

# Cognitive and Neural Hippocampal Effects of Long-Term Moderate Recurrent Hypoglycemia

Ewan C. McNay,<sup>1</sup> Anne Williamson,<sup>2</sup> Rory J. McCrimmon,<sup>1</sup> and Robert S. Sherwin<sup>1</sup>

**Recurrent hypoglycemia is the most feared complication of intensive insulin therapy for type 1 diabetes. Study of the cognitive impact of recurrent hypoglycemia in humans has been hampered by difficulty in controlling for prior glycemic history and diabetes status; there have been no prospective studies. We used a rat model of recurrent hypoglycemia with hypoglycemia for 3 h, once weekly, from 1 month of age. At 4, 8, and 12 months of age, cohorts were tested on a hippocampally dependent spatial memory task, during which hippocampal extracellular fluid (ECF) glucose and lactate were measured using microdialysis. At 4 months, recurrent hypoglycemia improved euglycemic task performance ( $76 \pm 4$  vs.  $64 \pm 3\%$  for controls) and reversed the task-associated dip in ECF glucose seen in controls. However, recurrent hypoglycemia impaired performance in animals tested when hypoglycemic ( $45 \pm 4$  vs.  $55 \pm 2\%$ ). Recurrent hypoglycemia preserved euglycemic task performance across age: at 12 months, both task performance (62%) and ECF glucose changes in euglycemic recurrently hypoglycemic animals resembled those of 4-month-old control animals, whereas control animals' performance deteriorated to chance (44%) by 8 months. At 12 months, hippocampal slice physiology was assessed, with results paralleling the cognitive findings: slices from recurrently hypoglycemic rats showed improved  $\gamma$ -aminobutyric acid (GABA)ergic inhibition at euglycemia but much greater loss of this tone at low bath glucose. Our data show that moderate weekly hypoglycemia prevented age-related decline in hippocampally cognitive function and cognitive metabolism, at least when euglycemic. The impact of recurrent hypoglycemia on cognition is multifaceted and includes both metabolic and electrophysiological components. *Diabetes* 55:1088–1095, 2006**

**T**he brain is, under normal conditions, fueled by glucose; acute hypoglycemia impairs cognition. The most common cause of hypoglycemia is the use of exogenous insulin by individuals with type 1 diabetes. The incidence of recurrent hypoglycemia has increased with use of intensive insulin therapy (1), leading to hypoglycemia being the most feared consequence of such treatment (2) and many patients worrying about the impact of recurrent hypoglycemia on their future cognitive function. Reports of the impact of recurrent hypoglycemia on neural and cognitive function in

humans have varied widely, likely in large part because of difficulty in adequately controlling and determining prior hypoglycemic history as well as the competing influences of factors such as chronic exposure to hyperglycemia, cerebrovascular disease, and chronic illness per se; the literature contains reports of recurrent hypoglycemia producing enhanced, impaired, or unaffected subsequent cognitive function (3–17). Recently, we showed, using a rat model of short-term (3-day) recurrent hypoglycemia, that both cognitive function and hippocampal extracellular fluid (ECF) glucose were altered following recurrent hypoglycemia (18), in keeping with the importance of glucose supply in modulating hippocampally dependent cognitive performance (19–21); the hippocampus is a key brain area for many forms of learning and memory.

However, the 3-day recurrent hypoglycemia protocol used in our previous study (18) did not mimic the clinical situation, where hypoglycemic events are sporadic over a longer time. Further complicating the picture is the fact that aging itself appears to diminish hippocampal glucose supply, especially at times of cognitive challenge, which in turn leads to impaired cognitive performance (20). Therefore, we studied the impact of a more clinically relevant moderate hypoglycemia (50 mg/dl plasma glucose, 3 h, once weekly) for up to 11 months, beginning at 1 month of age. We started with young rats because the neural and cognitive consequences of recurrent hypoglycemia in humans may be more severe when beginning during childhood (22,23). Cognitive assessment used a hippocampally dependent spatial memory task known to be sensitive to glucose availability (19). Key to the design was the ability to examine the impact of recurrent hypoglycemia without confounds from long-term diabetes, which is often associated with accelerated cognitive decline, the underlying cause(s) of which are unclear.

We hypothesized that recurrent hypoglycemia might cause adaptation in the brain, perhaps including increased glucose supply and/or use of alternate fuels, leading to improved cognitive performance especially during subsequent hypoglycemia. This hypothesis was shown to be correct at euglycemia, but not during subsequent hypoglycemia, where in fact a significant performance deficit was found. Our findings suggest that recurrent hypoglycemia markedly affects subsequent cognition and (at least at euglycemia) may if anything enhance and preserve cognitive function. Moreover, because preservation of function was seen for at least 12 months (the equivalent of perhaps late middle-age in a rat), the mechanisms underlying such an attenuation of age-related cognitive decline will be of increasing interest both to diabetic subjects and to the general population.

