Published in final edited form as:

Neurobiol Learn Mem. 2008 February ; 89(2): 142–152.

HIPPOCAMPAL INFUSIONS OF GLUCOSE REVERSE MEMORY DEFICITS PRODUCED BY CO-INFUSIONS OF A GABA RECEPTOR AGONIST

Desiree L. Krebs-Kraft^{1,2} and Marise B. Parent¹

¹ Department of Psychology and Center for Behavioral Neuroscience Georgia State University, Atlanta Georgia USA

² Department of Physiology and Program in Neuroscience University of Maryland, Baltimore Maryland USA

Abstract

Although septal infusions of glucose typically have positive effects on memory, we have shown repeatedly that this treatment exacerbates memory deficits produced by co-infusions of γ -aminobutyric acid (GABA) receptor agonists. The present experiments tested whether this negative interaction between glucose and GABA in the medial 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 reverse or exacerbate memory deficits produced by hippocampal co-infusions of the GABA receptor agonist muscimol. Fifteen minutes prior to either assessing 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 μ l/ 2 minutes), glucose (33 or 50 nmol), muscimol (0.3 μ g or 0.4 μ g, SA or 3 μ 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 medial septum impair memory, the present findings show that an opposite effect is observed in the hippocampus. Collectively, these findings suggest that the memory-impairing interaction between glucose and GABA in the medial septum is not a general property of the brain, but rather is brain region-dependent.

Keywords

Muscimol; GABA; Hippocampus; Spontaneous Alternation; Inhibitory Avoidance

Introduction

Brain glucose concentration is an essential variable that influences brain function. Elevations in brain glucose levels can occur in response to meals, drugs, stress, exercise, and diabetes (Bequet, Peres, Gomez-Merino, Berthelot, Satabin, Pierard, & Guezennec, 2000; Canal,

Correspondence concerning this article should be addressed to Desiree L. Krebs-Kraft, Department of Physiology, University of Maryland, Baltimore, 655 West Baltimore Street, Bressler Research Building 5-014, Baltimore, MD 21201. Phone 410-706-2654, Fax: 410-706-8341, email: dkrebskraft@gmail.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

McNay, & Gold, 2005; Fellows, Boutelle, & Fillenz, 1993; Jacob, Fan, Evans, Dziura, & Sherwin, 2002; Silver & Erecinska, 1994). Memory is one crucial function that is influenced by changes in brain glucose levels. Interestingly, extracellular brain glucose levels fluctuate during learning and memory (McNay, Fries, & Gold, 2000; McNay, McCarty, & Gold, 2001). Furthermore, infusions of glucose into the brain enhance memory and reverse memory deficits produced by some drugs (Ragozzino & Gold, 1995; Ragozzino, Pal, Unick, Stefani, & Gold, 1998; Ragozzino, Parker, & Gold, 1992; Stefani & Gold, 1998; Stefani & Gold, 2001; Stefani, Nicholson, & Gold, 1999), but not others (Parent, Laurey, Wilkniss, & Gold, 1997; Parent & Gold, 1997; Shah & Parent, 2003; Shah & Parent, 2004). Specifically, glucose administration into the medial septum reverses deficits produced by the opiate agonist morphine and the peptide galanin (Ragozzino & Gold, 1995; Ragozzino, Wenk, & Gold, 1994; Stefani & Gold, 1998). In contrast, septal co-infusions of glucose do not reverse the memory-impairing effects of γ -aminobutyric acid (GABA) agonists (Parent et al., 1997; Parent & Gold, 1997). Rather, septal infusions of glucose potentiate these memory-impairing effects of GABA_A agonists (Parent et al., 1997). Furthermore, septal 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; Shah & Parent, 2004).

In addition to being neurotransmitter-dependent, the memory-modulating effects of glucose also may be brain-region dependent. This hypothesis is based on several lines of evidence showing that the effects of glucose are not uniform throughout the brain and that glucose levels change in a brain region-dependent manner. For instance, brain activity is measured in a variety of species using two-deoxy-, 2-fluorodeoxyglucose, and positron emission tomography because these methods show regional differences in glucose metabolism and utilization. In addition, in rodents, 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, Beyer, Lin, McGonigle, Schechter, & Bach, 2004; McNay et al., 2001). In humans and rats, brain areas vary in terms of the amount of glucose they require to carry out their functions (Gage, Kelly, & Bjorklund, 1984; Gerber, Choki, Brunswick, Reivich, & Frazer, 1983; Kimbrell, Dunn, George, Danielson, Willis, Repella, Benson, Herscovitch, Post, & Wassermann, 2002; Kimbrell, Ketter, George, Little, Benson, Willis, Herscovitch, & Post, 2002; McNay et al., 2001). In rats, acute increases in blood glucose either increase or decrease cerebral blood flow depending on the brain region (Duckrow, 1988). Moreover, in rats and mice, the effects of glucose on electrochemical signaling also vary by neuronal phenotype, brain area, and glucose concentration (Fioramonti, Lorsignol, Taupignon, & Penicaud, 2004; Wang, Liu, Hentges, Dunn-Meynell, Levin, Wang, & Routh, 2004).

