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Age-related memory impairments due to reduced blood glucose responses to epinephrine

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Abstract

Increases in blood glucose levels are an important component of the mechanisms by which epinephrine enhances memory formation. The present experiments addressed the hypothesis that a dysfunction in the blood glucose response to circulating epinephrine contributes to age-related memory impairments. Doses of epinephrine and glucagon that significantly increased blood glucose levels in young adult rats were far less effective at doing so in two-year-old rats. In young rats, epinephrine and glucose were about equally effective in enhancing memory and in prolonging post-training release of acetylcholine in the hippocampus. However, glucose was more effective than epinephrine in enhancing both memory and acetylcholine release in aged rats. These results suggest that an uncoupling between circulating epinephrine and glucose levels in old rats may lead to an age-related reduction in the provision of glucose to the brain during training. This in turn may contribute to age-related changes in memory and neural plasticity.

Keywords

Aging; memory loss; rapid forgetting; memory enhancement; acetylcholine; inhibitory avoidance; memory impairment

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All animal protocols used in this study have been approved by our institutional animal care and use committee.

1. Introduction

Epinephrine, released from the adrenal medulla in response to many experiences, enhances memory in both rodents and humans (Cahill and Alkire, 2003; Gold, 2001, 2005; Gold and McCarty, 1995; Gold and McGaugh, 1975; Gold and van Buskirk, 1975; Korol and Gold, 2007; McGaugh and Roozendaal, 2002). Because epinephrine does not readily cross the blood-brain barrier, the hormone most likely enhances memory by engaging peripheral mechanisms that modify brain functions. Glucose, released from the liver in response to circulating epinephrine, enhances memory, with results similar to those of epinephrine in many respects (Gold, 1995, 2005; Korol and Gold, 2007; McNay and Gold, 2002; Messier, 2004). However, some important differences between the two treatments support the idea that increases in blood glucose mediate the effects of epinephrine on memory. First, peripheral adrenergic receptor antagonists block the memory-enhancing effects of peripheral epinephrine injections (Sternberg et al., 1986) but not of glucose (Gold et al., 1986), suggesting that glucose bypasses the need to activate peripheral adrenergic receptors to enhance memory. Second, glucose enhances memory in food-deprived rats but epinephrine does not (Talley et al., 2000), consistent with the diminished increases in blood glucose to epinephrine injections seen after food deprivation. Additionally, microinjections of glucose into the lateral ventricles, medial septum, hippocampus, and amygdala, enhance memory (Ragozzino et al., 1996, 1998; Schroeder and Packard, 2003; Krebs and Parent, 2005; Pych et al., 2006; Canal et al., 2005). These findings are consistent with the idea that glucose acts directly on the brain to modulate memory processing (Gold, 2005; Korol and Gold, 2007; Gold, 2008).

There are several mechanisms by which glucose might modulate brain functions, including regulating neural excitability and stimulus-secretion coupling by closing potassium-ATP channels (Stefani and Gold, 2001; Stefani et al., 1999; Rashidy-Pour, 2001) and regulating cell signaling mechanisms such as the mammalian target of rapamycin (TSC-mTOR) cascade (Dash et al., 2006). Additionally, both systemic and central injections of glucose appear to act on memory through cholinergic mechanisms (Kopf et al., 2001; Durkin et al., 1992; Gold, 1995). In particular, *in vivo* microdialysis studies show that glucose augments training-related release of acetylcholine in a manner related to enhancement of memory (Ragozzino et al., 1996, 1998; cf. McNay and Gold, 2002). Together with the relationships between loss of cholinergic functions and aging (cf.: Bartus et al., 1982; Mesulam, 2004), these findings suggest a possible link between glucose and ACh during aging. The present experiment adds support to these relationships.

Regional fluctuations in brain glucose concentrations in extracellular fluid (ECF) are evident during memory testing. ECF glucose levels in the hippocampus decrease substantially while rats are tested on a spatial working memory task (McNay et al., 2000; McNay and Gold, 2001). Systemic injections of glucose block this decrease when enhancing memory, suggesting that ECF glucose levels limit the efficacy of memory processing. The training-induced decreases in hippocampal ECF glucose are exaggerated in aged rats. Systemic administration of glucose blocks this decrease in ECF glucose and enhances memory, raising the scores of old rats to those of young rats. Blood glucose responses to behavioral testing are decreased in senescent rats, but circulating epinephrine responses to training or stress are actually increased (Gold, 2005; Mabry et al., 1995a,b,c). The increase in release of epinephrine without subsequent increases in circulating glucose levels suggests a breakdown in a neuroendocrine pathway important for modulating memory in old rats, potentially at the step in which glucose release from the liver is coupled to the binding of epinephrine. The uncoupling between peripheral epinephrine and glucose release may reduce the amount of glucose available to the brains of old rats during memory tasks and lead to the memory impairments and other cognitive changes seen in aged rats, mice and humans (Buckner,

2004; Chawla and Barnes, 2007; Disterhoft and Oh, 2006; Gazzaley and D'Esposito, 2007; Gold, 2005; Korol, 2002; Mattson et al., 2004). The present experiments examine the role of uncoupled epinephrine-glucose responses in modulation of memory and in augmenting training-related release of ACh in aged rats.

