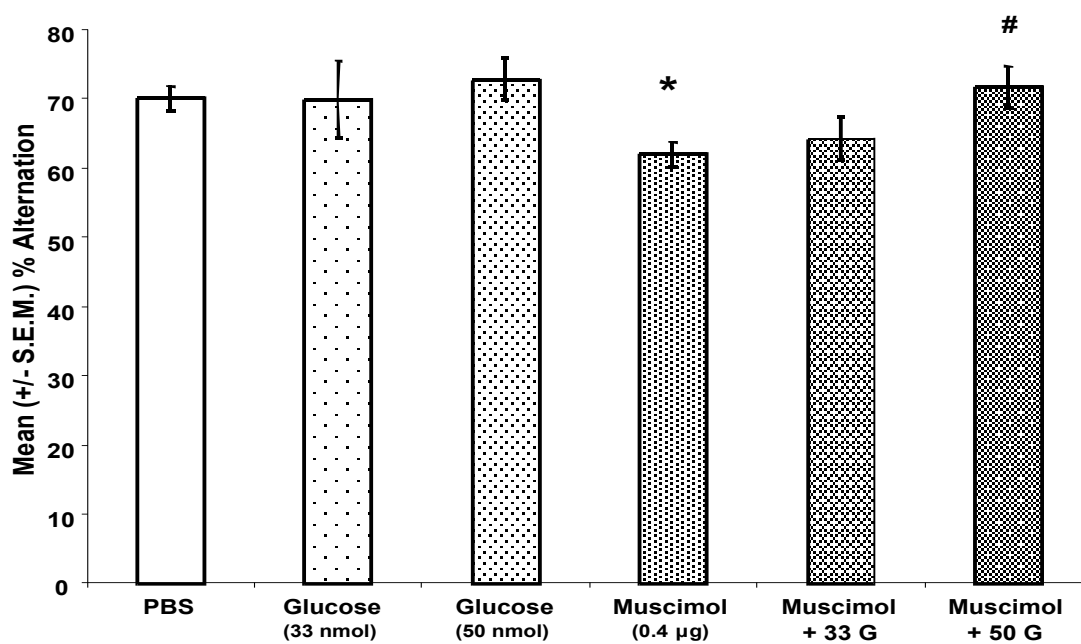


Figure 6.3A. Hippocampal infusions of a higher dose of muscimol significantly decreased mean (+/- S.E.M.) percent alternation scores (\* $p < .05$  vs. PBS). Hippocampal co-infusions of glucose attenuated (33 nmol;  $p > .05$  vs. PBS) or reversed (50 nmol; # $p < .05$  vs. muscimol,  $p > .05$  vs. PBS) the muscimol-induced alternation deficits.

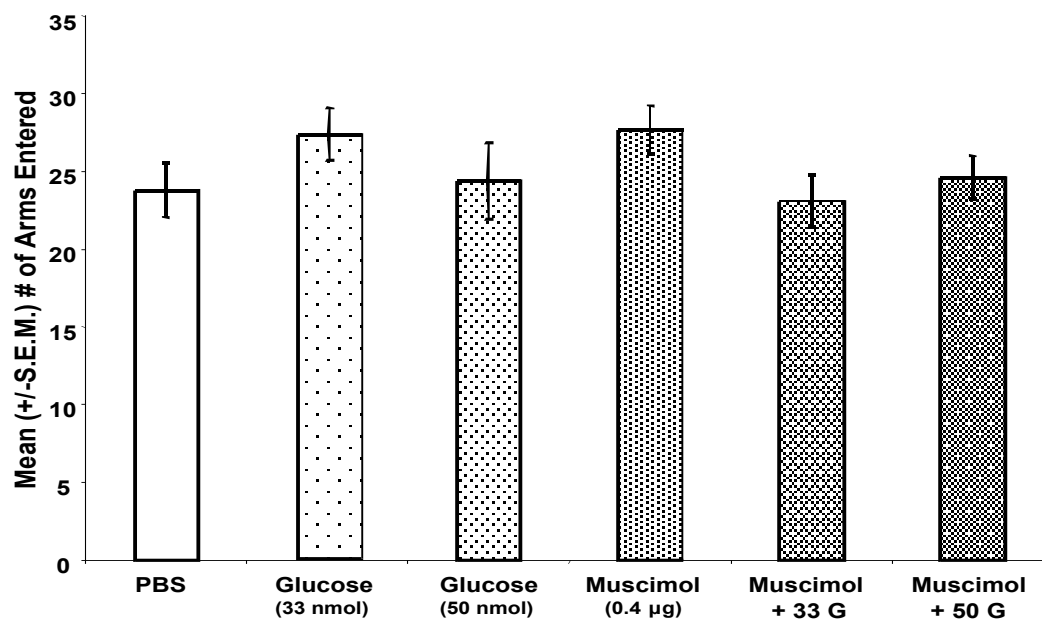


Figure 6.3B. Hippocampal infusions did not significantly affect the mean (+/-S.E.M.) number of arm entries ( $p > .05$  vs. PBS).

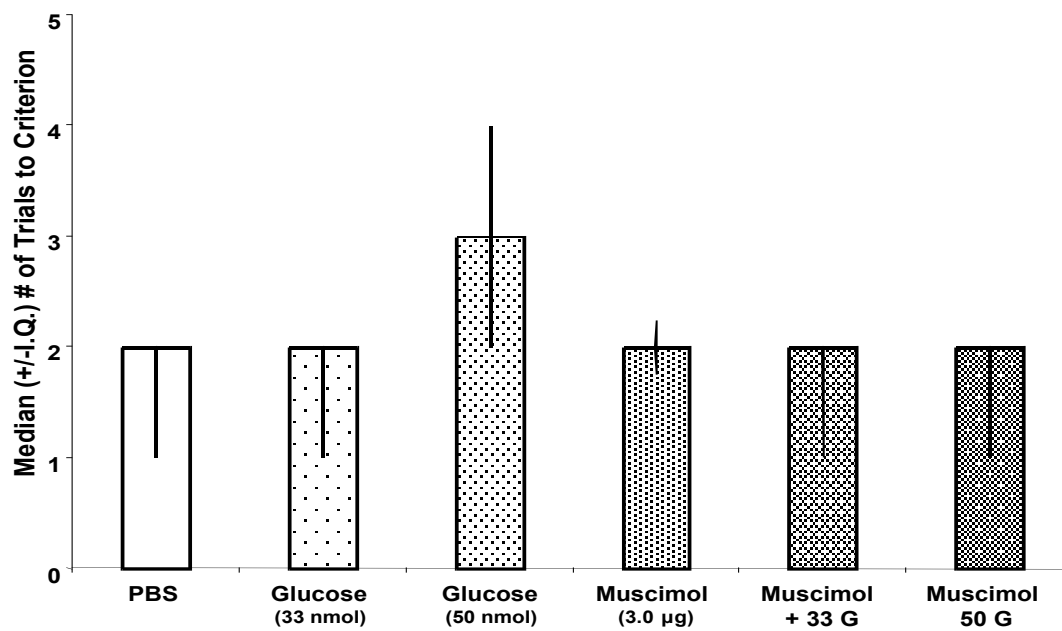


Figure 6.4A. Hippocampal infusions did not significantly affect the median ( $\pm$  I.Q.) number of trials to criterion ( $p > .05$  vs. PBS).

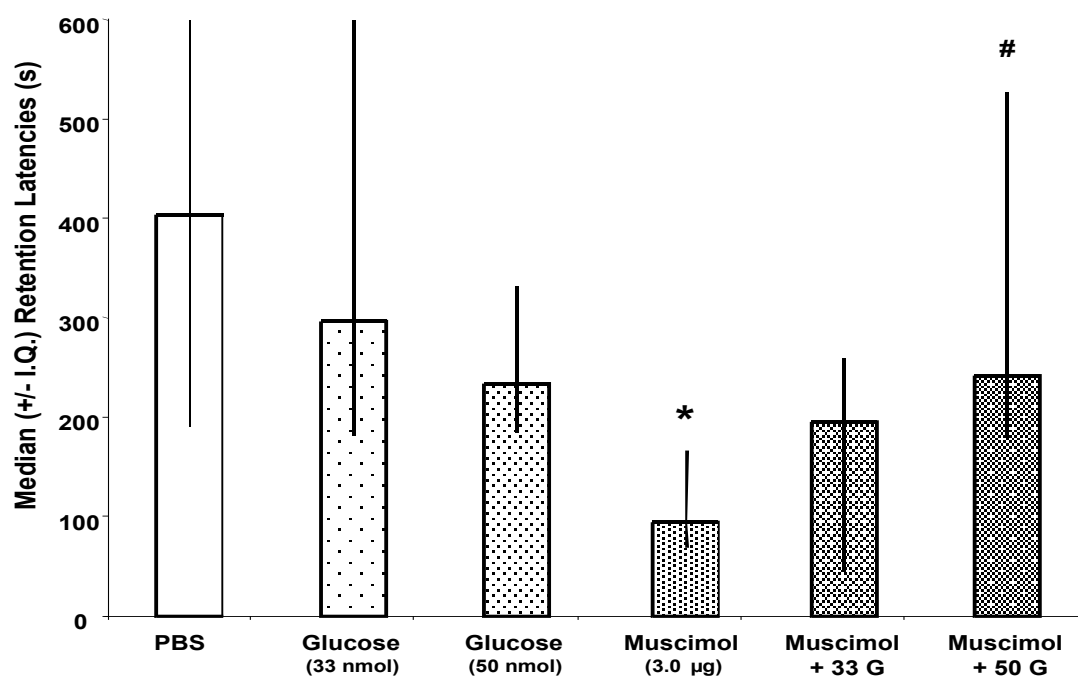


Figure 6.4B. Hippocampal infusions of 3.0 µg muscimol significantly decrease median (+/- I.Q.) retention latencies (\* $p < .05$  vs. PBS). Hippocampal co-infusions of glucose attenuated (33 nmol;  $p > .05$  vs. PBS) or reversed (50 nmol; # $p < .05$  vs. muscimol,  $p > .05$  vs. PBS) the alternation deficits.



**CHAPTER 7****REGIONAL DIFFERENCES IN EXTRACELLULAR GLUCOSE LEVELS DO NOT  
ACCOUNT FOR THE BRAIN REGION-DEPENDENT EFFECTS OF GLUCOSE ON  
MEMORY**

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### Abstract

We have demonstrated repeatedly that glucose infusions into the medial septum (MS) exacerbate memory deficits produced by co-infusions of  $\gamma$ -aminobutyric acid (GABA) receptor agonists. In contrast to these negative effects, we showed recently that hippocampal glucose infusions reverse the impairing effects of co-infusions of the GABA agonist muscimol. Emerging evidence suggests that extracellular (ECF) glucose concentrations vary by brain region, raising the possibility that there may be differences in ECF glucose levels in the MS versus the hippocampus. Such differences could affect the dose-response properties of glucose and contribute to its brain region-dependent effects on memory. Experiment 1 quantified ECF glucose levels in the MS and hippocampus of freely-moving rats using dual-probe, zero net flux *in vivo* microdialysis procedures. Experiment 2 estimated ECF glucose levels, using zero net flux *in vivo* microdialysis procedures, in the MS and hippocampus at different times. The results indicated that ECF glucose levels in the hippocampus and MS are comparable ( $\sim 1.0$ - $1.2$  mM). Specifically, ECF glucose concentrations in the MS were estimated to be  $1.22 \pm .25$  (Experiment 1) or  $1.39 \pm .36$  (Experiment 2). The ECF glucose concentrations in the hippocampus were estimated to be  $.96 \pm .15$  (Experiment 1) and  $1.27 \pm .13$  (Experiment 2). Collectively, these findings suggest that the opposite effects of glucose in the MS and hippocampus are not likely due to regional differences in basal ECF glucose concentrations.