Importantly, extracellular brain glucose levels change in a regional manner during memory in animals with normal blood glucose levels. For instance, performance in a memory task decreases brain extracellular glucose levels in the hippocampus, without affecting extracellular glucose levels near the hippocampus or in the striatum (McNay et al., 2000; McNay et al., 2001). This decrease is presumably due to increased neural uptake, which is supported by the finding that performance in a learning task is associated with a selective increase in hippocampal glucose transporter expression (Choeiri et al., 2005). Critically, glucose that is administered systemically when rats are performing in a hippocampal-dependent memory task is transported preferentially to the hippocampus and enhances memory (McNay et al., 2000; McNay et al., 2001). This suggests that memory-induced regional decreases in extracellular glucose can be selectively replenished when peripheral glucose levels are elevated. Exogenous infusions of glucose into discrete brain areas can thus be used as a tool to mimic this brain-region dependent replenishment.

The goal of the present experiments was to test whether the memory-impairing interaction between glucose and GABA receptor activation that occurs in the medial septum would be observed in the hippocampus, a brain region where glucose typically has positive effects on memory (Krebs & Parent, 2005a; Parent et al., 1997; Ragozzino et al., 1998; Ragozzino, Unick, & Gold, 1996). To do so, we determined whether hippocampal infusions of glucose would reverse or exacerbate memory deficits produced by hippocampal co-infusions of the GABA receptor agonist muscimol in two memory tasks that are dependent on the septo-hippocampal system: spontaneous alternation (SA) and inhibitory avoidance.

Materials and Methods

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 septum.

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 (20×40×20 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/minute 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/minute 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/minute oxygen to the face-mask. 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 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 $\pm$ 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 identification of 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 by removing and wiping the dummy cannula a minimum of three times. Betadine was also 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/minute of oxygen and given an additional injection of penicillin (1500 units im). These rats were included in the behavioral testing, which took place 5 or more days after the injection.

Drug Preparation and Intracranial Infusions—Two days prior to behavioral testing, the experimenter handled each rat for 2 minutes. Behavioral testing was conducted between 7:00 a.m. and 7:00 p.m. Before and after all handling and behavioral testing, the rats were allowed a minimum of 30 minutes to habituate to the laboratory environment. 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 μ l, 0.5 μ l/minute; phosphate-buffered saline [PBS], pH = 7.4; n = 14), glucose (33 or 50 nmol; n = 9 for both groups), muscimol (0.3 μ g, Sigma; n = 19) or muscimol combined with glucose in one solution (n = 12 or 10). 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 medial septum (Parent & Krebs, 2004; Parent et al., 1997; Parent & Gold, 1997; Shah & Parent, 2003; Shah & Parent, 2004). The 50 nmol dose of glucose was selected because it reverses memory deficits when infused into the hippocampus (Krebs & Parent, 2005a; Parent et al., 1997). These doses produce increases in extracellular glucose (33 nmol/1.0 μ 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 et al., 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 μ 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 septal 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 μ g/1.0 μ l concentrations and aliquoted (200 μ l) into microcentrifuge tubes and frozen (2–8 °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 μ 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 the arms in the maze, the rats must remember their visits to previous arms. This interpretation 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 et al., 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 $\times$ 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 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 minute 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 μ 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 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 higher doses of muscimol. The effects of co-infusing glucose with sub-effective doses of muscimol versus memory-impairing doses of muscimol were examined in separate experiments ($k=6$ per experiment) in order to prevent the large reduction in statistical power that results from having all treatment conditions in one experiment ($k=10$; Bakeman & McArthur, 1999). The same procedures were used as in Experiment 1 with the following exceptions: Fifteen minutes prior to assessing SA, different groups of rats were given bilateral hippocampal infusions of vehicle (1 μ l, 0.5 μ l/minute; PBS, pH = 7.4; $n = 13$), glucose (33 or 50 nmol; $n = 9$ or 10), muscimol (0.4 μ g, Sigma; $n = 16$) or muscimol combined with glucose in one solution ($n = 17$ or 18). The higher 0.4 μ g dose of muscimol was used for this experiment 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 continuous multiple trial inhibitory avoidance (CMIA) vary in motivational, temporal, and cognitive demands, the use of two behavioral tasks would also help 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 in a SA task in Experiment 1 or 2, rats were given bilateral hippocampal infusions of either vehicle (1 μ l, 0.5 μ l/minute; PBS, pH = 7.4; $n = 9$), glucose (33 or 50 nmol; $n = 10$ or 10), muscimol (3 μ g, Sigma; $n = 12$) or muscimol combined with glucose in one solution 15 minutes prior to

CMIA training ($n = 9$ or 11). 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. The drug treatments were counterbalanced across the SA and CMIA tasks. 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 hours) 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 ranges and the non-parametric Kruskal-Wallis and Mann-Whitney U tests were used to detect differences between treatment groups.

Results

Experiment 1

Figure 1 illustrates the approximate locations of the hippocampal infusions. Drug infusions into the hippocampus [$F(5, 72) = 1.99$; $p > .05$] did not significantly affect SA performance (see Figure 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 [$F(5, 72) = 2.30$; $p > .05$] also did not significantly affect the number of arms that the rats entered in the maze (see Figure 2B).