2. Materials and Methods

2.1. Subjects

Young adult (3 to 5 mo.) and old (24 to 26 mo.) male Fischer-344 rats from NIA colonies were individually housed in translucent cages with a 12-h light/dark cycle (lights on at 07:00 h) and *ad libitum* access to food and water. One set of rats was used for the blood glucose measurements. A second set of rats was used for the memory and neurochemistry experiments.

2.2. Blood Glucose Measurements

Rats were handled for 4–5 minutes each day for 5 consecutive days prior to blood glucose measurements. Epinephrine (0.1 mg/kg [Ns = 6 young, 5 old] or 0.3 mg/kg [Ns = 6 young, 4 old]) or glucagon (200 μg/kg or 400 μg/kg [all Ns = 4]) was injected subcutaneously, followed by monitoring of blood glucose levels by collecting blood drops from the tip of the tail with a Penlet® (Lifescan, Inc., Milpitas, CA) and measuring their glucose levels with a One Touch® glucometer (Lifescan, Inc., Milpitas, CA). Data were discarded for three old rats receiving epinephrine injections because their baseline blood glucose levels were markedly low.

2.3. Surgery

Rats were anesthetized with isoflurane and placed in a stereotaxic apparatus with skulls in a horizontal orientation. A CMA/11 guide cannula (CMA, North Chelmsford, MA) was implanted above the central portion of the ventral hippocampus in both young [coordinates: −5.5 mm from bregma; ± 4.8 mm lateral; −4.2 mm deep from skull] and old [coordinates: −5.8 mm from bregma; ± 5.0 mm lateral; −4.8 mm deep from skull] rats. Skull screws were inserted and the entire assembly was anchored in place with dental cement. Beginning 1 week after surgery, rats were handled 4 to 5 minutes each day for 5 consecutive days prior to microdialysis and behavioral training.

2.4. Microdialysis

On the day of training, a 4-mm CMA/11 microdialysis probe (CMA) was inserted into the ventral hippocampus. Brains were perfused continuously at a rate of 0.67 μl/min with artificial cerebral spinal fluid (aCSF: 128 mM NaCl, 2.5 mM KCl, 1.3 mM $CaCl_2$, 2.1 mM $MgCl_2$, 0.9 mM NaH_2PO_4, 2.0 mM Na_2HPO_4, 1.0 mM dextrose, pH 7.4) containing 200 nM of the acetylcholinesterase inhibitor neostigmine (Chang et al., 2006). The 1.0 mM dextrose in the aCSF was based on previous work showing that extracellular glucose levels in the hippocampus of awake young and aged rats are about 1.0 mM (McNay and Gold, 1999). Samples collected during the first hour of microdialysis were discarded to allow for baseline stabilization (Westerink and Timmerman, 1999). Ten total samples were collected in 10-min intervals immediately prior (samples B1-4), during (P1), and after (P2-6) training. The final volume of each sample was 6.7 μL.

2.5. Training

After 4 baseline samples (10 min each) were collected, rats were trained on a one-trial inhibitory avoidance task. The apparatus was a trough-shaped alleyway (91 cm long, 22.9 cm wide at the top, 7.6 cm wide at the bottom, and 15.2 cm deep) divided into lit (31 cm)

and dark (60 cm) compartments by a sliding door that could be lowered through the floor. Rats were placed in the lit chamber and the door was lowered. Upon entering the dark chamber, the door was closed and the rats received a brief mild footshock (0.2 mA, 0.4 sec). To facilitate comparisons of drug enhancement of memory at both ages, the shock level and training conditions were selected to match 48-hr memory across ages. Past evidence indicates that age-related differences in forgetting would likely be seen at longer training-test intervals (Gold et al., 1981), but this was not assessed here. Immediately after training, rats received a subcutaneous injection of saline (0.9%) (Ns = 18 young, 15 old), epinephrine (0.1 mg/kg) (Ns = 8 young, 8 old), or glucose (250 mg/kg) (Ns = 9 young, 7 old). The rats were then returned to the holding cage as microdialysis continued. Memory, measured as the latency to enter the dark compartment (maximum = 300 sec), was assessed 48 hours later.