**Keywords:** Zero Net Flux, *in vivo* microdialysis, glucose, memory,

## Introduction

Although glucose typically enhances memory (Lee, Graham, & Gold, 1988; Stefani, Nicholson, & Gold, 1999; Sunram-Lea, Foster, Durlach, & Perez, 2002) and reverses memory deficits (Craft et al., 1999; Gold, 1995; Korol, 2002; Korol & Gold, 1998; Manning, Ragozzino, & Gold, 1993; Newcomer et al., 1999; Petit, 1988), elevated glucose levels are also associated with memory dysfunction (Biessels et al., 1996; Convit, 2005; Flood, Mooradian, & Morley, 1990; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; Hagemann, Sartory, Hader, & Kobberling, 2005; McCall, 2002; Winocur et al., 2005). The mechanisms that dissociate these positive and negative effects of glucose on memory are poorly understood. It is known, though, that the impairing and enhancing effects of glucose are mediated, at least in part, by the brain (Krebs & Parent, 2005a; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Ragozzino & Gold, 1995b; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Shah & Parent, 2003, 2004; Stefani & Gold, 1998, 2001b).

The memory-modulating effects of glucose administration are neurotransmitter- and brain region-dependent. For instance, glucose reverses deficits produced by some neuroactive substances but not others. Specifically, glucose administration reverses deficits produced by morphine, galanin, and scopolamine (Ragozzino & Gold, 1994, 1995b; Ragozzino, Parker, & Gold, 1992; Rashidy-Pour, 2001; Stefani & Gold, 1998), but not the memory deficits produced by GABA agonists (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997). Rather, septal (MS) infusions of glucose potentiate the memory-impairing effects of GABA<sub>A</sub> agonists (Parent & Gold, 1997). Furthermore, MS co-infusions of glucose with the GABA agonist muscimol, the benzodiazepine chlordiazepoxide, or the GABA<sub>B</sub> agonist baclofen, at doses that individually do not affect memory performance, produce memory deficits (Erickson, Watts, &

Parent, 2006; Parent, Laurey, Wilkniss, & Gold, 1997; Shah & Parent, 2003, 2004).

Interestingly, the memory-impairing interaction between glucose and GABA receptor activation observed in the MS does not generalize to the hippocampus (Krebs & Parent, 2005b; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004), a brain region where glucose typically has positive effects on memory (Krebs & Parent, 2005a; Parent & Gold, 1997; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Ragozzino, Unick, & Gold, 1996). Specifically, hippocampal infusions of glucose do not exacerbate, but rather reverse memory deficits produced by hippocampal co-infusions of the GABA receptor agonist muscimol (Krebs & Parent, 2005b).

Several other lines of evidence indicate that the effects of glucose on brain function vary by brain area. Previously, glucose was assumed to be contained within one universal brain compartment and to have the same fate and effect in all neuronal cells (Lund-Anderson, 1979; Sokoloff et al., 1977). More recent evidence, however, suggests many characteristics of glucose are not uniform throughout the brain. For instance, there are brain region differences in glucose utilization (Gage, Kelly, & Bjorklund, 1984; Gerber, Choki, Brunswick, Reivich, & Frazer, 1983; Kimbrell et al., 2002b). In addition, basal extracellular (ECF) glucose concentrations and glucose transporter distribution, activation, and expression are brain region-dependent (Barros, Porras, & Bittner, 2005; Choeiri, Staines, Miki, Seino, & Messier, 2005; Khandelwal et al., 2004; McNay, McCarty, & Gold, 2001). Acute increases in blood glucose either increase or decrease cerebral blood flow depending on the brain region (Duckrow, 1988). The effects of glucose on electrochemical signaling also vary by neuronal phenotype, brain area, and glucose concentration (Fioramonti, Lorsignol, Taupignon, & Penicaud, 2004; Song, Levin, McArdle, Bakhos, & Routh, 2001; Wang et al., 2004). For instance, within the hypothalamus, glucose

increases the electrical activity of some cells and decreases the activity of others via an effect on different channels (Song, Levin, McArdle, Bakhos, & Routh, 2001). Importantly, changes in brain ECF glucose levels associated with performance in a memory task are brain region-specific. Specifically, performance in a spatial working memory task decreases brain ECF glucose levels in the hippocampus, without changing ECF glucose levels the striatum (McNay, McCarty, & Gold, 2001). Similarly, performance in an operant conditioning task produces selective increases in glucose transporter expression in the hippocampus (Choeiri, Staines, Miki, Seino, & Messier, 2005).

The finding that there are brain region-dependent differences in glucose concentration (Fray, Boutelle, & Fillenz, 1997; McNay & Gold, 1999; McNay, McCarty, & Gold, 2001) raises the possibility that the brain region-dependent effects of glucose on memory are related to differences in basal differences in ECF glucose concentrations. More specifically, these potential differences in ECF glucose concentrations may affect the dose-response properties of glucose and contribute to its brain region-dependent effects on memory. Although the ECF glucose concentrations of the hippocampus are estimated to be 1.0 -1.2 mM (McNay & Gold, 1999), the ECF glucose concentration of the MS is unknown. If basal MS ECF glucose levels are higher than those of the hippocampus, then smaller increases in glucose may be required to produce memory deficits in the MS than in the hippocampus. To test this hypothesis, the present set of experiments determined whether basal ECF glucose would differ between the MS and hippocampus. Specifically, the goal of Experiment 1 was to use dual-probe, zero-net-flux (ZNF) *in vivo* microdialysis procedures to estimate basal ECF glucose levels in the MS and hippocampus and determine whether they differ significantly. The MS and hippocampus are highly connected brain regions that interact as a system to influence memory (Borisjuk,

Denham, Denham, & Hoppensteadt, 1999; Gold, 2003a; Izquierdo & Medina, 1997; Parent & Baxter, 2004). This connectivity between the MS and the hippocampus raises the possibility that manipulations of the glucose concentration in one brain area during ZNF dual probe procedures, may influence the glucose concentration in the other. As a result, Experiment 2 determined whether basal ECF glucose concentrations would differ between the MS and hippocampus when ZNF *in vivo* microdialysis procedures were used to sample each brain area separately rather than simultaneously.

## Results

Figure 7.1 shows the approximate locations of MS and hippocampal dialysis probes for Experiment 1. The results show that ECF glucose levels in the MS and hippocampus are similar [ $t(1,16) = .326, p > .05$ ]. More specifically, these data demonstrate that the ECF glucose concentration is  $1.22 \pm .249$  mM in the MS and  $.96 \pm .15$  mM in the hippocampus (see Figure 7.2). The ECF glucose concentrations obtained when the data were expressed as the means of the individual ZNF regression values (see Figure 7.2 inset) were slightly different from the ECF glucose concentrations estimated using ZNF linear regression of all the rat's mean values at each concentration of glucose.

The approximate locations of the MS and hippocampal dialysis probes in Experiment 2 were similar to those in Experiment 1. As in the dual probe experiment, the results of Experiment 2 show that ECF glucose in the MS and hippocampus are similar, when each brain area was sampled on two separate occasions [ $t(1,25) = 1.11, p > .05$ ]. More specifically, these data demonstrate that the ECF glucose concentration is  $1.39 \pm .36$  mM in MS and  $1.27 \pm .13$  mM in the hippocampus (see Figure 7.2). Thus, collectively the data from the two experiments

indicated that the ECF glucose concentration is approximately 1.0-1.2 mM in both brain areas (see Figures 7.2 & 7.3).

The sampling procedure did not affect the mean ECF glucose concentrations obtained in Experiment 1 and 2 [ $F(1,44) = .247, p > .05$ ]. There was also no significant difference in terms of the brain region sampled in Experiment 1 and 2 [ $F(1,44) = .736, p > .05$ ]. More importantly, the sampling procedure did not significantly interact with the brain region to affect the mean ECF glucose concentrations found in the MS versus hippocampus in Experiment 1 and 2 [ $F(1,44) = .192, p > .05$ ]. Similar to the results obtained in Experiment 1, the ECF glucose concentrations obtained when the data were expressed as the means of the individual ZNF regression values (see Figure 7.3 inset) were slightly different from the ECF glucose concentration estimated based on ZNF determination with the linear regression of all the mean values for each rat at each concentration of glucose (see Figure 7.3).

### Discussion

These findings demonstrate that the basal ECF glucose concentrations do not significantly differ between the MS and hippocampus. These data replicate previous findings showing that the basal ECF glucose levels in the hippocampus are approximately 1.0 mM. This 1.0 mM concentration is not uniform across the brain. Specifically, striatal ECF glucose levels are approximately 30% lower than hippocampal glucose levels (Fray, Boutelle, & Fillenz, 1997; McNay, McCarty, & Gold, 2001). These findings extend previous finding by showing that the ECF glucose concentration of the MS is similar to the hippocampus rather than to levels observed in the striatum. As a result, these findings do not support the notion that the different effects of glucose in the MS versus hippocampus on memory are due to differences in basal ECF concentrations of glucose in these two brain areas. These findings also demonstrate whether the

two areas were sampled simultaneously or separately did not influence the values that were obtained. These findings are of methodological importance because they suggest that using a dual-probe dialysis design to sample ECF neurochemicals within an interacting system may not create an artifactual result. Furthermore, based on the finding that the ZNF determination was different depending on the statistical analyses used, these findings suggest that when comparing ZNF values obtained between different reports, it may be important to take into account the statistical methods used to analyze the ZNF data.

Collectively, these findings suggest that basal differences in ECF glucose concentration do not likely contribute to the different effects of glucose on memory in the MS versus hippocampus. Specifically, MS infusions of glucose impair memory when co-infused with the GABA receptor agonist muscimol (Parent & Krebs, 2004; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004). These same manipulations; however, reverse muscimol-induced deficits in the hippocampus (Krebs & Parent, 2005b). We postulated that differences in basal ECF glucose concentrations may affect the dose response properties of glucose in these two brain regions and account for these opposing, brain region-dependent effects of glucose on memory. This hypothesis was based in part, on the finding that peripheral administration of large amounts of glucose impairs memory, whereas small amounts of glucose enhance memory (Parsons & Gold, 1992). These present findings, however, do not support this hypothesis.