Experiment 2

Figure 3A shows that drug infusions into the hippocampus [$F(5, 72) = 2.98$; $p < .05$], using the higher dose of muscimol, significantly affected SA performance. Hippocampal infusions of muscimol ($0.4 \mu\text{g}$) significantly impaired SA performance. Specifically, the scores of rats given hippocampal infusions of muscimol ($0.4 \mu\text{g}$) 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 μ 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 [$F(5,72) = 1.68$; $p > .05$] did not significantly affect the number of arms that the rats entered in the maze (see Figure 3B).

Experiment 3

Hippocampal drug infusions [$\chi^2(5,62) = 9.05$; $p > .05$] did not significantly affect trials to criterion during CMIA training (see Figure 4A). Figure 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 muscimol (3 μ g) significantly impaired shock avoidance performance. The retention latencies of rats given hippocampal infusions of muscimol were significantly lower than those of rats given septal infusions of vehicle [$U(1,23) = 19.0$; $p < .05$]. 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 [$U(1,21) = 52.0$; $p > .05$] or [$U(1,21) = 38.0$; $p > .05$]. 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

The 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. Moreover, elevations in hippocampal glucose do not interact with sub-effective doses of muscimol to produce impairments. These findings are in direct contrast with our previous research showing that septal glucose administration exacerbates the impairing effects of muscimol or interacts with subeffective doses to produce memory deficits (Parent et al., 1997; Parent & Gold, 1997; Shah & Parent, 2003; Shah & Parent, 2004). Combined with these previous data, the present results indicate that the effects of glucose on memory are brain region-dependent.

The present findings are consistent with previous research indicating that hippocampal infusions of glucose reverse the memory-impairing effects of septal GABA receptor activation (Krebs & Parent, 2005a; Parent et al., 1997). In these earlier studies, glucose elevation and GABA receptor activation occurred in separate brain regions. Using co-infusions into the hippocampus, the present study shows that glucose also reverses memory deficits produced by

local increases in GABA receptor activation. Consequently, the present study adds to those previous findings by showing that the local memory-impairing interaction between glucose and GABA receptor activation in the medial septum is not a general property of the brain.

These findings are also consistent with data showing that hippocampal infusions of glucose interact with other manipulations to affect SA and inhibitory avoidance retention (Krebs & Parent, 2005a; Parent et al., 1997; Ragozzino et al., 1998). The present findings do contrast, however, with other reports showing that elevating glucose alone in the hippocampus enhances spontaneous alternation (Ragozzino et al., 1998; Stefani & Gold, 2001). This difference may be a result of differences in task complexity, because glucose typically enhances spontaneous alternation in a 4-armed maze (McNay et al., 2000; Ragozzino et al., 1998; Ragozzino et al., 1996; Stefani & Gold, 2001), but not in a 3-armed version (Krebs & Parent, 2005a; McNay et al., 2000; Parent et al., 1997; Ragozzino & Gold, 1995; Ragozzino, Hellems, Lennartz, & Gold, 1995). Successfully alternating between four arms is more difficult than alternating between three arms, as indicated by the lower control scores observed in the 4-armed version. Importantly, glucose is more likely to have an effect when performance is challenged by variables such as task difficulty, pathology, or pharmacological manipulations (Korol & Gold, 1998).

The motivational, temporal, and cognitive differences between the SA and the CMIA task support the hypothesis that glucose influenced memory rather than some other process that influences performance in a memory task. The finding that the hippocampal infusions of muscimol 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 et al., 2002; Johnson, Olton, Gage, & Jenko, 1977; Krebs & Parent, 2005a; Lovely, Grossen, Moot, Bauer, & Peterson, 1971; Parent et al., 1997). Specifically, the findings from the SA task suggest that the hippocampus is involved in on-line spatial associations. The findings from the CMIA task support the role of the hippocampus in long term stimulus-stimulus associations. 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 the newly formed memories.

The present findings add to the growing evidence indicating that glucose does not influence memory in a non-specific manner. Collectively, the evidence indicates that the effects of glucose on memory depend on the chemical systems that are activated, the brain region in which glucose is elevated, and the type of memory process that is involved. For instance, elevating glucose in the amygdala reverses deficits produced by septal infusions of morphine in spontaneous alternation, but not in inhibitory avoidance (McNay & Gold, 1998). Similarly, elevating glucose in the striatum impairs place learning, but has no effect on response learning (Pysh, Kim, & Gold, 2006). In the amygdala, glucose reverses memory deficits produced by the opioid morphine (Ragozzino & Gold, 1994), but not the beta-noradrenergic antagonist propranolol (Lennartz, Hellems, Mook, & Gold, 1996). Likewise, when infused into the septum, glucose reverses deficits produced by morphine (Ragozzino & Gold, 1995; Ragozzino et al., 1992), galanin (Stefani & Gold, 1998), but not those produced by the GABA agonist muscimol (Parent et al., 1997; Parent & Gold, 1997; Shah & Parent, 2003; Shah & Parent, 2004). The finding that glucose interacts with the effects of GABA receptor activation in an opposite manner in the septum (Parent et al., 1997; Parent & Gold, 1997; Shah & Parent, 2003; Shah & Parent, 2004) versus the hippocampus (present findings) is consistent with evidence indicating that the effects of intracranial infusions on memory are influenced by the location of the infusion (Gold, 1995; Lennartz et al., 1996; Ragozzino & Gold, 1994). Of particular relevance is the finding that infusing the same dose of glucose into the striatum or the hippocampus has a different effect on learning rates and strategy use in the same task (Canal, Stutz, & Gold, 2005). In addition, variations in brain glucose transport and brain