## RESEARCH DESIGN AND METHODS

A total of 98 male Sprague-Dawley rats (Charles River, Wilmington, MA) were studied starting at 1 month of age. Rats were individually housed, with food

From the <sup>1</sup>Section of Endocrinology, Yale School of Medicine, New Haven, Connecticut; and the <sup>2</sup>Department of Neurosurgery, Yale School of Medicine, New Haven, Connecticut.

Address correspondence and reprint requests to Ewan C. McNay, Section of Endocrinology, Yale School of Medicine, One Gilbert Street, TAC S147, P.O. Box 208020, New Haven, CT 06519. E-mail: ewan.mcnay@yale.edu.

Received for publication 9 October 2005 and accepted in revised form 5 January 2006.

aCSF, artificial cerebrospinal fluid; ECF, extracellular fluid; GABA,  $\gamma$ -aminobutyric acid; PPI, paired pulse inhibition.

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and water available ad libitum, on a 12:12-h light:dark schedule (lights on at 0700). The Yale University Institutional Animal Care and Use Committee approved all procedures. After a 1-week acclimatization period, animals received one injection per week intraperitoneally of either sterile saline (0.5 ml, control animals) or human insulin (Humulin, Eli Lilly; recurrently hypoglycemic animals). The insulin dose was initially 10 units/kg, given in 0.5 ml, which in our hands reliably induces moderate hypoglycemia of ~50 mg/dl (18,24). Insulin doses were gradually reduced to maintain this level of induced hypoglycemia; the recurrently hypoglycemic rats required progressively less insulin to achieve target hypoglycemia, presumably due to the loss of the counterregulatory response to induction of hypoglycemia (25; see RESULTS). By the end of the experiment, the dose of insulin used was 0.5 units/kg. Recurrently hypoglycemic animals were closely observed after induction of hypoglycemia, and food was withheld for 3 h, after which animals were given an intraperitoneal bolus of glucose, if necessary, to restore euglycemia. During a random subset of the weekly hypoglycemic episodes, blood glucose levels were monitored to confirm that hypoglycemic levels of ~50 mg/dl were consistently achieved. There were no instances of blood glucose levels <30 mg/dl; animals who became nonresponsive to a mild tail pinch received 50% dextrose intraperitoneally to achieve target hypoglycemia. No animal experienced coma or seizure.

**Surgery.** Two weeks before testing, rats received atropine sulfate (0.2 ml of 540 mg/ml solution i.p.) followed by anesthesia with a ketamine:xylazine mix. Microdialysis guide cannulae (CMA12, CMA/Microdialysis) were aimed at the hippocampus, as described previously (26). Rats were allowed to recover for at least 1 week and were handled extensively. About 5 days before testing, indwelling vascular catheters were implanted as described previously (24).

**Groups.** At testing, animals were randomly assigned to one of two conditions: 1) euglycemia or 2) acute hypoglycemia. This produced a total of 2 (control/recurrent hypoglycemia)  $\times$  2 (euglycemia/acute hypoglycemia)  $\times$  3 (age-groups) = 12 groups. However, at 8 or 12 months, animals made hypoglycemic became aknetic (not moving from their initial point of placement on the maze). Hence, we analyzed only euglycemic maze performance in the older animals. Thus, we analyzed performance data from four groups of animals at 4 months of age and two groups at each of 8 and 12 months.

Acutely hypoglycemic animals received intraperitoneal insulin 20 min before maze testing. This reduced their plasma glucose to ~50 mg/dl at the midpoint of the test period. Control acutely hypoglycemic animals received 10 units/kg insulin; the dose of insulin used in recurrently hypoglycemic animals was titrated to achieve this glucose level based on each animal's history. Animals studied at euglycemia received a control injection of saline.

**Microdialysis procedures.** A fresh probe was inserted, and animals were allowed to acclimate for 2 h. The dialysis membrane was 3 mm long and thus sampled across several regions of the hippocampus. Rats were allowed to move freely throughout. Probes were perfused at 1.5  $\mu$ l/min with artificial extracellular fluid as previously described (26). All reagents were obtained from Sigma (St. Louis, MO).

**Blood sampling.** On the day of testing, vascular catheters were opened. After the acclimation period, plasma glucose samples (100  $\mu$ l) were taken every 10 min. Additional samples (200  $\mu$ l) were taken during baseline and immediately after testing for hormone analysis. Vascular catheters on several animals became nonpatent during testing; behavioral and microdialysis data from these animals were included in analyses, and a thigh prick was used to confirm hypoglycemia post-testing, if the animal was part of an acutely hypoglycemic group.