The brain region-dependent effects of glucose may be related to differences in activity-dependent changes in ECF glucose concentrations in the MS versus hippocampus. In some cases, the effects of manipulations on neurochemical function differ in resting versus active animals, such as animals learning in a maze, walking on a treadmill, feeding, or mating (Etgen &



Morales, 2002; Rada, Mendiola, Hernandez, & Hoebel, 2003; Ragozzino, Pal, Unick, Stefani, & Gold, 1998a; Ragozzino, Unick, & Gold, 1996; Westerink, 1995). For example, although systemic and hippocampal infusions of glucose do not affect hippocampal ECF ACh levels in a rat in its home cage, the same injections produce a large increase when the rat is exploring a maze (Ragozzino, Pal, Unick, Stefani, & Gold, 1998a; Ragozzino, Unick, & Gold, 1996). Previous evidence has shown the hippocampal ECF glucose concentrations decrease by 30% while rats are performing a SA task. In contrast, ECF glucose concentrations do not decrease in the striatum, a brain area that does not appear to be involved in SA (McNay, Fries, & Gold, 2000; McNay, McCarty, & Gold, 2001). These previous findings raise the possibility that any differences in ECF glucose levels between the MS and hippocampus may only be observed when a rat is engaged in a memory task. This possibility is unlikely; however, because lesion studies show that both the MS and hippocampus are involved in SA performance (Chang & Gold, 2004; Dunnett, Low, Iversen, Stenevi, & Bjorklund, 1982; Jeltsch et al., 1994; Liu et al., 2002; Pallage, Toniolo, Will, & Hefti, 1986).

There are several alternative mechanisms that may contribute to the brain region-dependent effects of glucose on memory. One possibility is that differences in glucose utilization between the two brain regions may be involved. This possibility is unlikely, though, because glucose utilization between the MS and hippocampus is similar (Huang, Tsai, Huang, & Sim, 1999; Wree, Goller, & Beck, 1995). That finding is consistent with the current finding that basal ECF glucose levels also do not differ. Alternatively, the distribution of glucose transporters may be different between these two brain regions and may contribute to the brain region-dependent effects of glucose. For example, the dissociation constants for GLUT1, GLUT3, and GLUT4 transporter proteins differ (Hertz & Dienel, 2002). Moreover, the rate of

transport of glucose into the cell also differs between different types of transporters (Carruthers, 1990; Maher, Davies-Hill, & Simpson, 1996; Vannucci, Gibbs, & Simpson, 1997). Furthermore, previous results show that GLUT1 but not GLUT3 transporters are elevated in the hippocampus during a learning task (Choeiri, Staines, Miki, Seino, & Messier, 2005), suggesting different transporters may be involved in different processes. These findings raise the possibility that variation in the location or distribution of those transporter proteins between the MS and hippocampus may differentially affect glucose function, even when the total number of transporters are equivalent between brain areas.

Glucose may impair memory through regional distributions of transporters that are co-localized to different types of neurons. That is, GLUT transporters may interact with neurotransmitters that up-regulate memory in one brain area and with neurotransmitters that down-regulate in another. For instance, GLUT4 transporters are co-localized to cholinergic neurons in the basal forebrain but not the hippocampus (Apelt, Mehlhorn, & Schliebs, 1999). Furthermore, these same transporters are co-localized with GABAergic projection neurons but not with GABA interneurons in the MS (Apelt, Mehlhorn, & Schliebs, 1999). Interestingly, GLUT 4 transporters are co-localized with GABA neurons in the hippocampus (Apelt, Mehlhorn, & Schliebs, 1999), suggesting this neuron specific localization of GLUT transporters may also be brain region-dependent. Combined with previous findings suggesting that increasing ACh function in the MS could impair memory (Elvander et al., 2004; Pang, 1999), these findings suggest that glucose may impair memory through these regional distributions of transporters that are co-localized to different types of neurons. Moreover, some drugs such as brain-derived peptides, insulin growth factor, and adrenergic receptor agonists can alter glucose transport and GLUT transporter expression (Boado, 1998; Boado & Pardridge, 1990; Boado,

Wu, & Windisch, 1999; Bondy & Cheng, 2002; Cheng & Liu, 2000; Duelli, Staudt, Maurer, & Kuschinsky, 1998) and the effects of these substances could vary by brain area (Ori, Freo, Pizzolato, & Dam, 2002).

Although the amount of glucose utilization between the MS and hippocampus does not appear to differ between the MS and hippocampus, it is possible that the distribution and type of brain glucose transporters do. Facilitative glucose transporters (GLUT) are responsible for glucose uptake into the brain and neurons. They exhibit both cell type and brain region-specific localization (Apelt, Mehlhorn, & Schliebs, 1999; Zeller, Rahner-Welsch, & Kuschinsky, 1997), suggesting that the transport of glucose is different between brain areas. Furthermore, previous research has shown that glucose transporter plasticity is correlated with learning a task (Choeiri, Staines, Miki, Seino, & Messier, 2005). Specifically, training on an operant task increases the expression of glucose transporters in the hippocampus (Choeiri, Staines, Miki, Seino, & Messier, 2005). Collectively, these findings suggest that behavioral-induced alterations in the transporter quantity in the MS versus hippocampus may account for the brain region-dependent effects of glucose on memory. Another possibility is that the distribution of different types of glucose transporters between these two brain regions may contribute the brain region-dependent effects of glucose on memory. For example, the dissociation constants for GLUT1, GLUT3, and GLUT4 transporter proteins differ (Hertz & Dienel, 2002). Moreover, the rate of transport of glucose into the cell also differs between different types of transporters (Carruthers, 1990; Maher, Davies-Hill, & Simpson, 1996; Vannucci, Gibbs, & Simpson, 1997). Furthermore, previous results show that GLUT1 but not GLUT3 transporters are elevated in the hippocampus during a learning task (Choeiri, Staines, Miki, Seino, & Messier, 2005), suggesting different transporters may be involved in different processes. These finding raise the possibility that

variation in the location or distribution of those transporter proteins between the MS and hippocampus may differentially affect glucose function, even when the total number of transporters are equivalent between brain areas.

The opposite effects of glucose on memory in different brain regions could also involve to interactions between GLUT transporters and different neurotransmitter groups. For instance, GLUT transporters may interact with neurotransmitters that may have positive or negative consequences for memory in different brain regions. For instance, GLUT4 transporters are co-localized with cholinergic neurons in the basal forebrain but not the hippocampus (Apelt, Mehlhorn, & Schliebs, 1999). Furthermore, these same transporters are co-localized with GABAergic projection neurons, but not GABA interneurons in the MS but not with GABA interneurons in the MS (Apelt, Mehlhorn, & Schliebs, 1999). Interestingly, GLUT 4 transporters are co-localized with GABA neurons in the hippocampus (Apelt, Mehlhorn, & Schliebs, 1999), suggesting this neuron specific localization of GLUT transporters may also be brain region-dependent. Moreover, some drugs such as brain-derived peptides, insulin growth factor, and adrenergic receptor agonists can alter glucose transport and GLUT transporter expression (Boado, 1998; Boado & Pardridge, 1990; Boado, Wu, & Windisch, 1999; Bondy & Cheng, 2002; Cheng & Liu, 2000; Duelli, Staudt, Maurer, & Kuschinsky, 1998), suggesting that the effects of the interactions between glucose with different drugs may affect also contribute to the brain region-dependent effects of glucose. This is supported by the finding that the effects of drugs on glucose utilization can vary by brain region (Ori, Freo, Pizzolato, & Dam, 2002).

Another possibility is that different products of glucose metabolism in the MS versus the hippocampus contribute to the brain region-dependent effects of glucose on memory. Glucose metabolism yields two molecules of pyruvate and two molecules of adenosine-tri-phosphate

(ATP; Hertz & Dienel, 2002). Pyruvate metabolism, in turn, yields by-products necessary for glutamate and GABA synthesis (Hertz & Dienel, 2002). Pyruvate also contributes to the synthesis of acetylcholine (ACh). Specifically, decarboxylation of pyruvate produces acetyl coenzyme A (CoA), one of the biosynthetic precursors for ACh (Tucek, 1983), and preventing pyruvate oxidation decreases ACh synthesis (Gibson, Jope, & Blass, 1975; Lefresne, Guyenet, & Glowinski, 1973). Previous research has demonstrated that infusions of the glycolytic metabolite pyruvate mimic both the memory-impairing and memory-enhancing effects of glucose (Krebs & Parent, 2005c; Shah & Parent, 2003, 2004).

Glucose could enhance memory through a process that involves ACh or glutamate in the hippocampus, but impair memory by increasing GABA function in the MS. Manipulations that increase hippocampal ACh enhance memory (Degroot & Parent, 2000, 2001; Disterhoft et al., 1999; Scali, Giovannini, Prosperi, Bartolini, & Pepeu, 1997). Furthermore, glucose administration enhances hippocampal ACh and memory when rats are performing in a memory task (Ragozzino & Gold, 1995b; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Ragozzino, Unick, & Gold, 1996). Glucose may also enhance memory by increasing glutamate in the hippocampus, because glucose administration also elevates hippocampal glutamate (Burke & Nadler, 1989; Gruetter, 2002; Hamberger, Chiang, Nylen, Scheff, & Cotman, 1979), which in turn can enhance memory (O'Donnell, Stemmelin, Nitta, Brouillette, & Quirion, 2003; Teyler, 1987). In contrast, the evidence showing the negative interaction between glucose and GABA in the MS (Parent & Krebs, 2004; Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004), raises the possibility that MS infusions of glucose may impair memory by increasing GABA levels in the MS. This is supported by the finding that, acute administration of large amounts of glucose and experimentally induced chronic hyperglycemia

increase GABA levels in the brain (Amoroso, Schmid-Antomarchi, Fosset, & Lazdunski, 1990; During, Leone, Davis, Kerr, & Sherwin, 1995; Fink & Gothert, 1993; Fink, Zentner, & Gothert, 1994; Ohtani, Ohta, & Sugano, 1997; Schmid-Antomarchi, Amoroso, Fosset, & Lazdunski, 1990).

Another possibility is that glucose both enhances and impairs memory via an influence on the same neurotransmitter in different brain regions. For instance, glucose may both enhance and impair memory via an influence on ACh in both brain areas. Although increasing ACh activity typically has positive consequences for learning and memory (Chang & Gold, 2003a; Degroot, Kornecook, Quirion, DeBow, & Parent, 2003; Ragozzino, Unick, & Gold, 1996), including the MS (Decker, Majchrzak, & Anderson, 1992; Givens & Olton, 1995; Givens & Olton, 1990; Olton et al., 1991), some studies have shown that MS administration of the cholinergic agonist oxotremorine can produce memory deficits (Elvander et al., 2004; Pang, 1999). Furthermore, oxotremorine infusions into the striatum impair striatal long-term-potential (Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998), which would be expected to impair memory. Elevations in ACh could have different effects on two brain regions due to difference in their connectivity. For example, MS elevations in ACh may enhance the activity of GABA interneurons in the MS and thus decrease the function of the cholinergic, GABAergic, and/or glutamatergic septo-hippocampal projections, which would be expected to impair memory (Durkin, 1992b; Parent & Baxter, 2004). In contrast, elevating these neurotransmitters in the hippocampus would be expected to enhance pyramidal cell function in the hippocampus, which would be expected to enhance memory (Farr, Flood, & Morley, 2000; Izquierdo, 1994; Izquierdo & Medina, 1995; Teyler, 1987).