extracellular glucose levels associated with performance on a memory task are brain region-specific (Choeiri et al., 2005; Fellows & Boutelle, 1993; Fellows, Boutelle, & Fillenz, 1992; McNay et al., 2000; McNay et al., 2001). Specifically, SA performance decreases hippocampal extracellular glucose levels, without changing extracellular glucose levels the striatum (McNay et al., 2001). These differences could contribute to the differential effects of glucose on memory in the medial septum 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 medial septum than the hippocampus, then smaller increases in glucose may be required to produce memory deficits in the medial septum 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) in the hippocampus (Degroot, Kornecook, Quirion, DeBow, & Parent, 2003; Kopf, Buchholzer, Hilgert, Loffelholz, & Klein, 2001; Messier, 2004; Ragozzino et al., 1998). In contrast, the fact the glucose produces memory deficits when combined with GABA agonists in the medial septum (Parent & Krebs, 2004; Parent et al., 1997; Parent & Gold, 1997; Shah & Parent, 2003; Shah & Parent, 2004) suggests that glucose may have negative effects on memory in the medial septum by increasing GABA synthesis or release. This possibility is supported by the finding that acute administration of glucose and experimentally induced chronic hyperglycemia can increase GABA levels in other regions of 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).

A number of mechanisms could underlie such brain region dependent effects of glucose on neurotransmitter release. For instance, glucose can affect neurotransmitter release via K⁺ATP channels; importantly, these channels appear to be located on neurons that release different neurotransmitters in different brain areas (Amoroso et al., 1990; During et al., 1995; Lee, Dixon, Rowe, Ashford, & Richardson, 1996; Levin, 2000). Regional variation in glucose transporter distribution and/or activation also could be involved in brain region-dependent effects of glucose on neurotransmitter release (Barros et al., 2005; Choeiri et al., 2005). Previous research has demonstrated that infusions of the glycolytic metabolite pyruvate mimic both the memory-impairing and memory-enhancing effects of glucose (Krebs & Parent, 2005b; Shah & Parent, 2003; Shah & Parent, 2004). Glucose 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 glucose concentration or brain region.

Another possibility is that glucose enhances and impairs memory via an influence on the same neurotransmitter in different brain areas. The same neurotransmitter could hypothetically have the same effect in the medial septum versus the hippocampus given the differences in chemical composition and neural connectivity of these brain areas. For instance, glucose may increase ACh in the medial septum as it does in the hippocampus (Degroot et al., 2003; Ragozzino et al., 1998). Although increasing ACh function in the brain, including the medial septum, typically has positive consequences for learning and memory (Gold, 2003a; Gold, 2003b), there are cases where septal administration of cholinergic agonists can produce memory deficits (Bunce, Sabolek, & Chrobak, 2003; Bunce, Sabolek, & Chrobak, 2004a; Bunce, Sabolek, & Chrobak, 2004b; Elvander, Schott, Sandin, Bjelke, Kehr, Yoshitake, & Ogren, 2004; Pang & Nocera, 1999; Sabolek, Bunce, & Chrobak, 2005). It is not clear why increasing septal ACh activity is sometimes detrimental for memory. It is likely that the deleterious effects of septal

infusions of ACh agonists are due to their effects on hippocampal physiology, because ACh receptors are present on both septo-hippocampal GABA and ACh projection neurons (Alreja, Wu, Liu, Atkins, Leranath, & Shanabrough, 2000; Rouse & Levey, 1996). Activation of septal ACh receptors increases hippocampal theta (Givens & Olton, 1990; Lee, Chobak, Sik, Wiley, & Buzsak, 1994; Monmaur & Breton, 1991), which would be expected to enhance encoding (Hasselmo, Bodelon, & Wyble, 2002). However, prolonged increases in theta may not be beneficial to memory, because theta activity typically alternates with sharp wave activity, and sharp wave activity may be necessary for memory consolidation (Bunce Sabolek, & Chrobak, 2004). Furthermore, cholinergic agonists can have negative consequences for long-term potentiation (Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998), which can also have negative implications for memory.

Another possibility is that elevations in glucose in the medial septum impair memory via increases in medial septal glutamate activity. Glucose administration also elevates glutamate levels in the brain (Burke & Nadler, 1989; Gruetter, 2002; Hamberger, Chiang, Nylen, Scheff, & Cotman, 1979). Although elevations in hippocampal glutamate typically have positive effects on memory, there is some evidence suggesting that medial septal infusions of glutamate may have negative consequences for memory. For example, medial septal infusions of low concentrations of glutamate tend to impair memory (Parent et al., 1997). In addition, medial septal 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, 2003; Gold, 2003a; Parent & Baxter, 2004). Furthermore, medial septal infusions of glutamate antagonists increase SDHACU (Marighetto et al., 1994).

In summary, administration of glucose into the hippocampus reverses memory deficits produced by hippocampal GABA receptor activation. These 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 medial septum produce memory deficits, these findings show that the memory-impairing interaction between glucose and GABA in the medial septum is not a general property of the brain, but rather is brain region-dependent.

Acknowledgements

This research was supported in part by grants from NINDS-HIDDK-JDF (RO1NS41173-02), the STC Program of the National Science Foundation under Agreement No. IBN-9876754, and the Georgia State University Brains and Behavior Program.