2.6. HPLC

Microdialysis samples were assayed for ACh using high performance liquid chromatography (HPLC) with electrochemical detection (BAS; Bioanalytical Systems, West Lafayette, IN). 5 μl of each sample were manually injected into the system via an injection valve with a 10 μl loop (Rheodyne, Rohnert Park, CA). Samples were separated using an ion-exchange microbore analytical column (BAS P/N MF 8904, 530 × 1 mm) followed by a microbore ACh/choline immobilized enzyme reactor containing acetylcholinesterase and choline oxidase (BAS P/N MF-8903, 50 × 1mm). Electrochemical detection was performed by a 3 mm glassy fiber working electrode (BAS P/N MF 1095) coated with a redox polymer film containing horseradish peroxidase and an auxiliary electrode with a radial flow electrochemical thin-layer cell and a 13-mm thin-layer gasket (BAS P/N MF 1091). The working electrode held a 100 mV potential relative to an Ag/AgCl reference electrode (BAS P/N MF 2078). A Shimadzu LC-10ADvp pump (Tokyo, Japan) with microstep plunger maintained the flow rate at 140 μL/min. The mobile phase contained 50 mM Na_2HPO_4 and 0.005% ProClin (BAS P/N CF-2150) and was adjusted to a pH of 8.5. Assays were completed within 12.5 min. The detection limit of this system was approximately 5 fmol.

2.7. Histology

Rats were deeply anesthetized with sodium pentobarbital prior to decapitation. Brains were removed and placed into 4% paraformaldehyde in 0.1 M phosphate buffer for at least 24 hrs. The brains were transferred to 20% glycerol in 0.1 M PB for at least 24 hours. Frozen sections (50 μM) were collected with a Leica 1800 cryostat. Sections containing the guide cannulae tracts were mounted on slides, dried, stained with cresyl violet, and visualized under a microscope (Figure 1). Behavioral and chemical data were discarded for those rats (N = 3) with probe sites outside of the ventral hippocampus.

2.8. Data Analyses

All analyses were performed using Statview software. Blood glucose results and HPLC data were analyzed using repeated measures ANOVA with post hoc Fisher PLSD and t-tests where appropriate. Behavioral results were analyzed using unpaired t-tests. Absolute levels of ACh release were calculated using recovery and internal HPLC standards.

3. Results

3.1. Epinephrine and glucagon injections raise blood glucose levels less in old than in young rats

Figure 2 shows the levels of blood glucose following subcutaneous injections of two different doses of epinephrine. Injections of 0.1 mg/kg epinephrine, a dose previously shown to enhance memory in young rats (Gold and van Buskirk, 1978), produced significant

increases in blood glucose levels in young rats ($F_{(1,6)} = 17.11$, $P < 0.0001$), with peak blood glucose levels increasing by 96% above baseline at 1 hr after injection. In contrast, the same dose of epinephrine resulted in very modest but statistically significant ($F_{(1,6)} = 3.54$, $P < 0.05$) increases in blood glucose levels in old rats, with peak increases of only 21% above baseline evident at 30 min after injection. The difference in the increases in blood glucose levels in young versus old rats was statistically significant ($F_{(1,6)} = 5.62$, $P < 0.05$).

In young rats, injection of a higher dose (0.3 mg/kg) of epinephrine resulted in a significant increase in blood glucose levels ($F_{(1,6)} = 24.18$, $P < 0.0001$) that was comparable to that seen at the lower dose ($F_{(1,6)} = 0.17$, $P > 0.5$). Peak blood glucose levels were evident at 1 hr after injection, reaching 99% above baseline. In contrast to the results in young rats, the epinephrine-induced increases in blood glucose levels were dose-dependent in old rats. Injection of the higher dose (0.3 mg/kg) of epinephrine in old rats resulted in significant increases in blood glucose levels compared to the lower dose ($F_{(1,6)} = 7.78$, $P < 0.05$), with peak increases of 49% above baseline evident at 1 hr after injection. The difference in the increases in blood glucose levels in young vs. old rats at the higher dose of epinephrine was not significant ($F_{(1,6)} = 1.47$, $P > 0.1$).

Glucagon injections in young and old rats resulted in similar but more rapid increases in blood glucose levels compared to epinephrine, with peak levels evident at 15 minutes after injection (Figure 3). At both doses and both ages, glucagon injections resulted in significant increases in blood glucose levels ($Fs_{(1,3)} = 16.53$ to 29.34, $Ps < 0.01$). Similar to epinephrine, there was a significant effect of age at a low (200 μg/kg) but not a high (400 μg/kg) dose of glucagon ($F_{(1,3)} = 140.81$, $P < 0.0001$ for low dose; $F_{(1,3)} = 3.35$, $P > 0.1$ for high dose). Unlike epinephrine, there was a significant effect of treatment in young but not old rats ($F_{(1,3)} = 14.34$, $P < 0.01$ in young; $F_{(1,3)} = 1.87$, $P > 0.1$ in old).

3.2. Glucose is more effective than epinephrine at enhancing memory in old rats

As shown in Figure 4, post-training injections of either epinephrine or glucose significantly increased 48-hr test latencies in the inhibitory avoidance task in young rats (t-tests: $Ps < 0.05$ for epinephrine and for glucose vs. saline). The difference in test latencies in rats injected with glucose and epinephrine did not differ significantly ($P > 0.05$).