In summary, ECF glucose concentrations are estimated to be ~1.0-1.2 mM in both the MS and the hippocampus. This finding was shown under conditions when both brain areas were sampled simultaneously or separately. Collectively, these findings suggest that differences in basal ECF glucose concentration do not likely contribute to the brain region-dependent effects of glucose on memory.

## **Experimental Procedure**

### **Experiment 1**

#### **Subjects**

Five male Sprague-Dawley derived rats weighing 200-250 g upon arrival (Charles River, Wilmington, MA) were used. The rats were housed individually in polycarbonate cages (20x40x20 cm) with corncob bedding on a 12 hour light-dark cycle (lights on at 7:00 a.m.) in a temperature-controlled colony room (70-74°F). Animals had free access to food and water. The rats were acclimated to lab conditions for approximately 1 week prior to surgery. The Georgia State University Institutional Animal Care and Use Committee (IACUC) approved all procedures involving rats.

#### **Surgery**

Prior to surgery, rats were placed in a clear, plastic gas induction chamber and anesthetized with 5% isoflurane (Baxter, Deerfield, IL) delivered in 1000 ml/min medical grade oxygen. After the rat was no longer ambulatory, it was removed from the chamber and placed on a face-mask that delivered 3% isoflurane. Rats were then given injections of atropine sulfate (0.4 mg/kg, ip, Baxter, Deerfield, IL) and penicillin (1500 units, im, Hanfords US Vet, Syracuse, NY). The future incision site was shaved with a #50 electric clipper blade (Oster) and betadine

solution was applied to the surgical area. The percentage of isoflurane given to the rats was adjusted (from 1-3%) to maintain a surgical plane of anesthesia as determined by the toe pinch test. A stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) that was equipped with an anesthesia mask was used to implant one 15 mm inert, biocompatible plastic dialysis probe guide cannulae (Bioanalytical Systems, Inc., Roanoke, VA) aimed at the MS (0.5 mm anterior to bregma [AP] and 4.9 mm ventral to dura [DV]) and one aimed at the dorsal hippocampus (-5.0 mm; AP, +/-4.5 from the interaural line, and -1.6 mm; DV; Paxinos & Watson, 1998). The hemisphere in which the unilateral hippocampal dialysis probe guide was implanted was counterbalanced across rats. The incision site was anesthetized locally with a 2% lidocaine/.001% epinephrine cocktail (0.5-2.0 cc, sc, Abbott Labs, Chicago, IL). The 2% lidocaine/.001 % epinephrine cocktail solution (.05- 1.0 cc) was then applied topically to the skull to facilitate seeing lambda and bregma. The cannula was secured to the skull with three jeweler's screws and cranioplastic cement and a stylet was inserted to keep the cannula free of debris. Immediately after surgery, the rats were given an injection of 0.9 % sterile saline (3.0 cc, sc) and the non-steroidal anti-inflammatory flunixin meglumine (2.5 mg/kg, ip, Fort Dodge Animal Health, Fort Dodge, IA) and then wrapped with a paper towel and kept under a warm lamp until recovery from anesthesia. Two days following surgery, the patency of each cannula was checked and betadine was applied to the surgical wound. If signs of infection were evident, the rats were anesthetized with isoflurane gas (5 %) and given an additional injection of penicillin (1500 units, im).

### ***In Vivo* Microdialysis Procedures**

Each rat was handled for 2 minutes on two separate occasions prior to microdialysis procedures. Each rat participated in two microdialysis sessions separated by at least 4 days.



Before and after all handling and *in vivo* microdialysis procedures, the rats were allowed a minimum of 30 minutes to habituate to the laboratory environment. *In vivo* microdialysis was conducted between 7:00 a.m. and 7:00 p.m. On the day of microdialysis, the rat was placed in a round Plexiglas containment bowl (BAS) and attached to a tether (BAS) that permitted him to move in the microdialysis containment bowl. Following a 1 hour habituation period, a polyacrylonitrile microdialysis probe (320  $\mu\text{m}$  OD; BAS) that extended 1 mm (MS) or 3 mm (hippocampus) beyond the guide cannula was inserted into the guide cannula of both brain areas at the same time. The 1 mm probe length was selected because it would restrict the probe to the MS area and allow for sampling of the D-V extent of the MS. The 3 mm probe length was selected for the hippocampus based on previous research using this length to investigate ECF glucose levels in the hippocampus (Canal, McNay, & Gold, 2005; McNay, Canal, Sherwin, & Gold, 2006; McNay, Fries, & Gold, 2000; McNay & Gold, 2001; McNay, McCarty, & Gold, 2001). Furthermore, this length would permit the sampling from a more representative extent of the D-V portions of the hippocampus. Using a microinfusion pump (BAS), the probes were perfused at the rate of 2  $\mu\text{l}/\text{min}$  with artificial cerebrospinal fluid (aCSF; mM: NaCl 128.0, KCl 3.0,  $\text{CaCl}_2$  1.3,  $\text{MgCl}_2$  1.0,  $\text{NaH}_2\text{PO}_4$  1.3,  $\text{NaHCO}_3$  21.0, pH 7.3; filtered [0.2  $\mu\text{M}$ ] and degassed). The flow rate of 2  $\mu\text{l}/\text{min}$  was chosen to permit the collection of sample volumes (10  $\mu\text{l}$ ) needed to assay glucose at 5 min sample periods. This is the sample period used in previous research estimating hippocampal ECF glucose levels (McNay & Gold, 1999; McNay, McCarty, & Gold, 2001). After a 2 h stabilization period, the ECF space of the MS and hippocampus were sampled using zero-net flux (ZNF) dual-probe *in vivo* microdialysis procedures. The two brain areas were perfused simultaneously with seven different quasi-randomly assigned concentrations of

glucose (0-3 mM) each for 6 sample periods. The range of glucose concentrations was based on the range of ECF glucose values observed in previous research (Fellows, Boutelle, & Fillenz, 1992; Forsyth et al., 1996; Fray, Boutelle, & Fillenz, 1997; Lund-Anderson, 1979; McNay, Fries, & Gold, 2000; McNay & Gold, 1999). The samples were kept on dry ice during the experiment and then transferred to a -80 °C freezer for storage until analysis. Half-way through each sample period, the overall motor activity of the rat was rated using a 5-point scale (0 = no obvious movement, 1 = head movement, 2 = head and fore-limb movement, 3 = infrequent movement of all 4 limbs [e.g., burrowing into bedding], and 4 = movement of all four paws with locomotion and/or rearing; Moore, Sarter, & Bruno, 1993).

### **Glucose Assay**

To increase the likelihood that the ECF glucose levels had reached steady state, only the last three of the six samples from each glucose concentration were assayed. The amount of glucose in each sample was measured using a glucose hexokinase kit (Pointe Scientific, Inc. Canton, MI) and a spectrophotometer (Molecular Devices; Spectra Max 340PC) with SOFTmax Pro software. Dialysate (8 µL) was diluted by adding 1mL of glucose hexokinase (HK) reagent (Pointe Scientific, Inc., Canton, MI) containing buffered (pH= 7.5) hexokinase (100 U/L), glucose-6-phosphate dehydrogenase (G<sub>6</sub>PDH;1000U/L), ATP (1.0 mM), and nicotinamide adenine dinucleotide (NAD; 1.0 mM) that was reconstituted with deionized water (dH<sub>2</sub>O). The solution was mixed and allowed to stand for a minimum of 3 min at room temperature. The spectrophotometer was zeroed with 200 µL dH<sub>2</sub>O at 340 nm. HK catalyzes the phosphorylation of glucose by ATP. The product, glucose-6-phosphate (G<sub>6</sub>P) is then oxidized with the concomitant reduction of NAD to NADH in the reaction catalyzed by G<sub>6</sub>PDH. The formation of NADH causes an increase in absorbance at 340 nm, and the increase is directly proportional to

the amount of glucose in the dialysate. The amount of NADH in each 200  $\mu$ L solution of sample was measured, in triplicate, and used as an index of the glucose concentration (mM). Fresh standards (0- 10 mM) were run for each assay and showed a linear response to glucose concentration.

## **Histology**

After behavioral testing, the rats were euthanized with an overdose of sodium pentobarbital (400 mg/kg, ip) and perfused intracardially with 0.9% saline followed by 10% formalin. Their brains were stored in a 10% formalin solution for at least 2 days before sectioning. All brains were sectioned on a cryostat (Leica CM 30510 S) and 45-60  $\mu$ m sections were taken through the MS cannulae tracts. The brain sections were stained with thionin and an unbiased observer determined the cannulae placement using a light microscope (Olympus BX41). Acceptable MS cannulae placement was defined as MS dialysis probe sites located within the MS, but not within the lateral septum or the ventral diagonal band of Broca. Moreover, the cannula must not have penetrated the fimbria. Acceptable placement for hippocampal cannula was defined as dialysis probe membranes located within hippocampal fields CA1, CA2, CA3, or dentate gyrus. Only rats with acceptable cannulae placements in both brain regions were included in the statistical analyses.

## **Statistical Analysis**

The change in concentration between the sample and perfusate in the mean of the last three samples of each glucose concentration (y-axis) was plotted against the perfusate concentration alone (x-axis) for each individual rat. Using regression analyses, the point at which the concentration of glucose perfused into the brain matched the glucose concentration in

the ECF (i.e., point of zero net flux) was determined (x-intercept when  $y = 0$ ) and used as a measure of ECF glucose levels. The ZNF data were expressed as mean intercept  $\pm$  SEM. The data from each concentration of glucose were also expressed as mean ( $\pm$ S.E.M.) glucose gain or loss. The resultant ZNF data from the MS and hippocampus were analyzed using paired t-tests to detect differences between the group ZNF intercept means. An alpha level of 0.05 was used as the criterion for statistical significance.

### Experiment 2

The same procedures as those used in Experiment 1 were used in the present experiment with the following exceptions: The current experiment determined whether basal ECF glucose concentrations would differ between the MS and hippocampus when ZNF *in vivo* microdialysis procedures were used to sample each brain area separately rather than simultaneously. Fourteen rats were used for Experiment 2.