References

- Alreja M, Wu M, Liu W, Atkins J, Leranath C, Shanabrough M. Muscarinic tone sustains impulse flow in the septohippocampal GABA but not cholinergic pathway: Implications for learning and memory. *The Journal of Neuroscience* 2000;20:8103–8110. [PubMed: 11050132]
- Amoroso S, Schmid-Antomarchi H, Fosset M, Lazdunski M. Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K⁺ channels. *Science* 1990;247:852–854. [PubMed: 2305257]
- Bakeman R, McArthur D. Determining the power of multiple regression analyses both with and without repeated measures. *Behavioral Research Methods, Instruments and Computers* 1999;31:150–154.
- Barros LF, Porras OH, Bittner CX. Why glucose transport in the brain matters for PET. *Trends in Neuroscience* 2005;28:117–119.
- Bequet F, Peres M, Gomez-Merino D, Berthelot M, Satabin P, Pierard C, et al. Simultaneous NMR microdialysis study of brain glucose metabolism in relation to fasting or exercise in the rat. *Journal of Applied Physiology* 2000;88:1949–1954. [PubMed: 10846004]

- Bunce JG, Sabolek HR, Chrobak JJ. Intraseptal infusion of oxotremorine impairs memory in a delayed-non-match-to-sample radial maze task. *Neuroscience* 2003;121:259–267. [PubMed: 14521985]
- Bunce JG, Sabolek HR, Chrobak JJ. Intraseptal infusion of the cholinergic agonist carbachol impairs delayed-non-match-to-sample radial arm maze performance in the rat. *Hippocampus* 2004a;14:450–459. [PubMed: 15224982]
- Bunce JG, Sabolek HR, Chrobak JJ. Timing of administration mediates the memory effects of intraseptal carbachol infusion. *Neuroscience* 2004b;127:593–600. [PubMed: 15283959]
- Burke SP, Nadler JV. Effects of glucose deficiency on glutamate/aspartate release and excitatory synaptic responses in the hippocampal CA1 area in vitro. *Brain Research* 1989;500:333–342. [PubMed: 2575005]
- Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G. Blockade of M2-like muscarinic receptors enhances long-term potentiation at corticostriatal synapses. *European Journal of Neuroscience* 1998;10:3020–3023. [PubMed: 9758172]
- Canal CE, McNay EC, Gold PE. Increases in extracellular fluid glucose levels in the rat hippocampus following an anesthetic dose of pentobarbital or ketaminexylazine: an in vivo microdialysis study. *Physiology and Behavior* 2005;84:245–250. [PubMed: 15708776]
- Canal CE, Stutz SJ, Gold PE. Glucose injections into the dorsal hippocampus or dorsolateral striatum of rats prior to T-maze training: modulation of learning rates and strategy selection. *Learning and Memory* 2005;12:367–374. [PubMed: 16027177]
- Chang Q, Gold P. Switching memory systems during learning: Changes in patterns of brains acetylcholine release in the hippocampus and striatum in rats. *The Journal of Neuroscience* 2003;23:3001–3005. [PubMed: 12684487]
- Chang Q, Gold PE. Impaired and spared cholinergic functions in the hippocampus after lesions of the medial septum/vertical limb of the diagonal band with 192 IgG-saporin. *Hippocampus* 2004;14:170–179. [PubMed: 15098723]
- Choeiri C, Staines W, Miki T, Seino S, Messier C. Glucose transporter plasticity during memory processing. *Neuroscience* 2005;130:591–600. [PubMed: 15590143]
- Deacon RM, Bannerman DM, Kirby BP, Croucher A, Rawlins JN. Effects of cytotoxic hippocampal lesions in mice on a cognitive test battery. *Behavioral Brain Research* 2002;133:57–68.
- Degroot A, Kornecook T, Quirion R, DeBow S, Parent M. Glucose increases hippocampal extracellular acetylcholine levels upon activation of septal GABA receptors. *Learning and Memory* 2003;979:71–77.
- Dember, W. Spontaneous alternation behavior. Springer-Verlag; New York: 1989.
- Dolezal V, Tucek S. Utilization of citrate, acetylcarnitine, acetate, pyruvate and glucose for the synthesis of acetylcholine in rat brain slices. *Journal of Neurochemistry* 1981;36:1323–1330. [PubMed: 6790669]
- Duckrow RB. Regional cerebral blood flow and glucose utilization during hyperinsulinemia. *Brain Research* 1988;462:363–366. [PubMed: 3191397]
- During MJ, Leone P, Davis KE, Kerr D, Sherwin RS. Glucose modulates rat substantia nigra GABA release in vivo via ATP-sensitive potassium channels. *Journal of Clinical Investigation* 1995;95:2403–2408. [PubMed: 7738204]
- Elvander E, Schott P, Sandin J, Bjelke B, Kehr J, Yoshitake T, et al. Intraseptal muscarinic ligands and galanin: Influence on hippocampal acetylcholine and cognition. *Neuroscience* 2004;126:541–557. [PubMed: 15183504]
- Fellows LK, Boutelle MG. Rapid changes in extracellular glucose levels and blood flow in the striatum of the freely moving rat. *Brain Research* 1993;604:225–231. [PubMed: 8457850]
- Fellows LK, Boutelle MG, Fillenz M. Extracellular brain glucose levels reflect local neuronal activity: a microdialysis study in awake, freely moving rats. *Journal of Neurochemistry* 1992;59:2141–2147. [PubMed: 1431898]
- Fellows LK, Boutelle MG, Fillenz M. Physiological stimulation increases nonoxidative glucose metabolism in the brain of the freely moving rat. *Journal of Neurochemistry* 1993;60:1258–1263. [PubMed: 8455025]