**Sample analysis.** Microdialysis samples were assayed for glucose and lactate using a CMA600 analyzer. Measurements were corrected for in vivo probe recovery using the slope of a hippocampal ECF zero-net-flux plot for glucose under the same experimental conditions (26). In vitro pilot experiments showed probe recovery for glucose and lactate to be similar. Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA). Catecholamine analysis was performed by high-performance liquid chromatography (ESA, Acton, MA).

**Behavioral procedures.** Rats were placed into the center of a four-arm maze and allowed to explore for 20 min. Rats spontaneously alternate between maze arms, using spatial working memory to retain knowledge of arms previously visited. Spontaneous alternation has been extensively used as a spatial working memory task (27–30) that examines acute learning and memory. Specifically, the measure of memory performance used was percent 4/5 alternation. An alternation is counted when the rat visits all four arms within any span of five consecutive arm choices; the maximum number of alternations is  $N-4$ , where  $N$  is the total number of arms entered (19). The actual number of alternations made is expressed as a percentage of this number. Chance level on this measure is 44% (31).

**Histology.** After maze testing, rats were killed by overdose of sodium pentobarbital, brains were removed, and sections were taken for confirmation

of probe placement; only data from animals with correct probe placements were included in analyses.

**Slice recording methods.** In a subset of 12-month-old rats ( $n = 9$ , 4 control and 5 recurrently hypoglycemic rats), electrophysiological measurements were made in the hippocampus contralateral to that in which microdialysis was performed. Rats were anesthetized with sodium pentobarbital and decapitated. The brains were immersed in ice-cold oxygenated artificial cerebrospinal fluid (aCSF), in which the NaCl was replaced with sucrose. The standard aCSF contained (in mmol/l) 124 NaCl, 3 KCl, 2 MgSO<sub>4</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 2.0 CaCl<sub>2</sub>, and 10 glucose, pH 7.4. After 2 min, 400  $\mu$ m transverse hippocampal slices were prepared. Slices were oxygenated and maintained at 35°C in an interface type recording chamber (Fine Science Tools, Foster City, CA). The tissue was perfused with aCSF at 1 ml/min.

Extracellularly recorded population spikes were measured in CA1 using patch pipettes filled with aCSF. The amplitude of the population spike (Fig. 6A) provides a measure of the number of cells firing in response to a synaptic stimulus. The recording electrode was placed in the cell body layer, and a twisted bipolar stimulating electrode was placed in the stratum radiatum. Two levels of bath glucose were used to simulate euglycemia and hypoglycemia: 10 and 2 mmol/l, respectively. The levels of glucose needed to support synaptic function are higher than those needed in vivo because the glucose must permeate into the tissue without the aid of a functioning microvasculature. Under our recording conditions, tissue glucose is on the order of 5 mmol/l with 10 mmol/l glucose in the bath and 0.5 mmol/l at 2 mmol/l bath glucose (32). A total of 2 mmol/l glucose was chosen as being "hypoglycemic" based on pilot studies in slices from young adult rats showing a 25% decrease in the amplitude of the evoked population spike after repetitive stimulation at this glucose level.

In a subset of slices, we examined the IPSP (inhibitory postsynaptic potential) conductance in CA1 pyramidal cells using intracellular recordings using sharp microelectrodes filled with 4 mol/l K-acetate. The polysynaptic IPSP was evoked at several membrane potentials, and the IPSP conductance was taken as the difference between the slope of the current voltage relationship at the peak of the response and at rest (33). This measure allows for direct confirmation and quantification of effects on inhibitory synaptic function.

**Statistical analysis.** Significance for all tests was set at an  $\alpha$  level of 0.05. Data analysis was performed using Axograph or Excel; Statview or Excel was used for statistical analyses. Data are expressed as means  $\pm$  SE. ECF glucose data were analyzed with planned  $t$  tests on the first maze sample, on the basis of past results (18–21,34) and further post hoc tests as indicated.

## RESULTS

**Long-term hypoglycemia.** Plasma glucose during weekly hypoglycemia averaged  $46 \pm 1$  mg/dl. Hypoglycemia produced in recurrently hypoglycemic animals was the result of administered insulin rather than conditioning; injections of saline to recurrently hypoglycemic animals did not affect plasma glucose (data not shown).

**Systemic glucose during maze testing.** Baseline plasma glucose did not vary significantly, with group means between 120 and 133 mg/dl. All euglycemic groups showed a similar slight rise in plasma glucose during maze testing (as seen previously [20]; data not shown). Plasma glucose during hypoglycemic testing did not vary significantly between groups, averaging  $47 \pm 1$  mg/dl (recurrent hypoglycemia) and  $50 \pm 4$  mg/dl (control) (Fig. 1).