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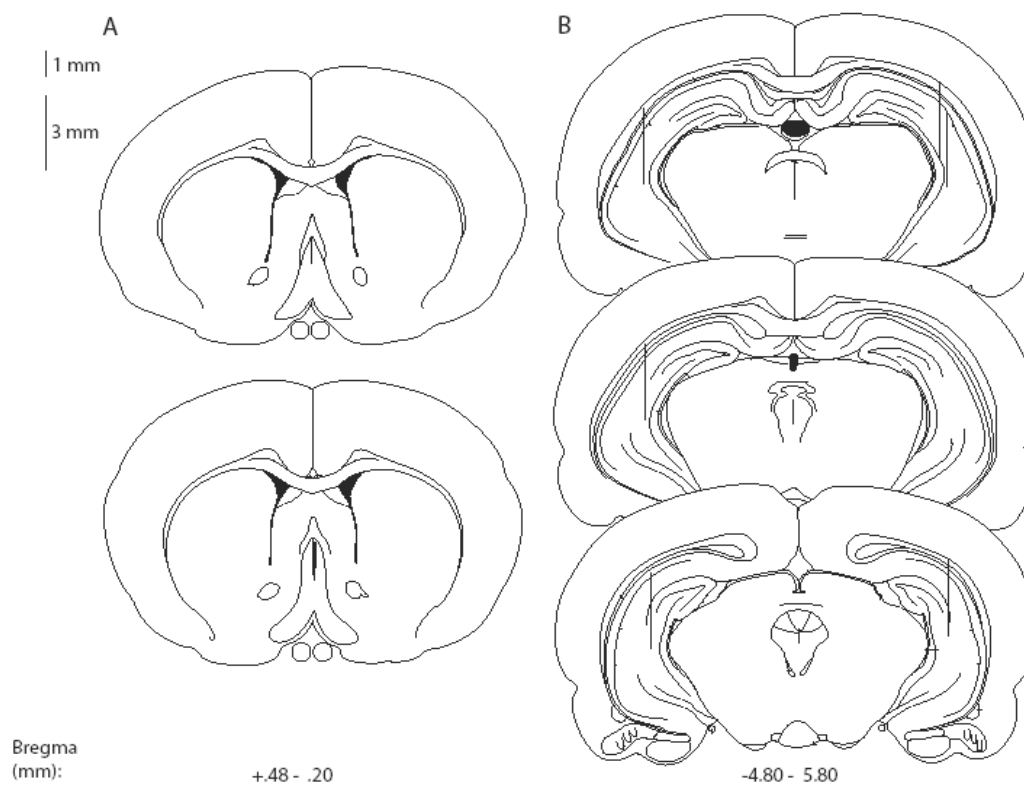


Figure 7.1. Schematic illustration of coronal sections of the rat brain showing the approximate locations of (A) MS and (B) hippocampal dialysis probe and infusion sites. Atlas plates were adapted from Paxinos and Watson (1998).

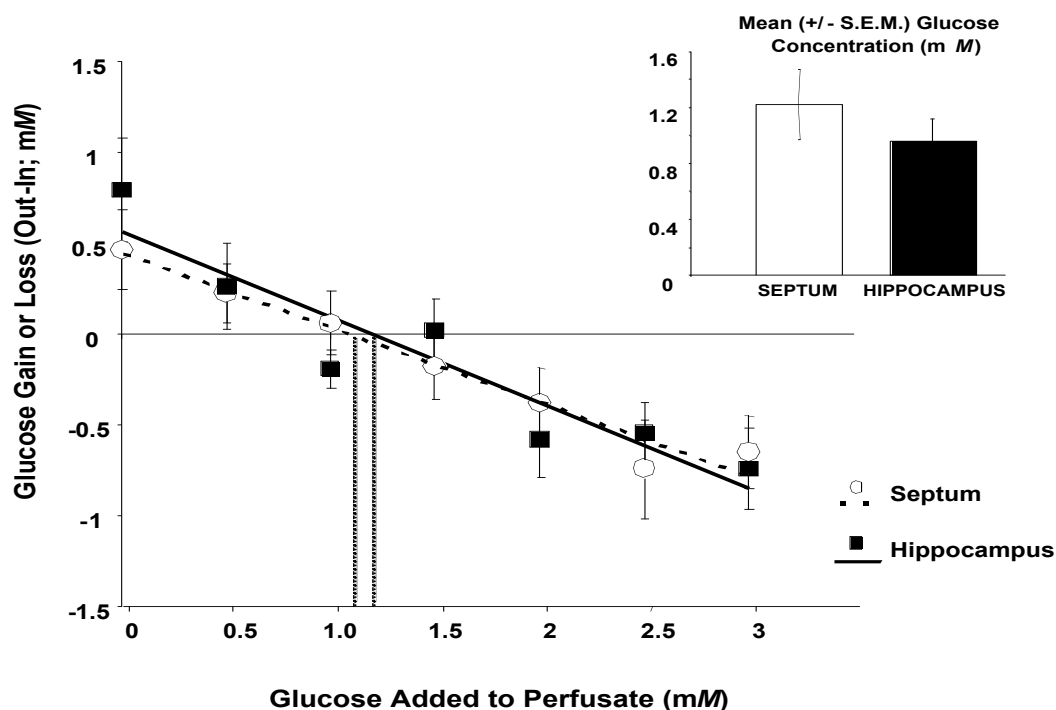


Figure 7.2. Glucose gain or loss to the brain as a function of perfusate concentration (0-3 mM) in the MS or hippocampus of rats using dual-probe microdialysis procedures. The point of zero net flux (ZNF) is the estimated ECF concentration of glucose. The solid (hippocampus) and dashed (MS) lines are the line of best fit for the point of ZNF determination. The insert shows the mean (+/-S.E.M.) estimated glucose concentrations based on the individual regression analyses of each rat. The ECF glucose concentration in the MS (1.22 +/- .25 mM) and the hippocampus (0.96 +/- .16 mM) do not significantly differ ( $p > .05$ ).

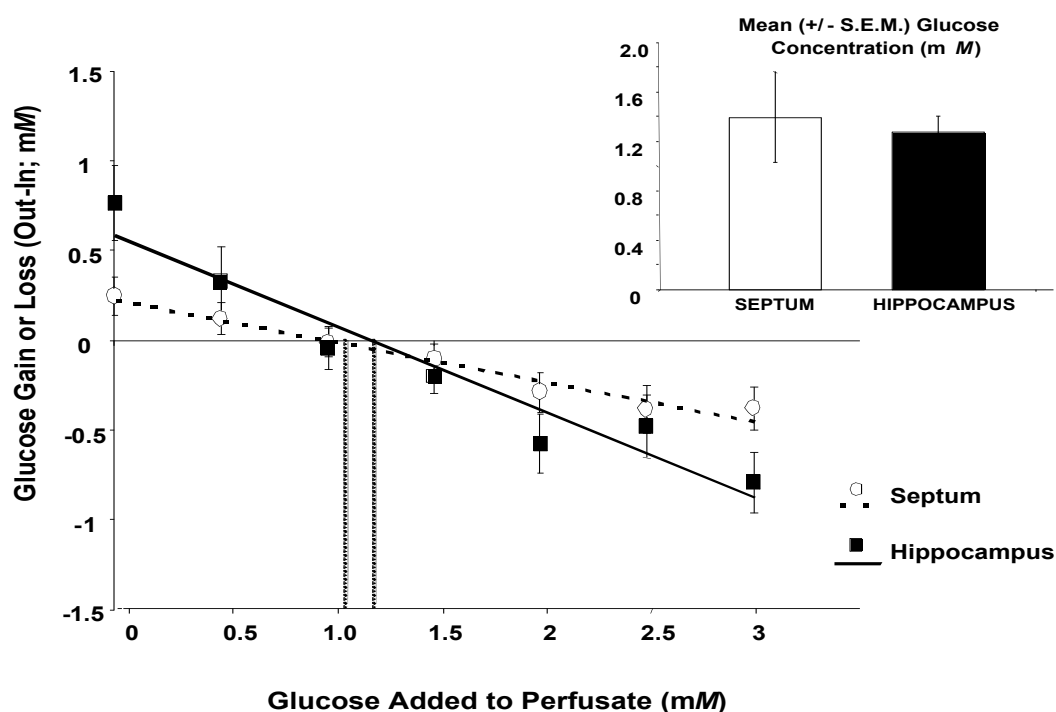


Figure 7.3. Glucose gain or loss to the brain as a function of perfusate concentration (0-3 mM) when the MS and hippocampus are sampled separately rather than simultaneously. The point of zero net flux is the estimated ECF concentration of glucose. The solid (hippocampus) and dashed (MS) lines are the line of best fit to the data for the point of ZNF determination. The insert shows the mean (+/-S.E.M.) estimated glucose concentrations based on the individual regression analyses of each rat. The estimated ECF glucose concentration in the MS (1.39 +/- .36 mM) and hippocampus (1.27 +/- .13 mM) did not significantly differ ( $p > .05$ ).

## CHAPTER 8

### GENERAL DISCUSSION

The overall goal of this dissertation was to test hypotheses regarding the neurochemical mechanisms underlying the effects of acute increases in glucose in two brain regions important for memory. This dissertation attempted to determine what conditions dissociated the memory-enhancing and -impairing effects of glucose. Specifically, we tested whether the memory-impairing effects of glucose are due to increases in septal (MS) GABA synthesis or release and are specific to GABA receptor activation, whether this negative effect of glucose on memory is a general property of the brain, or is brain region-specific, and if differences in extracellular glucose levels between brain areas could contribute to the different effects of glucose on memory. These results provided evidence showing that the positive and negative effects of glucose are both neurotransmitter- and brain region- dependent. These data also demonstrated that the memory-impairing effects of glucose are not concentration-dependent. Moreover, the impairing effects of MS infusions of glucose are not due to increases in MS GABA synthesis or release. Finally, these data eliminated the possibility that differences in basal extracellular glucose levels between the MS and hippocampus dissociate the positive and negative effects of glucose on memory.

#### **The memory-modulating effects of MS glucose are neurotransmitter- but not concentration-dependent**

The data from Aim 1 showed that MS co-infusions of glucose with morphine do not produce memory deficits when glucose is combined with sub-effective doses of morphine. These results also showed that MS co-infusions of glucose impair memory when combined with the benzodiazepine agonist CDP. Combined with the data that shows that glucose impairs memory

when combined with the GABA receptor agonist muscimol (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2004), these results demonstrated that MS co-infusions of glucose impair memory when combined with a drug that specifically modulates GABA<sub>A</sub> receptor activation. These findings suggest that GABA<sub>A</sub> receptors are involved in the memory-impairing effects of MS infusions of glucose. Combined together, these findings suggest that the memory-impairing effects of MS infusions of glucose are not concentration-dependent but are rather neurotransmitter-specific. Specifically, these data suggest that MS co-infusions of glucose impair memory through an influence on the GABA neurotransmitter system.

**MS infusions of glucose do not impair memory via an influence on extracellular GABA synthesis or release**

The findings from Specific Aim 2 demonstrated that MS infusions of muscimol with glucose impaired spatial working memory. More importantly, the results of this aim showed that MS infusions of glucose or glucose with muscimol did not increase MS extracellular GABA levels. Furthermore, these data showed that MS perfusions with glucose for longer periods of time, at a concentration that increases hippocampal extracellular ACh levels (Ragozzino, Pal, Unick, Stefani, & Gold, 1998b), also did not increase MS extracellular GABA levels. As a positive control, we did show that MS perfusion of K<sup>+</sup> increased MS extracellular GABA levels. Collectively, these findings suggest that glucose-induced increases in GABA synthesis or release are not likely involved in the impairing effects of glucose on memory in the MS. Combined with the finding of Specific Aim 1, these results suggest that, although the memory-impairing effects of MS infusions of glucose do not involve glucose-induced increases in GABA synthesis or release, they do involve an interaction with the GABA<sub>A</sub> receptor.