- Fink K, Gothert M. High D-glucose concentrations increase GABA release but inhibit release of norepinephrine and 5-hydroxytryptamine in rat cerebral cortex. *Brain Research* 1993;618:220–226. [PubMed: 8397048]
- Fink K, Zentner J, Gothert M. Increased GABA release in the human brain cortex as a potential pathogenetic basis of hyperosmolar diabetic coma. *Journal of Neurochemistry* 1994;62:1476–1481. [PubMed: 8133276]
- Fioramonti X, Lorsignol A, Taupignon A, Penicaud L. A new ATP-sensitive K⁺ channel-independent mechanism is involved in glucose-excited neurons of mouse arcuate nucleus. *Diabetes* 2004;53:2767–2775. [PubMed: 15504956]
- Gage FH, Kelly PA, Bjorklund A. Regional changes in brain glucose metabolism reflect cognitive impairments in aged rats. *Journal of Neuroscience* 1984;4:2856–2865. [PubMed: 6502208]
- Gerber JC 3rd, Choki J, Brunswick DJ, Reivich M, Frazer A. The effect of antidepressant drugs on regional cerebral glucose utilization in the rat. *Brain Research* 1983;269:319–325. [PubMed: 6309323]
- Gibson GE, Jope R, Blass JP. Decreased synthesis of acetylcholine accompanying impaired oxidation of pyruvic acid in rat brain minces. *Biochemistry Journal* 1975;148:17–23.
- Givens B, Olton D. Cholinergic and GABAergic modulation of medial septal area: Effect on working memory. *Behavioral Neuroscience* 1990;104:849–855. [PubMed: 2178347]
- Gold, PE. Modulation of emotional and non-emotional memories: Same pharmacological systems, different neuroanatomical systems.. In: McGaugh, J.L.; Weinberger, N.; Lynch, G., editors. *Brain and Memory: Modulation and mediation of neuroplasticity*. Oxford University Press; New York: 1995. p. 41–74.
- Gold PE. Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiology of Learning and Memory* 2003a;80:194–210. [PubMed: 14521863]
- Gold PE. Acetylcholine: cognitive and brain functions. *Neurobiology of Learning and Memory* 2003b; 80:177. [PubMed: 14521861]
- Gruetter R. In vivo ¹³C NMR studies of compartmentalized cerebral carbohydrate metabolism. *Neurochemistry International* 2002;41:143–154. [PubMed: 12020614]
- Hamberger AC, Chiang GH, Nylen ES, Scheff SW, Cotman CW. Glutamate as a CNS transmitter. I. Evaluation of glucose and glutamine as precursors for the synthesis of preferentially released glutamate. *Brain Research* 1979;168:513–530. [PubMed: 435980]
- Hasselmo ME, Bodelon C, Wyble BP. A proposed function for hippocampal theta rhythm: separate phases of encoding and retrieval enhance reversal of prior learning. *Neural Computation* 2002;14:793–817. [PubMed: 11936962]
- Hertz, L.; Dienel, G. Energy metabolism in the brain.. In: Dwyer, D., editor. *Glucose Metabolism in the Brain*. 51. Academic Press; San Diego: 2002. p. 2–102.
- Jacob RJ, Fan X, Evans ML, Dziura J, Sherwin RS. Brain glucose levels are elevated in chronically hyperglycemic diabetic rats: no evidence for protective adaptation by the blood brain barrier. *Metabolism* 2002;51:1522–1524. [PubMed: 12489061]
- Johnson CT, Olton DS, Gage FH 3rd, Jenko PG. Damage to hippocampus and hippocampal connections: effects on DRL and spontaneous alternation. *Journal of Comparative Physiology and Psychology* 1977;91:508–522.
- Khandelwal P, Beyer CE, Lin Q, McGonigle P, Schechter LE, Bach AC 2nd. Nanoprobe NMR spectroscopy and in vivo microdialysis: new analytical methods to study brain neurochemistry. *Journal of Neuroscience Methods* 2004;133:181–189. [PubMed: 14757359]
- Kimbrell TA, Dunn RT, George MS, Danielson AL, Willis MW, Repella JD, et al. Left prefrontal-repetitive transcranial magnetic stimulation (rTMS) and regional cerebral glucose metabolism in normal volunteers. *Psychiatry Research* 2002;115:101–113. [PubMed: 12208488]
- Kimbrell TA, Ketter TA, George MS, Little JT, Benson BE, Willis MW, et al. Regional cerebral glucose utilization in patients with a range of severities of unipolar depression. *Biological Psychiatry* 2002;51:237–252. [PubMed: 11839367]
- Kopf S, Buchholzer M, Hilgert M, Löffelholz K, Klein J. Glucose plus choline improve passive avoidance behavior and increases hippocampal acetylcholine release in mice. *Neuroscience* 2001;103:365–371. [PubMed: 11246151]