Although both epinephrine and glucose resulted in higher memory scores in young rats, only glucose significantly increased latencies on the memory tests in old rats. In old rats receiving post-training epinephrine injections, the median latency on the test trials increased from 74 seconds in the saline controls to 159 seconds in the epinephrine-treated rats, but these latencies did not differ significantly from each other ($P > 0.1$). In contrast, old rats in the glucose group had median retention latencies at the maximum of 300 seconds, scores significantly higher than those of the saline controls ($P < 0.05$). There was no significant difference in test latencies between glucose- and epinephrine-treated old rats or between glucose-treated old rats and either epinephrine-or glucose-treated young rats ($Ps > 0.2$).

3.3. Glucose modulates training-related release of ACh similarly in young and old rats, but epinephrine is more effective in young than in old rats

Figure 5 shows the ACh results obtained with *in vivo* microdialysis in young and old rats. The baseline values shown are percent change from the mean baseline levels, which were obtained for each rat by averaging the 4 samples (20 min each) collected prior to training. Thus, the data are presented as percent change from baseline levels in 10-minute intervals immediately prior to (B1-4 = Baseline), during (P1), and after (P2-6) training. In both young and old rats, training in the saline-treated rats resulted in increases from baseline ACh release of ~100 – 150%, which gradually returned to baseline in the 40 minutes or so after

training. In young rats, there was a significant effect of treatment ($F_{(2,9)} = 3.66$, $P < 0.05$), in which post-training administration of epinephrine or glucose significantly enhanced ACh release (Fisher's PLSD: Ps < 0.05 vs. saline).

As was seen in young rats, there was a significant effect of treatment on ACh release ($F_{(2,9)} = 6.76$, $P < 0.01$) in old rats, with glucose significantly enhancing ACh release compared to saline ($P < 0.0001$). However, unlike in young rats, epinephrine did not significantly increase ACh release in old rats ($P > 0.05$), although it did result in modest non-significant increases in the duration of ACh release. These results parallel those shown in Figures 2 and 4, in which epinephrine was deficient at increasing blood glucose levels and at enhancing memory in old rats, respectively.

3.4. Baseline ACh release in the ventral hippocampus is lower in old than in young rats

While the percent changes in ACh release immediately after training are similar in young and old rats, as in Figure 5, there are substantial age-related differences in baseline and peak levels of ACh concentrations in the dialysates. Figure 6 shows the absolute baseline and training-induced levels of ACh release in the ventral hippocampus of young and old rats. Baseline levels were significantly lower in old rats than in young rats, with values about half those seen in young rats (t-test: $P < 0.0001$). Training-related levels of ACh release were reduced in old compared to young rats with post-training injections of both epinephrine and glucose (Fisher's PLSD: Ps < 0.05). Thus, it is clear that the increases in concentration and duration of ACh release concentrations induced by glucose administered to aged rats bring the levels toward those of young baseline values but do not result in full amelioration of age-related decreases in release.

4. Discussion

These findings suggest that dysfunctions in blood glucose responses to epinephrine contribute significantly to age-related memory loss. During aging, epinephrine develops reduced efficacy in increasing blood glucose levels, enhancing memory, and augmenting training-initiated increases in release of ACh in the hippocampus.

4.1. Impaired blood glucose responses to epinephrine and glucagon in aged rats

Doses of epinephrine and glucagon that significantly increased blood glucose levels in young rats were far less effective at doing so in old rats, in which higher doses were needed to increase blood glucose levels. These findings are consistent with others showing that a wide variety of hormones, including insulin and ACTH, show reduced functionality with age (Fink et al., 1983; Giordano et al., 2001; Parker et al., 2000).

Previous work has shown that basal levels of plasma epinephrine are similar in young and aged rats, but that aged rats exhibit higher circulating epinephrine levels after placement in a novel environment, footshock, or immersion in water (Mabry et al., 1995a,b,c, 1996). The higher responses to stimulation may reflect a futile physiological attempt to generate increases in blood glucose levels. The present results support and extend these findings by showing that old rats have substantially blunted increases in blood glucose following subcutaneous injections of epinephrine.

In old rats, the uncoupling between circulating epinephrine and glucose suggests a dysfunction in the ability of the liver to produce glucose in response to epinephrine binding. There is substantial evidence for age-related changes in hepatic adrenoreceptors, including in their regulation of hepatic glucose production in both rats and humans (Ebstein et al., 1985; Graham et al., 1987; Katz et al., 1993; Podolin et al., 1996; Van Ermen et al., 1992). Blockade of peripheral adrenoreceptors blocks the effects of systemic injections of

epinephrine but not glucose injections on blood glucose levels and on enhancement of memory (cf. Gold, 2005).