### **The memory-impairing effects of MS GABA receptor activation involve the GABAergic SH projection**

The results from Aim 3 show that hippocampal infusions of bicuculline prevented the memory-impairing effects of MS GABA receptor activation. The findings also showed that MS co-infusions of sub-effective doses of scopolamine, a drug that influences the GABAergic rather than the cholinergic SH projection (Alreja et al., 2000), with muscimol impaired memory. Collectively, these findings provide converging evidence supporting the hypothesis that the memory-impairing effects of MS GABA receptor activation are mediated through a process involving the GABAergic SH projection.

### **MS infusions of glucose involve an influence on the hippocampus: implications for compromised hippocampal function**

The results of Aim 4 demonstrated that the memory-enhancing effects of hippocampal infusions of glucose override the memory-impairing effects of MS infusions of glucose, when both phenomena happen simultaneously. Specifically, hippocampal infusions of glucose reversed deficits produced by MS co-infusions of the GABA receptor agonist muscimol with glucose. Combined, these findings suggest that MS infusions of glucose likely impair memory by influencing the hippocampus. Furthermore, these data suggest that hippocampal infusions of glucose prevent these deficits by augmenting hippocampal function. This leads to the prediction that the memory-impairing effects of hyperglycemia would only be observed when hippocampal function is impaired. This is consistent with the common finding that the impairing effects of diabetes on memory are most robustly observed in populations with impaired hippocampal function (Ryan & Geckle, 2000). These results are also consistent with possibility that the mnemonic effects of glucose may also be brain region-dependent. Specifically, glucose has

positive effects on memory in the hippocampus (Krebs & Parent, 2005a; Krebs & Parent, 2005b; Parent, Laurey, Wilkniss, & Gold, 1997; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b), but has negative effects in the MS (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003, 2004).

### **The memory-impairing effects of glucose are brain region-dependent**

These results demonstrate that hippocampal infusions of the GABA receptor agonist muscimol impair memory. More importantly, these findings show that hippocampal co-infusions of muscimol and glucose, at doses that have no effect alone, do not exacerbate or produce memory deficits. Moreover, these findings demonstrate that hippocampal co-infusions of glucose reverse the memory deficits produced by hippocampal GABA receptor activation. These data show that the memory-impairing interaction between glucose and GABA in the MS is not a general property of the brain, but rather is brain region-dependent.

### **The positive and negative effects of glucose in the MS and hippocampus are not due to differences in basal extracellular glucose levels between both brain regions**

The results of Aim 6 showed that ECF glucose concentrations are estimated to be comparable (1.0 - 1.2 mM) in both the MS and the hippocampus. Furthermore, the results of Aim 6 demonstrated that sampling from the MS and hippocampus simultaneously or on two separate occasions did not significantly impact the estimated ECF glucose concentrations of these two brain regions. Collectively, these findings suggest that basal differences in ECF glucose concentration do not likely contribute to the brain region-dependent effects of glucose on memory.

### **The role of the septo-hippocampal system in memory**

The most recent theories in learning and memory today are focused on the hypothesis that there are multiple brain regions that serve as different memory systems. Specifically, each of these different brain areas subserves a different type of memory, although each of these brain systems likely interacts to influence memory. Memory likely depends on the involvement of multiple brain systems that process different aspects of an experience (Poldrack et al., 2001; Poldrack & Packard, 2003; White & McDonald, 2002). For instance, there are multiple types of associations that can be formed in an experience and these associations can involve different brain regions. Specifically, stimulus-stimulus associations (e.g., place learning) are mediated by the hippocampus (Compton, 1993; Compton, Griffith, McDaniel, Foster, & Davis, 1997; Eichenbaum, 1992; Eichenbaum, Fagan, Matthews, & Cohen, 1988; Eichenbaum, Matthews, & Cohen, 1989; Eichenbaum, Stewart, & Morris, 1991; Hirsh, 1974; Sutherland & Rudy, 1989), stimulus-affect associations (e.g., fear conditioning) are mediated by the amygdala (Compton, 1995; Izquierdo & Medina, 1997; McDonald & White, 1995a; McGaugh, McIntyre, & Power, 2002; Salinas & White, 1998), and stimulus-response associations (e.g., procedural memory) are mediated by the striatum (Kirkby, Polgar, & Coyle, 1981; Packard, Hirsh, & White, 1989; Packard & McGaugh, 1996; Packard & White, 1990; Petri & Mishkin, 1993; Phillips & Carr, 1987; Salinas & White, 1998; Squire & Butters, 1984). More often, these memory systems work in parallel (McDonald & White, 1993, 1994; Salinas & White, 1998).

No one particular brain region likely mediates memory all on its own because of interactions with different brain regions; therefore, these memory systems work in cooperation or competition with each other. Previous evidence supports this idea because lesions of one brain area can enhance acquisition of tasks associated with other brain regions. For example, lesions

of the hippocampus enhance amygdala-dependent (Ferbinteanu & McDonald, 2001; McDonald & White, 1995b) and striatum-dependent memories (Matthews, Simson, & Best, 1995).

Moreover, lesions of the SH projection neurons impair hippocampal-dependent memory but enhance striatum-dependent memory (Packard, Hirsh, & White, 1989; Schroeder, Wingard, & Packard, 2002). An elegant example of such cooperation and competition between brain systems was demonstrated by a series of experiments investigating the role of ACh in memory (Gold, 2003a). For instance, hippocampal activity correlated negatively with performance in an amygdala-dependent task, suggesting that the hippocampus competes with the amygdala in an amygdala-dependent task (McIntyre, Pal, Marriott, & Gold, 2002). In contrast, amygdala activity is correlated positively with performance in a hippocampal-dependent task, suggesting that the amygdala cooperates with the hippocampus in hippocampal-dependent tasks (McIntyre, Marriott, & Gold, 2003). Interestingly, the brain region's involvement in solving the task changes with the strategy used to solve the tasks (Chang & Gold, 2003a).

Although it is not clear exactly what role the hippocampus plays in memory, it is generally assumed that the hippocampus mediates memory formation that involves spatial associations and temporal coding (Compton, 1993; Eichenbaum, 1996; Eichenbaum, Stewart, & Morris, 1991; Huxter, Burgess, & O'Keefe, 2003; O'Keefe, 1993). For example, lesions of the hippocampus impair spatial working memory (Bannerman, Matthews, Deacon, & Rawlins, 2004; Jarrard, 1993). Furthermore, electrophysiological correlates of memory such as theta (Hasselmo, 2005; Hasselmo, Hay, Ilyn, & Gorchetnikov, 2002; Huxter, Burgess, & O'Keefe, 2003; O'Keefe, 1993) and long-term potentiation (LTP; Izquierdo, 1994; Izquierdo & Medina, 1995; Redman, 1996; Teyler, 1987) occur in the hippocampus (deToledo-Morrell, Geinisman, & Morrell, 1988; Hasselmo, 2005; Hasselmo, Hay, Ilyn, & Gorchetnikov, 2002; Huxter,

Burgess, & O'Keefe, 2003; Lynch, Kessler, Arai, & Larson, 1990; Mesches, Fleshner, Heman, Rose, & Diamond, 1999; Nilsson, Shapiro, Gage, Olton, & Bjorklund, 1987; O'Keefe, 1993; Redman, 1996). Theta is a rhythmic 4-8 Hz waveform in the hippocampus associated with hippocampal excitation (Bland, Oddie, & Colom, 1999) and is present when a rat explores its environment (Bland & Colom, 1993; Kramis, Vanderwolf, & Bland, 1975). Furthermore, the theta rhythm appears to facilitate induction of LTP (Larson, Wong, & Lynch, 1986), and different phases of theta are more likely to produce LTP (Huerta & Lisman, 1993), suggesting that theta acts as a gating mechanism for synaptic plasticity. For example, theta is observed in the hippocampus during memory tasks (Kahana, Seelig, & Madsen, 2001; Kahana, Sekuler, Caplan, Kirschen, & Madsen, 1999; Raghavachari et al., 2001) and may be involved in the neural coding of place (O'Keefe & Recce, 1993; Winson, 1978). LTP is the continual enhancement of synaptic efficacy, resulting from a brief electrical stimulation (Bliss & Lomo, 1973; Teyler, 1987; Winder & Schramm, 2001), suggesting it serves a mechanism of memory. The associative nature of LTP, because of coincidence detection required by the NMDA receptor to depolarize the postsynaptic neuron and produce LTP, further supports its role in memory (Bender, Bender, Brasier, & Feldman, 2006; Duguid & Sjostrom, 2006; Schrader, Perrett, Ye, & Friedlander, 2004; Sjostrom, Turrigiano, & Nelson, 2003; Xu, Ye, Poo, & Zhang, 2006). Specifically, the NMDA receptor requires prior depolarization via the action of nearby  $\alpha$ -amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA) receptors and the presence of glutamate for activation (Bender, Bender, Brasier, & Feldman, 2006; Duguid & Sjostrom, 2006; Schrader, Perrett, Ye, & Friedlander, 2004; Sjostrom, Turrigiano, & Nelson, 2003; Xu, Ye, Poo, & Zhang, 2006).

There is extensive evidence to suggest that the MS modulates hippocampal activity and influences memory. Lesions of both the MS and hippocampus impair memory (Bannerman, Matthews, Deacon, & Rawlins, 2004; Bast, Zhang, & Feldon, 2001; Berger-Sweeney, Stearns, Frick, Beard, & Baxter, 2000; Farber, 1996). Furthermore, the effects of MS lesions mimic the effects of hippocampal lesions on memory (Bannerman, Matthews, Deacon, & Rawlins, 2004). Furthermore, the MS influences memory by affecting hippocampal activity important for memory. For example, MS neurons are responsible for influencing the hippocampal theta rhythm (Allen & Crawford, 1984; Smythe, Colom, & Bland, 1992; Vertes & Kocsis, 1997). Specifically, MS neurons provide the optimal levels of excitatory and inhibitory inputs to maintain hippocampal theta (Bland, Oddie, & Colom, 1999; Colom, 2006; Smythe, Colom, & Bland, 1992). The production of hippocampal theta rhythm is determined by the proportion of MS neurons involved in the rhythmic process, and that the frequency of theta rhythm is determined by the frequency of theta bursts from the MS (Bland & Colom, 1993; Vinogradova, 1995). Furthermore, lesions of the MS or the SH projection neurons prevent theta and impair memory (Amassari-Teule, Maho, & Sara, 1991; Apartis, Poindessous-Jazat, Lamour, & Bassant, 1998; Bannerman, Matthews, Deacon, & Rawlins, 2004; Hepler, Olton, Wenk, & Coyle, 1985; Leutgeb & Mizumori, 1999; Vinogradova, 1995; Winson, 1978). Furthermore, LTP is enhanced when the MS is stimulated prior to the hippocampus (Fantie & Goddard, 1982). In addition, MS drug manipulations affect neurotransmitters important for memory in the hippocampus (Allen & Crawford, 1984; Giovannini, Mutolo, Bianchi, Michelassi, & Pepeu, 1994; Imperato, Dazzi, Obinu, Gessa, & Biggio, 1994; Moor, Auth, DeBoer, & Westerink, 1996; Walsh, Stackman, Emerich, & Taylor, 1993). For example, MS infusions of drugs that decrease hippocampal ACh impair memory (Allen & Crawford, 1984; Brioni, Decker, Gamboa,

Izquierdo, & McGaugh, 1990; Chrobak, Stackman, & Walsh, 1989; Durkin, 1992b; Moor, DeBoer, & Westerink, 1998; Ragozzino & Gold, 1995b; Ragozzino, Parker, & Gold, 1992; Walsh, Stackman, Emerich, & Taylor, 1993).