- Korol DL, Gold PE. Glucose memory and aging. *American Journal Of Nutrition* 1998;67:764S–771S.
- Krebs DL, Parent MB. The enhancing effects of hippocampal infusions of glucose are not restricted to spatial working memory. *Neurobiology of Learning and Memory* 2005a;83:168–172. [PubMed: 15721802]
- Krebs DL, Parent MB. Hippocampal infusions of pyruvate reverse the memory-impairing effects of septal muscimol infusions. *European Journal of Pharmacology* 2005b;520:91–99. [PubMed: 16150437]
- Lalonde R. The neurological basis of spontaneous alternation. *Neuroscience and Biobehavioral Reviews* 2002;26:91–104. [PubMed: 11835987]
- Lee K, Dixon AK, Rowe IC, Ashford ML, Richardson PJ. The high-affinity sulphonylurea receptor regulates KATP channels in nerve terminals of the rat motor cortex. *Journal of Neurochemistry* 1996;66:2562–2571. [PubMed: 8632183]
- Lee M, Chobak J, Sik A, Wiley R, Buzsak G. Hippocampal theta activity following selective lesion of the septal cholinergic system. *Neuroscience* 1994;62:1033–1047. [PubMed: 7845584]
- Lefresne P, Guyenet P, Glowinski J. Acetylcholine synthesis from (2–14 C)pyruvate in rat striatal slices. *Journal of Neurochemistry* 1973;20:1083–1097. [PubMed: 4697871]
- Lennartz RC, Hellems KL, Mook ER, Gold PE. Inhibitory avoidance impairments induced by intra-amygdala propranolol are reversed by glutamate but not glucose. *Behavioral Neuroscience* 1996;110:1033–1039. [PubMed: 8919006]
- Levin BE. Glucose-regulated dopamine release from substantia nigra neurons. *Brain Research* 2000;874:158–164. [PubMed: 10960600]
- Lovely RH, Grossen NE, Moot SA, Bauer RH, Peterson JJ. Hippocampal lesions and inhibition of avoidance behavior. *Journal of Comparative Physiological Psychology* 1971;77:345–352.
- Marighetto A, Micheau J, Jaffard R. Effects of intraseptally injected glutamatergic drugs on hippocampal sodium-dependent high-affinity choline uptake in “naive” and “trained” mice. *Pharmacology Biochemistry and Behavior* 1994;49:689–699.
- McNay EC, Fries TM, Gold PE. Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task. *Proceedings of the National Academy of Sciences U S A* 2000;97:2881–2885.
- McNay EC, Gold PE. Memory modulation across neural systems: intra-amygdala glucose reverses deficits caused by intraseptal morphine on a spatial task but not on an aversive task. *Journal of Neuroscience* 1998;18:3853–3858. [PubMed: 9570814]
- McNay EC, McCarty RC, Gold PE. Fluctuations in brain glucose concentration during behavioral testing: dissociations between brain areas and between brain and blood. *Neurobiology of Learning and Memory* 2001;75:325–337. [PubMed: 11300738]
- Messier C. Glucose improvement of memory: a review. *European Journal of Pharmacology* 2004;490:33–57. [PubMed: 15094072]
- Monmaur P, Breton P. Elicitation of hippocampal theta by intraseptal carbachol injection in freely moving rats. *Brain Research* 1991;544:150–155. [PubMed: 1855135]
- Ohtani N, Ohta M, Sugano T. Microdialysis study of modification of hypothalamic neurotransmitters in streptozotocin-diabetic rats. *Journal of Neurochemistry* 1997;69:1622–1628. [PubMed: 9326291]
- Pacteau C, Einon D, Sinden J. Early rearing environment and dorsal hippocampal ibotenic acid lesions: long-term influences on spatial learning and alternation in the rat. *Behavioral Brain Research* 1989;34:79–96.
- Pang KC, Nocera R. Interactions between 192-IgG saporin and intraseptal cholinergic and GABAergic drugs: role of cholinergic medial septal neurons in spatial working memory. *Behavioral Neuroscience* 1999;113:265–275. [PubMed: 10357451]
- Parent, M.; Krebs, D. The memory-enhancing effects of hippocampal glucose infusions override the memory-impairing effects of septal glucose infusions; Paper presented at the XXVIII Winter Conference on the Neurobiology of Learning and Memory; Park City, UT. 2004.
- Parent M, Laurey P, Wilkniss S, Gold P. Intraseptal infusions of muscimol impair spontaneous alternation performance: Infusions of glucose into the hippocampus, but not the medial septum, reverse the deficit. *Neurobiology of Learning and Memory* 1997;68:75–85. [PubMed: 9195592]
- Parent MB, Baxter MG. Septohippocampal acetylcholine: involved in but not necessary for learning and memory? *Learning and Memory* 2004;11:9–20. [PubMed: 14747512]