The finding that blood glucose responses to both glucagon and epinephrine were reduced in old rats suggests involvement of shared cell signaling mechanisms beyond the respective receptors. Considerable evidence demonstrates age-related dysfunctions in cell signaling pathways involved in hepatic glucose production, including activity of hepatic adenylate cyclase, involved in both epinephrine and glucagon-mediated signal transduction (Ebstein et al., 1985; Podolin et al., 2001), and mitochondrial damage, which may also be responsible for reduced rates of gluconeogenesis in hepatocytes of old rats (Liu et al., 2002; Sastre et al., 1996).

Cross-sectional studies in humans generally show a reduction in basal and stress-associated epinephrine in older individuals (Esler et al., 1995; Kjeldsen et al., 1982; Seals and Esler, 2000). However, interpretation of these cross-sectional studies is complicated by attrition (Woodruff-Pak, 1997), a particular concern here because epinephrine levels are positively associated with hypertension and other conditions (Floras, 1992; Rand and Majewski, 1984). A relatively short-term longitudinal study of aging human subjects found that increases in urinary epinephrine levels in elderly individuals over a three-year period were associated with declines in a variety of cognitive measures (Karlamangla et al., 2005). Based on this, the authors suggest that chronic stress may be an important predictor of cognitive decline in elderly humans. Alternatively, the elevated epinephrine levels may reflect disruption of a negative feedback mechanism due to an impaired glucose response, with cognitive impairments related to loss of glucose modulation of brain functions as is seen in rats.

4.2. Epinephrine and glucose effects on memory in aged rats

A glucose dose that is effective in enhancing memory in young rats retains that efficacy in aged rats. However, an epinephrine dose effective in enhancing memory in young rats is not as effective in old rats. Together with the blood glucose results, there is likely an age-related shift to higher doses of epinephrine needed to enhance memory in aged rats. Direct glucose injections may enhance memory in old rats by circumventing the functional impairment of epinephrine-induced increases in blood glucose levels.

Unlike the lower dose of epinephrine, a higher dose did significantly enhance circulating blood glucose in old rats. Given this, one might expect that the higher dose would significantly enhance memory for the inhibitory avoidance task, consistent with an age-related shift in the dose-response curve for memory facilitation by epinephrine. However, in attempting tests with the higher dose in aged rats, the dose resulted in apparent discomfort at the injection site that interfered with the behavioral tests, and the tests were therefore discontinued.

4.3. Glucose effects on brain neurochemical responses to training

Extracellular glucose levels, and the maintenance of these levels by increases in blood glucose levels (Fellows and Boutelle, 1993; Fellows et al., 1993; Korf et al., 1993), appear to be important to memory functions. ECF glucose levels in the hippocampus decrease while rats perform a spatial working memory task; the magnitude of the decrease increases as a function of task difficulty (McNay et al., 2000). Systemic injections of glucose reverse the depletion of ECF glucose and improve memory scores. The decrease in hippocampal ECF glucose levels is exaggerated in magnitude and duration in aged rats, declining by as much as 50% during memory testing (McNay and Gold, 2001). Of interest, the more rapid recovery of ECF glucose levels in young rats is associated with a rise in blood glucose levels, a rise that does not occur in aged rats.

The effects of epinephrine-glucose uncoupling on brain functions may be exacerbated by other age-related dysfunctions in mechanisms of glucose uptake into the brain. For instance, changes with age in the efficiency and plasticity of glucose transporters may result in local reductions in glucose uptake (Messier, 2004; Messier and Teutenberg, 2005). GLUT-1 is a high-affinity glucose transporter expressed in capillary endothelial cells with a principle role in mediating the facilitated uptake of glucose through the blood-brain barrier (Maher et al., 1994). GLUT-1 is reduced in specific regions of the brain, including the hippocampus, during normal aging (Gschanes et al., 2000; Vorbrodt et al., 1999) and in Alzheimer's Disease (Horwood and Davies, 1994; Kalaria and Harik, 1989; Simpson et al., 1994).

There are several mechanisms by which dynamic changes in ECF glucose might regulate memory functions (cf. McNay and Gold, 2002). One possibility is that ECF glucose may regulate neural excitability by actions at K-ATP channels. In pancreatic β-cells, glucose controls insulin release by regulating intracellular ATP/ADP ratios and closing K-ATP channels (Ashcroft, 2005; Hansen, 2006). These inwardly rectifying potassium channels are present throughout the brain and expressed at high levels in the hippocampus (Dou et al., 2003; Mourre et al., 1990, 1991), offering a potential mechanism by which glucose might regulate neural excitability and stimulus-secretion coupling. Administration of drugs that close and open K-ATP channels have effects on memory similar to those of glucose availability and depletion, respectively (Banchelli et al., 2000; Ghelardinia et al., 1998; Rashidy-Pour, 2001; Stefani and Gold, 2001; Stefani et al., 1999).