### **Mechanisms that could contribute to the memory-enhancing versus -impairing effects of glucose**

There are multiple potential mechanisms that contribute the memory-modulating effects of glucose on memory. The present dissertation provided evidence showing that the memory-impairing interaction between glucose and GABA agonists in the MS is observed with different types of GABAergic drugs including the GABA<sub>A/C</sub> agonist muscimol and the BZD GABA<sub>A</sub> receptor modulator CDP. These negative effects of glucose with GABA agonists on memory are not likely due to extracellular hyperosmolarity, because previous research has shown that equiosmolar concentrations of other sugars do not produce memory deficits when combined with muscimol (Shah & Parent, 2003, 2004). Furthermore, the present findings showed that infusions of similar concentrations of glucose into the MS did not produce memory deficits when combined with morphine, suggesting that the memory-impairing effects of glucose are not concentration-dependent. The fact that the interaction between elevated glucose and GABA receptor activation in the MS is synergistic suggests that both are acting on a common mechanism to impair memory (Seeley & Moran, 2002). One candidate is glucose metabolism, because GABA agonists inhibit brain glucose metabolism (Ishizuka et al., 1989; Ito et al., 1994). This possibility seems unlikely, though, because both morphine and muscimol inhibit glucose metabolism (Cohen, Kimes, & London, 1991; Ito et al., 1994), yet glucose potentiates deficits produced by muscimol, but prevents deficits produced by morphine. A second possibility is that the synergistic interaction is somehow due to decreases in the membrane potential produced by

the various GABA agonists. This is also not likely because glucose reverses deficits produced by galanin and morphine (Ragozzino & Gold, 1995a; Ragozzino, Parker, & Gold, 1992; Stefani & Gold, 1998), two peptides that also hyperpolarize neurons (Hokfelt et al., 1999; Inturrisi, 2002). A third candidate mechanism is that glucose enhances memory when combined with drugs that enhance hippocampal ACh levels. MS infusions of the opiate morphine, the GABA agonist muscimol, and the BZD CDP decrease hippocampal ACh levels (Ragozzino & Gold, 1995b; Ragozzino, Wenk, & Gold, 1994; Walsh, Stackman, Emerich, & Taylor, 1993); whereas MS infusions of galanin increase extracellular ACh levels (Elvander et al., 2004) or decrease in extracellular ACh levels (Fisone, Bartfai, Nilsson, & Hokfelt, 1991; McDonald, Gleason, Robinson, & Crawley, 1998). The effects of these drugs on hippocampal ACh; however, do not parallel their effect on memory when combined with glucose. Specifically, MS co-infusions of glucose reverse the memory-impairing effects of infusions of morphine or galanin but not muscimol or CDP in the MS.

There are several other mechanisms that may contribute to the brain region-dependent effects of glucose. One possibility is that glucose affects the functioning of different neurotransmitters in different brain areas. Extensive evidence indicates that glucose may have positive effects on memory via increases in ACh in the hippocampus (Durkin, Messier, de Boer, & Westerink, 1992; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Ragozzino, Unick, & Gold, 1996; Stefani & Gold, 2001b). The present findings showed that the memory-impairing effects of glucose in MS do not likely involve increases in GABA synthesis or release. Glucose metabolism yields two molecules of pyruvate and two molecules of adenosine-tri-phosphate (Hertz & Dienel, 2002). Previous research has demonstrated that infusions of the glycolytic metabolite pyruvate mimic both the memory-impairing and memory-enhancing effects of



glucose (Krebs & Parent, 2005c; Shah & Parent, 2003, 2004). Pyruvate metabolism yields by-products necessary for GABA and ACh synthesis (Dolezal & Tucek, 1981; Gibson, Jope, & Blass, 1975; Hertz & Dienel, 2002; Lefresne, Guyenet, & Glowinski, 1973). Collectively, these findings raise the possibility that different products of glycolytic metabolism could mediate the positive versus negative effects of glucose on memory. These different products could vary as a function of brain region.

Another possibility is that glucose both enhances and impairs memory via an influence on the same neurotransmitter in different brain areas. For instance, glucose may increase ACh in the MS as it does in the hippocampus. Extensive evidence has shown that glucose enhances memory in the hippocampus via ACh (Gold, 1995; Kopf, Buchholzer, Hilgert, Loffelholz, & Klein, 2001; Messier, Durkin, Mrabet, & Destrade, 1990; Ragozzino, Pal, Unick, Stefani, & Gold, 1998b; Ragozzino, Unick, & Gold, 1996; Ragozzino, Wenk, & Gold, 1994). In contrast, increasing ACh activity in the MS can have positive (Givens & Olton, 1995; Givens & Olton, 1990; Olton et al., 1991) or negative (Elvander et al., 2004; Pang, 1999) effects on memory. Furthermore, oxotremorine has negative consequences for long-term potentiation in the striatum (Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998), suggesting that ACh can have negative effects on memory. Increases in ACh in other parts of the brain, such as the striatum and amygdala, typically facilitate memory processes (Chang & Gold, 2003b; Gold, 2003a; McIntyre, Marriott, & Gold, 2003), suggesting that it is not likely that glucose-induced increases in MS ACh would lead to memory deficits.

Another possibility is that elevations in glucose in the MS impair memory via increases in MS glutamate activity. Glucose administration also elevates glutamate levels in the brain (Burke & Nadler, 1989; Gruetter, 2002; Hamberger, Chiang, Nylen, Scheff, & Cotman, 1979).

Furthermore, there is some evidence that suggesting that MS infusions of glutamate may have negative consequences for memory. For example, MS infusions of low concentrations of glutamate tend to impair memory (Parent, Laurey, Wilkniss, & Gold, 1997). In addition, MS infusions of glutamate decrease hippocampal makers for ACh. Specifically, MS infusions of low doses of glutamate decrease sodium-dependent high affinity choline uptake (SDHACU; (Marighetto, Micheau, & Jaffard, 1994) in the hippocampus, which would be expected to impair memory (Chang & Gold, 2003a; Gold, 2003a; Parent & Baxter, 2004). Furthermore, MS infusions of glutamate antagonists increase SDHACU (Marighetto, Micheau, & Jaffard, 1994).

Differences in the neuroanatomical connections of the MS and hippocampus may account for how glucose-induced increases in ACh and glutamate could produce opposite effects on memory in the MS versus the hippocampus. For example, MS elevations in ACh or glutamate may enhance the activity of GABA interneurons in the MS and thus decrease the function of the cholinergic, GABAergic, and/or glutamatergic septo-hippocampal projections, which would be expected to impair memory (Durkin, 1992b; Parent & Baxter, 2004). In contrast, elevating these neurotransmitters in the hippocampus would be expected to enhance pyramidal cell function in the hippocampus (Cobb, Bulters, & Davies, 2000; Cohen, Raymond, & Abraham, 1998; Jouvenceau, Dutar, & Billard, 1998; Liu, Yang, Zhang, & Liu, 2003; Miura, Watanabe, Offermanns, Simon, & Kano, 2002), which would be expected to enhance memory (Farr, Flood, & Morley, 2000; Izquierdo, 1994; Izquierdo & Medina, 1995; Teyler, 1987).

### **Other potential mechanisms underlying the memory-impairing effects of glucose**

Glucose may impair memory via other mechanisms. For instance, glucose may modulate memory by directly influencing the binding properties of the GABA receptor to either promote

or attenuate binding of GABA agonists to the receptor, although there is no evidence to date showing that glucose can do so. There is evidence, though, showing that glucose does reduce the binding of morphine to opioid receptors (Brase, Han, & Dewey, 1987). Another possibility is that adenosine tri-phosphate (ATP)-dependent potassium (K<sup>+</sup>) channels (K<sup>+</sup>-ATP) are involved in the negative effects of glucose on memory. K-ATP channels, which can affect neurotransmitter release (Amoroso, Schmid-Antomarchi, Fosset, & Lazdunski, 1990; During, Leone, Davis, Kerr, & Sherwin, 1995), are closed by glucose (Larsson, Kindmark, Branstrom, & Berggren, 1997; Straub, James, Dunne, & Sharp, 1998; Valdeolmillos, Nadal, Contreras, & Soria, 1992). It is not clear how K-ATP channels could participate in for the memory-impairing effects of glucose; however, because the opening of the MS K-ATP channels impairs memory (Rashidy-Pour, 2001; Stefani & Gold, 1998, 2001a; Stefani, Nicholson, & Gold, 1999) and blocking MS K-ATP channels with glucose enhances memory and reverses memory deficits (Rashidy-Pour, 2001; Stefani & Gold, 1998, 2001a; Stefani, Nicholson, & Gold, 1999).

### **Differences in glucose utilization do not likely contribute to the brain region-dependent effects of glucose on memory**

The results of this dissertation show that differences in basal ECF glucose levels between the MS and hippocampus do not likely contribute to the brain-region dependent effects on glucose. This finding is consistent with the finding that local cerebral glucose utilization based on measures of [<sup>14</sup>C] 2-deoxy-D-glucose are similar in both brain regions (Huang, Tsai, Huang, & Sim, 1999; Ori, Freo, Pizzolato, & Dam, 2002; Wree, Goller, & Beck, 1995). One possibility is that any differences in extracellular glucose levels between the MS and hippocampus; therefore, may only been seen when a rat is engaged in a memory task. The effects of manipulations on neurochemical function differ in resting versus active animals, such as animals

learning in a maze, walking on a treadmill, feeding, or mating (Etgen & Morales, 2002; Rada, Mendiola, Hernandez, & Hoebel, 2003; Ragozzino, Pal, Unick, Stefani, & Gold, 1998a; Ragozzino, Unick, & Gold, 1996; Westerink, 1995). Furthermore, systemic infusions of glucose do not affect hippocampal extracellular ACh levels in a rat in its home cage; however, the same injections produce a large increase when the rat is exploring a maze (Ragozzino, Pal, Unick, Stefani, & Gold, 1998a; Ragozzino, Unick, & Gold, 1996). Interestingly, previous research has shown differences in ECF glucose concentrations and behavioral-induced changes in ECF glucose levels between the hippocampus and striatum (McNay, McCarty, & Gold, 2001). The possibility that there are activity-dependent differences in the MS and hippocampus is unlikely, however, because the local cerebral glucose utilization values of the MS and hippocampus are similar but lower than those seen in the striatum (Huang, Tsai, Huang, & Sim, 1999).