- Parent MB, Gold PE. Intra-septal infusions of glucose potentiate inhibitory avoidance deficits when co-infused with the GABA agonist muscimol. *Brain Research* 1997;745:317–320. [PubMed: 9037425]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Fourth Edition. Academic Press; San Diego: 1998.
- Pych JC, Kim M, Gold PE. Effects of injections of glucose into the dorsal striatum on learning of place and response mazes. *Behavioral Brain Research* 2006;167:373–378.
- Ragozzino ME, Gold PE. Task-dependent effects of intra-amygdala morphine injections: attenuation by intra-amygdala glucose injections. *Journal of Neuroscience* 1994;14:7478–7485. [PubMed: 7996189]
- Ragozzino ME, Gold PE. Glucose injections into the medial septum reverse the effects of intraseptal morphine infusions on hippocampal acetylcholine output and memory. *Neuroscience* 1995;68:981–988. [PubMed: 8545004]
- Ragozzino ME, Hellems K, Lennartz RC, Gold PE. Pyruvate infusions into the septal area attenuate spontaneous alternation impairments induced by intraseptal morphine injections. *Behavioral Neuroscience* 1995;109:1074–1080. [PubMed: 8748958]
- Ragozzino ME, Pal SN, Unick K, Stefani MR, Gold PE. Modulation of hippocampal acetylcholine release and spontaneous alternation scores by intrahippocampal glucose injections. *The Journal of Neuroscience* 1998;18:1595–1601. [PubMed: 9454864]
- Ragozzino ME, Parker ME, Gold PE. Spontaneous alternation and inhibitory avoidance impairments with morphine injections into the medial septum. Attenuation by glucose administration. *Brain Research* 1992;597:241–249. [PubMed: 1472996]
- Ragozzino, ME.; Unick, KE.; Gold, PE. Hippocampal acetylcholine release during memory testing in rats: augmentation by glucose; *Proceedings of National Academy of Sciences United States of America*; 1996. p. 4693–4698.
- Ragozzino ME, Wenk GL, Gold PE. Glucose attenuates a morphine-induced decrease in hippocampal acetylcholine output: an in vivo microdialysis study in rats. *Brain Research* 1994;655:77–82. [PubMed: 7812793]
- Richman C, Dember W, Kim P. Spontaneous alternation behavior: A review. *Current Psychological Research & Review* 1987;5:358–391.
- Rouse ST, Levey AI. Expression of m1-m4 muscarinic acetylcholine receptor immunoreactivity in septohippocampal neurons and other identified hippocampal afferents. *Journal of Comparative Neurology* 1996;375:406–416.
- Sabolek HR, Bunce JG, Chrobak JJ. Intraseptal tacrine-induced disruptions of spatial memory performance. *Behavioral Brain Research* 2005;158:1–7.
- Shah AA, Parent MB. Septal infusions of glucose or pyruvate, but not fructose, produce avoidance deficits when co-infused with the GABA agonist muscimol. *Neurobiology of Learning and Memory* 2003;79:243–251. [PubMed: 12676523]
- Shah AA, Parent MB. Septal infusions of glucose or pyruvate with muscimol impair spontaneous alternation. *Brain Research* 2004;996:246–250. [PubMed: 14697502]
- Silver IA, Erecinska M. Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. *Journal of Neuroscience* 1994;14:5068–5076. [PubMed: 8046468]
- Stefani MR, Gold PE. Intra-septal injections of glucose and glibenclamide attenuate galanin-induced spontaneous alternation performance deficits in the rat. *Brain Research* 1998;813:50–56. [PubMed: 9824666]
- Stefani MR, Gold PE. Intrahippocampal infusions of k-atp channel modulators influence spontaneous alternation performance: relationships to acetylcholine release in the hippocampus. *Journal of Neuroscience* 2001;21:609–614. [PubMed: 11160440]
- Stefani MR, Nicholson GM, Gold PE. ATP-sensitive potassium channel blockade enhances spontaneous alternation performance in the rat: a potential mechanism for glucose-mediated memory enhancement. *Neuroscience* 1999;93:557–563. [PubMed: 10465439]
- Stevens R, Cowey A. Effects of dorsal and ventral hippocampal lesions on spontaneous alternation, learned alternation and probability learning in rats. *Brain Research* 1973;52:203–224. [PubMed: 4700704]

Wang R, Liu X, Hentges ST, Dunn-Meynell AA, Levin BE, Wang W, et al. The regulation of glucose-excited neurons in the hypothalamic arcuate nucleus by glucose and feeding-relevant peptides. *Diabetes* 2004;53:1959–1965. [PubMed: 15277373]

Figure 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 2A.

Hippocampal co-infusions of glucose with a low, sub-effective dose of muscimol (0.3 µg) did not significantly affect the mean (+/-S.E.M.) percent alternation scores ($p > .05$ vs. vehicle).

Figure 2B.

Hippocampal co-infusions of glucose with a low, sub-effective dose of muscimol (0.3 µg) did not interact to affect the mean (+/-S.E.M.) number of arm entries ($p > .05$ vs. vehicle).

Figure 3A.

Hippocampal infusions of a higher dose of muscimol (0.4 µg) significantly decreased mean (+/- S.E.M.) percent alternation scores (* $p < .05$ vs. vehicle). Hippocampal co-infusions of glucose attenuated (33 nmol; $p > .05$ vs. vehicle) or reversed (50 nmol; # $p < .05$ vs. muscimol [0.4 µg], $p > .05$ vs. vehicle) the muscimol-induced alternation deficits.

Figure 3B.
Hippocampal infusions did not significantly affect the mean (+/-S.E.M.) number of arm entries ($p > .05$ vs. PBS).

Figure 4A.
Hippocampal infusions did not significantly affect the median ($\pm$ I.Q.) number of trials to criterion ($p > .05$ vs. vehicle).

Figure 4B.

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