Changes in glucose availability, from blood glucose or by alterations in glucose transporter functions, have effects on neurotransmitters related to memory processing. Extensive evidence suggests that alterations in the cholinergic neurotransmitter system contribute to age-related and pathological memory impairments (Müller et al., 1991; Terry and Buccafusco, 2003). The finding here that baseline levels of release of ACh in the hippocampus decline with age is consistent with past results (Feuerstein et al., 1992; Terry and Buccafusco, 2003; Wu et al., 1988). The augmentation of ACh release in aged rats by glucose did not result in full amelioration of age-related declines in release even as the enhancement of memory was as robust, or more so, in aged rats. Together, the behavioral and neurochemical findings may reflect actions of glucose on neural functions other than ACh release or up-regulation of receptor and cell signaling responses to ACh in response to the decreased levels of release.

While past reports showed that glucose treatment can augment ACh release during memory testing (Ragozzino et al., 1996, 1998), the present study is the first to demonstrate an effect of a post-training memory-enhancing treatment on neurotransmitter release after an experience. Glucose was effective at augmenting training-related increases in the duration of ACh release in both young and old rats, in concert with enhancement of memory. Epinephrine also augmented the duration of ACh release and enhanced memory in young rats but, in contrast to glucose, failed to do so significantly in aged rats.

5. Conclusions

The findings of these experiments support the hypothesis that age-related memory impairments may arise from disruptions in the neuroendocrine regulators that engage central memory processes. According to this view, the release of epinephrine and the subsequent elevation in blood glucose levels provide a crucial component of a process that up-regulates brain mechanisms responsible for the formation of new memories. In young rats, trivial events accompanied by small physiological responses are quickly forgotten. Salient events that induce a relatively large physiological response lead to more enduring memories (Gold and McGaugh, 1975). The results here indicate that in aged rats, many experiences may be

handled as “trivial” events, due to an impaired glucose response, and are therefore forgotten more quickly. Thus, the more rapid forgetting observed in old rats may reflect a chain of events, beginning with an uncoupling of the epinephrine and glucose response as a component of dysfunctions leading to deficiencies in ECF glucose levels. In turn, this deficit may depress neural excitability and release of neurotransmitters including ACh, ultimately negatively impacting memory formation and maintenance. It will be important to extend these findings by examining the effects of treatments that enhance cognitive and other brain function during aging, including caloric restriction (e.g.: Adams et al., 2008; Fontan-Lozano et al., 2007; Patel and Finch, 2002) and exercise (e.g.: Erickson et al., 2007; van Praag et al., 2005), on central and peripheral regulation of glucose levels and utilization.

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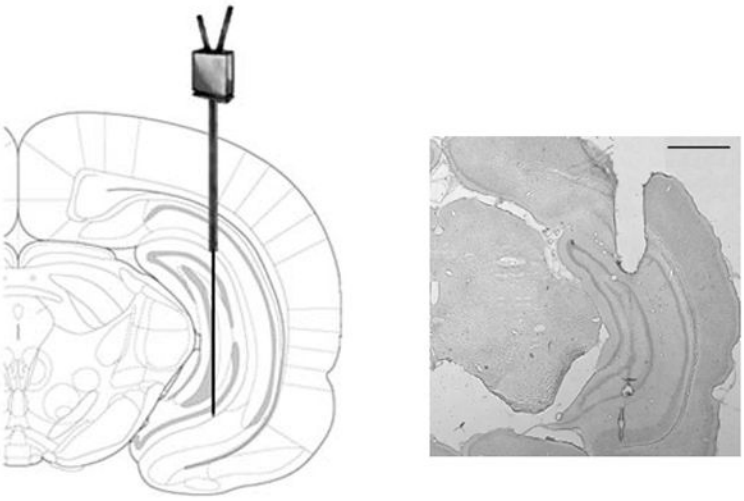

Figure 1.
(Left) Illustration of the area targeted in this study, showing insertion of a CMA microdialysis probe into the ventral hippocampus (adapted from Paxinos and Watson, 1998). (Right) Photomicrograph of a cresyl violet-stained section showing a representative cannula placement in the ventral hippocampus of an old rat. Scale bar = 1.6 mm.