Furthermore, the MS and hippocampus are involved in the SA task (Johnson, Olton, Gage, & Jenko, 1977); whereas the striatum is not (Annett, McGregor, & Robbins, 1989; Thullier, Lalonde, Mahler, Joyal, & Lestienne, 1996), suggesting that activity-dependent decreases in extracellular glucose are expected in both the MS and hippocampus. Local cerebral glucose utilization measurements, however, are highly controversial because different estimates of local cerebral glucose utilization are obtained with different techniques (Gruetter, 2002; Gruetter, Novotny, Boulware, Rothman, & Shulman, 1996; Harada, Okuda, Sawa, & Murakami, 1992; Harada, Sawa, Okuda, Matsuda, & Tanaka, 1993; Hu & Wilson, 1997a, 1997b; Netchiporouk, Shram, Jaffrezic-Renault, Martelet, & Cespuglio, 1996; Ronne-Engstrom, Carlson, Liu, Ungerstedt, & Hillered, 1995; Silver & Erecinska, 1994).

### **The potential role of glucose transporters in the brain region-dependent effects of glucose on memory**

Although the amount of glucose utilization does not appear to differ between the MS and hippocampus, it is possible that the distribution and type of brain glucose type transporters do. Facilitative glucose transporters (GLUT) are responsible for glucose uptake into the brain and neurons. They exhibit both cell type and brain region-specific localization (Apelt, Mehlhorn, & Schliebs, 1999; Zeller, Rahner-Welsch, & Kuschinsky, 1997), suggesting that the transport of glucose is different between brain areas. Furthermore, previous research has shown that glucose transporter plasticity is correlated with learning a task (Choeiri, Staines, Miki, Seino, & Messier, 2005). Specifically, training on an operant task increases the expression of glucose transporters in the hippocampus (Choeiri, Staines, Miki, Seino, & Messier, 2005). Collectively, these findings suggest that behavioral-induced alterations in transporter quantity in the MS versus hippocampus may account for the brain region-dependent effects of glucose on memory. Another possibility is that the distribution of different types of glucose transporters between these two brain regions may contribute the brain region-dependent effects of glucose on memory. For example, the dissociation constants for GLUT1, GLUT3, and GLUT4 transporter proteins differ (Hertz & Dienel, 2002). Moreover, the rate of transport of glucose into the cell also differs between different types of transporters (Carruthers, 1990; Maher, Davies-Hill, & Simpson, 1996; Vannucci, Gibbs, & Simpson, 1997). Furthermore, previous results show that GLUT1 but not GLUT3 transporters are elevated in the hippocampus during a learning task (Choeiri, Staines, Miki, Seino, & Messier, 2005), suggesting different transporters may be involved in different processes. These finding raise the possibility that variation in the location or distribution of

those transporter proteins between the MS and hippocampus may differentially affect glucose function, even when the total number of transporters are equivalent between brain areas.

The opposite effects of glucose on memory in different brain regions could also involve interactions between GLUT transporters and different neurotransmitter groups. For instance, GLUT transporters may interact with neurotransmitters that may have positive or negative consequences for memory in different brain regions. For instance, GLUT4 transporters are co-localized with cholinergic neurons in the basal forebrain but not the hippocampus (Apelt, Mehlhorn, & Schliebs, 1999). Furthermore, these same transporters are co-localized with GABAergic projection neurons, but not GABA interneurons in the MS (Apelt, Mehlhorn, & Schliebs, 1999). Interestingly, GLUT 4 transporters are co-localized with GABA neurons in the hippocampus (Apelt, Mehlhorn, & Schliebs, 1999), suggesting this neuron specific localization of GLUT transporters may also be brain region-dependent. Moreover, some drugs such as brain-derived peptides, insulin growth factor, and adrenergic receptor agonists can alter glucose transport and GLUT transporter expression (Boado, 1998; Boado & Pardridge, 1990; Boado, Wu, & Windisch, 1999; Bondy & Cheng, 2002; Cheng & Liu, 2000; Duelli, Staudt, Maurer, & Kuschinsky, 1998), suggesting that the effects of the interactions between glucose with different drugs may affect also contribute to the brain region-dependent effects of glucose. This is supported by the finding that the effects of drugs on glucose utilization can vary by brain region (Ori, Freo, Pizzolato, & Dam, 2002).

### **Insulin and insulin receptor signaling**

Insulin signaling may contribute to the memory-modulating effects of glucose and possibly the brain region-dependent effects of glucose. For example, previous research has shown that the memory-enhancing effects of glucose are prevented when insulin levels are

suppressed (Craft et al., 1999; Fucetola, Newcomer, Craft, & Melson, 1999). The insulin receptor stimulates GLUT 4 translocation to the plasma membrane and increases glucose uptake and utilization (Saltiel & Pessin, 2002). Insulin (Banks & Kastin, 1998) and the insulin receptor (Adamo, Raizada, & LeRoith, 1989; Unger, Livingston, & Moss, 1991; Zhao et al., 1999) are heterogeneously distributed throughout discrete brain regions. Furthermore, the insulin receptor and GLUT 4 transporters have an overlapping distribution (El Messari, Ait-Ikhlef, Ambroise, Penicaud, & Arluison, 2002; El Messari et al., 1998), suggesting they work in cooperation to regulate glucose transport. The memory-enhancing effects of insulin may be mediated via an influence on insulin receptors in the hippocampus (Adamo, Raizada, & LeRoith, 1989; Kar, Baccichet, Quirion, & Poirier, 1993). For example, training in spatial memory task is associated with increases in hippocampal insulin receptor expression and signaling (Zhao et al., 1999). Furthermore, memory impairments and deficits in hippocampal plasticity associated with experimentally-induced diabetes are reversed by insulin administration (Biessels et al., 1998; Magarinos et al., 2001). Furthermore, brain glucose transport and utilization are impaired in elderly patients with mild cognitive impairments (De Santi et al., 2001) and Alzheimer's disease (De Santi et al., 2001; Duara et al., 1986; Friedland et al., 1989; Harr, Simonian, & Hyman, 1995; Piert, Koeppe, Giordani, Berent, & Kuhl, 1996). More importantly, both glucose or insulin administration reverses the cognitive dysfunction in these populations (Craft et al., 1999; Gold, 1995; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; Manning, Ragozzino, & Gold, 1993). Collectively, these findings suggest the distribution of insulin or insulin-dependent GLUT transporters could contribute to the brain region-dependent effects of glucose.

### **The memory-impairing effects of glucose involve compromised hippocampal function**

The memory-impairing effects of glucose are associated with compromised hippocampal function. For example, elevations in blood glucose levels associated with diabetes are accompanied by generalized increases in brain extracellular glucose levels (Biessels, Bravenboer, & Gispen, 2004; Hofer & Lanier, 1991) and memory deficits (Biessels et al., 1996; Flood, Mooradian, & Morley, 1990; Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003; Messier & Gagnon, 1996; Ryan & Geckle, 2000). Furthermore, the hippocampus is especially vulnerable to damage during aging and diabetes (Gispen & Biessels, 2000; Magarinos et al., 2001). The hippocampal damage seen in Alzheimer's disease is associated with abnormal glucose regulation. Specifically, Alzheimer's patients have poor glucose tolerance (de Leon et al., 1988), have impairments in brain glucose utilization, and poorly regulate peripheral glucose levels (de Leon et al., 1997). Furthermore, diabetes produces structural changes in the hippocampus and alterations in hippocampal synaptic plasticity (Gispen & Biessels, 2000). Specifically, diabetes impairs the function of the hippocampus (Stewart & Liolitsa, 1999; Weir, Murray, Dyker, & Lees, 1997). For example, experimentally-induced diabetes impairs memory and hippocampal synaptic plasticity (Biessels, Bravenboer, & Gispen, 2004; Biessels et al., 1996; Gispen & Biessels, 2000; Kamal, Biessels, Urban, & Gispen, 1999). Finally, impaired glucose tolerance is associated with both memory deficits and hippocampal atrophy (Convit, Wolf, C, & de Leon, 2003). Interestingly, the memory-impairing effects of glucose are most evident in diabetics that are old (Ryan & Geckle, 2000). Older adults show decreased hippocampal brain volume (Mu, Xie, Wen, Weng, & Shuyun, 1999), which impairs hippocampal function (Gazzaley, Siegel, Kordower, Mufson, & Morrison, 1996; Morrison & Gazzaley, 1996; Morrison & Hof, 1997) and is correlated with memory deficits (Golomb et al., 1993; Golomb et al., 1996;



Visser et al., 1999). Collectively, these data indicate that the impairing effects of glucose involve impaired hippocampal function.

The present results show that elevating glucose in the MS impairs memory only when MS GABA receptors are activated. This is important because MS GABA receptor activation impairs hippocampal function (Allen & Crawford, 1984; Bland, Trepel, Oddie, & Kirk, 1996; Brioni, Decker, Gamboa, Izquierdo, & McGaugh, 1990). Furthermore, the finding that hippocampal infusions of glucose, which elevate hippocampal function, prevent the hippocampal-dependent memory deficits produced by glucose also suggests elevating glucose in the MS impairs memory via an influence on the hippocampus. Collectively, these findings suggest that the memory-impairing effects of glucose involve an interaction between additive effects of glucose and those produced by manipulations that impair hippocampal function.

### **Future directions**

The experiments in this dissertation have set the foundation for future experiments aimed at determining whether glucose-induced neurochemical changes are also brain region-dependent. As a result, it will be important to examine the effects of glucose on ACh, GABA, and glutamate levels in both brain areas when rats are engaged in a memory task. Furthermore, examining the distribution of different types of GLUT type transporters may also help explain the brain region-dependent effects of glucose on memory. Importantly, it is not clear whether the memory-modulating effects of glucose involve glial or neuronal cells or both. Indeed, glucose metabolism is compartmentalized in both neurons and astrocytes (Maher, 1995). Furthermore, neurons and astrocytes utilize different GLUT transporters so it may be important to determine whether the memory-modulating effects of glucose are mediated through its effects on different

types GLUT transporters that are colocalized with different types of brain cells. These findings are important for understanding how MS infusions of glucose impair memory and how the MS and hippocampus interact during learning and memory. The current research also suggested that the memory-impairing effects of glucose likely involve compromised hippocampal function. It may be important, therefore, to determine whether the impairing effects of glucose are observed in aged rats where hippocampal function is compromised, in the absence of MS GABA receptor activation.

### **Overall summary**

In summary, these combined findings have provided important information regarding the neurochemical and behavioral consequences of acute increases in glucose in different brain regions important for memory. The findings from this dissertation excluded some of the potential neurochemical mechanisms through which MS infusions of glucose could impair memory, and proposed other prospective mechanisms. The results showed that the memory-impairing effects of MS infusions of glucose are not concentration-dependent. Rather, these data indicated that the memory-impairing effects of MS glucose are specific to GABA receptor activation but not dependent on increases in MS GABA synthesis or release. Importantly, we showed that the memory-impairing interaction between MS glucose and GABA agonists does not generalize to the hippocampus, suggesting the memory-modulating effects of glucose are brain region-dependent. These brain region-dependent effects of glucose are not due to differences in basal extracellular glucose levels. Collectively, these findings are the first to suggest that the effects of glucose on memory are both neurotransmitter- and brain region-dependent. The present results also suggested that the memory-impairing effects of MS GABA receptor activation are through an influence on the GABAergic SH projection. Moreover, these

findings suggested that the memory-impairing interaction between MS glucose and GABA was via an influence on the hippocampus.

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