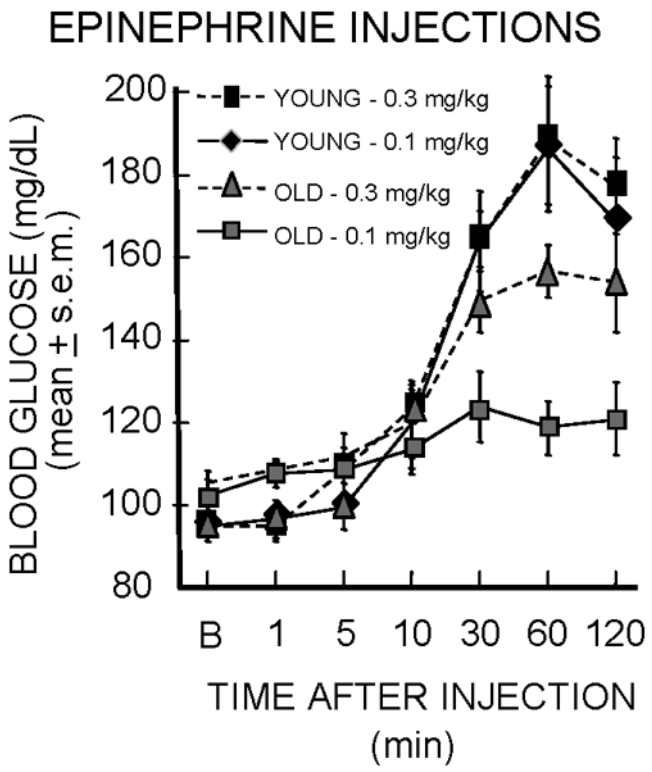

Figure 2.
Blood glucose measurements in young and old rats following injections of epinephrine. Age-related impairments in blood glucose responses were observed following a lower but not a higher dose of epinephrine.

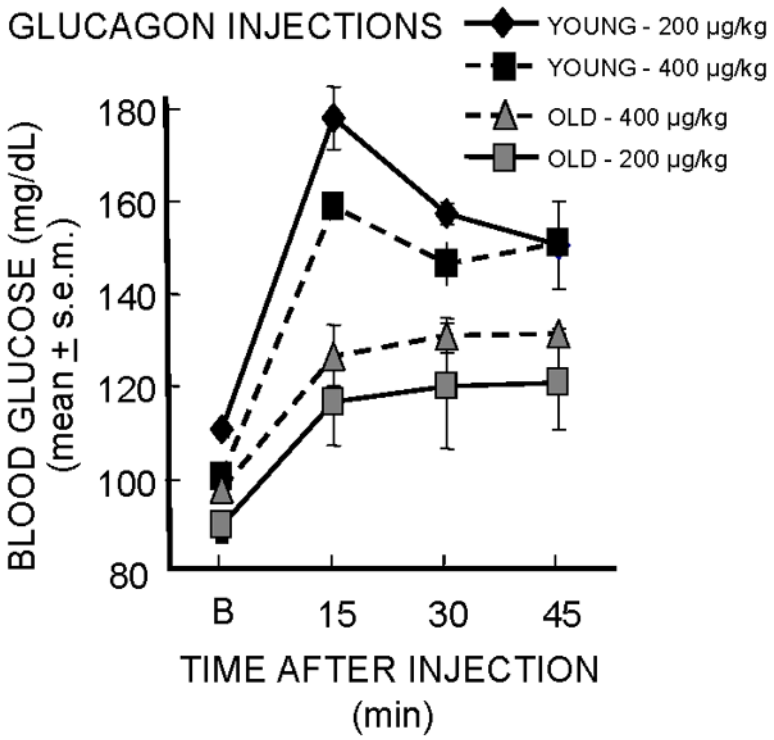

Figure 3.
Blood glucose measurements in young and old rats following injections of glucagon. Similar to epinephrine, age-related impairments in blood glucose responses were observed following a lower but not a higher dose of glucagon.

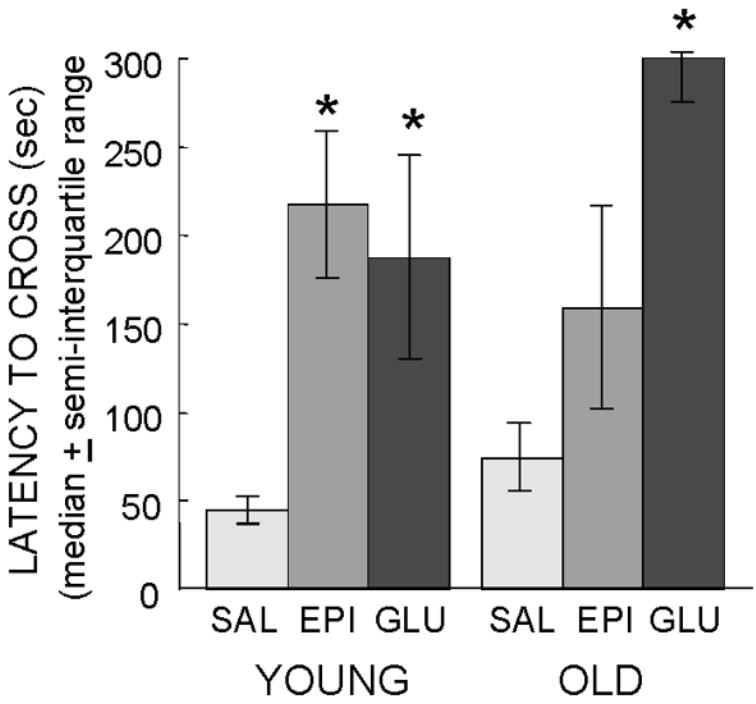

Figure 4.

Effects of post-training injections of epinephrine (0.1 mg/kg) and glucose (250 mg/kg) on memory tested 48 hours after inhibitory avoidance training in young and old rats. Post-training injections of glucose, but not epinephrine, significantly enhanced memory in old rats. (*) Ps < 0.05 vs. saline controls.

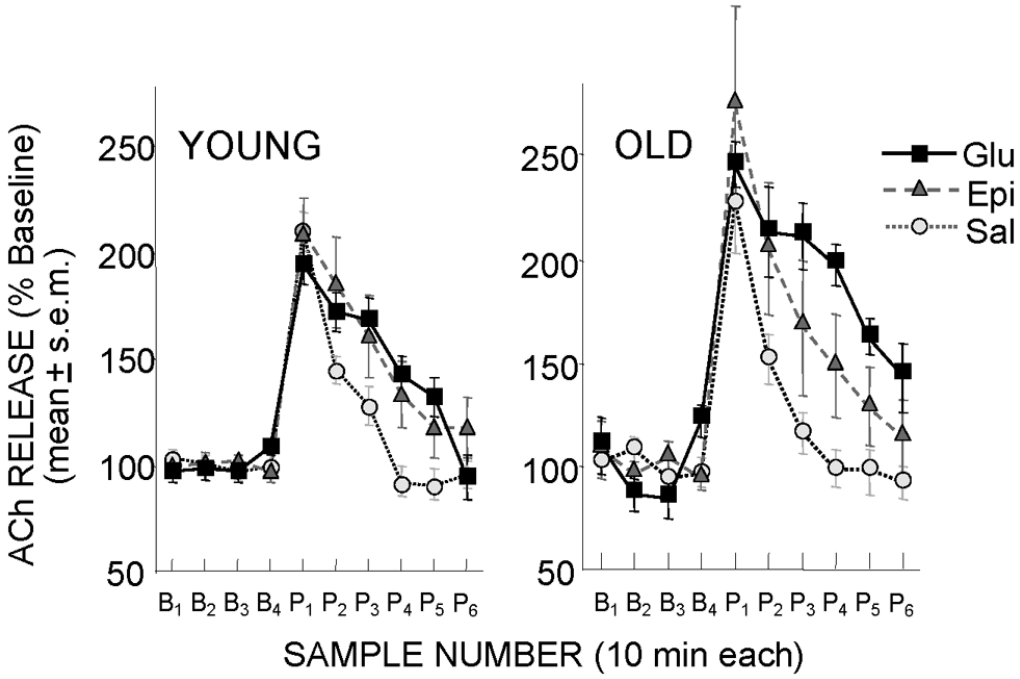

Figure 5.

Percent changes in ACh release in the ventral hippocampus accompanying inhibitory avoidance training in young (left graph) and old (right graph) rats. Compared to saline controls, post-training injections of glucose, but not epinephrine, significantly enhanced training-related release of ACh in old rats ($P < 0.05$). B = baseline samples; P = posttraining samples.

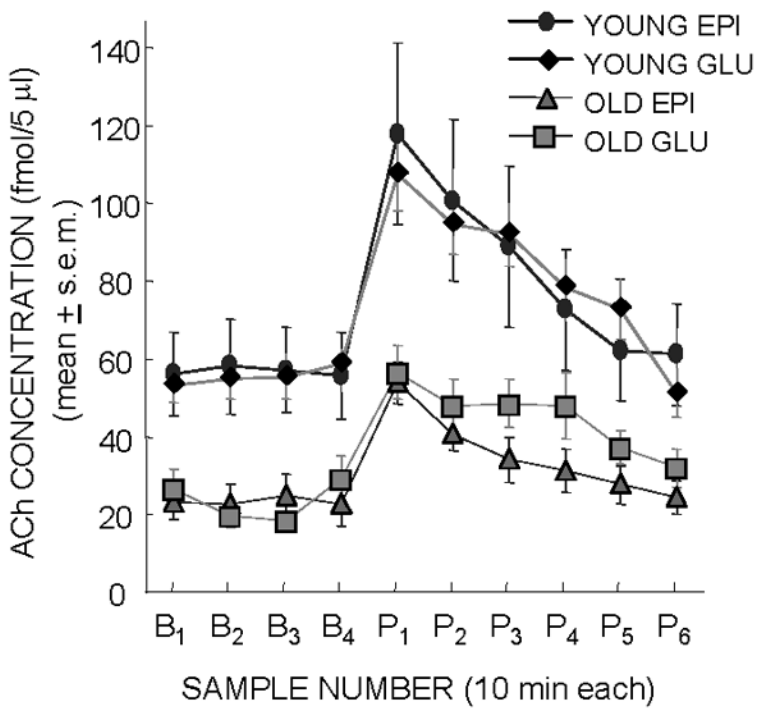

Figure 6.
Absolute levels of ACh release in the ventral hippocampus accompanying inhibitory avoidance training in young and old rats. Baseline and training-associated increases in ACh release were significantly lower in old compared to young rats (Ps < 0.05 young vs. old). B = baseline samples; P = posttraining samples.