**Plasma epinephrine.** We expected that recurrent hypoglycemia would suppress the epinephrine response to further hypoglycemia, and this hypothesis was confirmed. At the end of maze testing (i.e., hypoglycemic; Fig. 1), the plasma epinephrine of recurrently hypoglycemic-acutely hypoglycemic 4-month-old animals averaged 1,126 vs. 4,986 pg/ml in control animals [ $t(11) = 5.47$ ,  $P < 0.0002$ ]. Data from 8- and 12-month-old animals measured at hypoglycemia (without maze testing) confirmed this result. At 8 months, recurrently hypoglycemic animals averaged 829 vs. 4,936 pg/ml for controls [ $t(9) = 4.22$ ,  $P < 0.005$ ]; at 12 months, recurrently hypoglycemic animals averaged 566 vs. 2,963 pg/ml for controls [ $t(10) = 6.32$ ,  $P < 0.0001$ ]. The

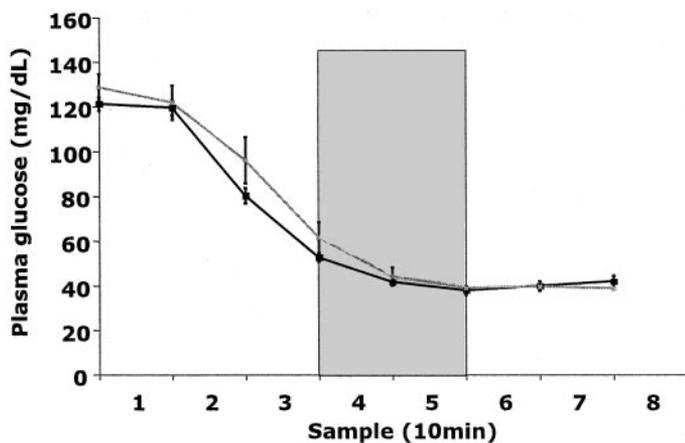


FIG. 1. Plasma glucose concentrations in the two acutely hypoglycemic groups of animals at 4 months old. The black line denotes recurrently hypoglycemic animals; the gray line denotes control animals. The shaded rectangle represents the maze-testing period. Data are means  $\pm$  SE and are point readings at the beginning of each sample period. No significant differences were observed between the groups.

decrease with age was significant (linear trend  $P < 0.02$ ) but did not vary significantly between recurrently hypoglycemic and control animals.

**Behavioral testing.** At euglycemia, 4-month-old recurrently hypoglycemic animals showed improved alternation performance ( $76 \pm 4$  vs.  $64 \pm 3\%$ ,  $P < 0.04$ ) compared with control animals (Fig. 2). Unexpectedly, when tested at acute hypoglycemia, recurrent hypoglycemia produced a performance deficit, with recurrently hypoglycemic-acutely hypoglycemic animals performing no better than chance on the maze task ( $45 \pm 4$  vs.  $55 \pm 2\%$  for control-acutely hypoglycemic animals,  $P < 0.03$ ). Performance differences were not due to altered locomotor activity; hypoglycemic animals entered fewer arms than did euglycemic animals, but no effect of recurrent hypoglycemia on locomotor activity was seen (data not shown).

At euglycemia, control animals at either 8 or 12 months of age performed at chance level (44 and 41%, respectively; Fig. 3). However, recurrent hypoglycemia improved task performance in all age-groups (Fig. 3): 8-month-old recurrently hypoglycemic animals performed at the same level as 4-month-old recurrently hypoglycemic animals ( $79\%$ , NS vs. 4-month-old recurrently hypoglycemic), while even at

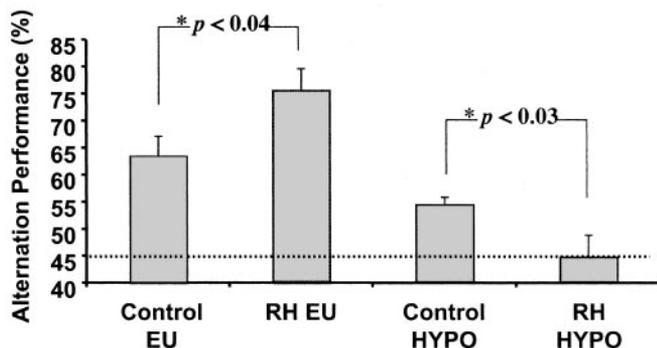


FIG. 2. Maze alternation performance in 4-month-old animals. Recurrent hypoglycemia (RH) produces an enhancement of performance at euglycemia (EU), but a decrement in performance at hypoglycemia (HYPO). Data are means  $\pm$  SE. Chance level performance, indicated by a dotted line, is 44%. In addition to the significant effects of recurrent hypoglycemia indicated, acutely hypoglycemic animals were impaired in general, as would be expected.

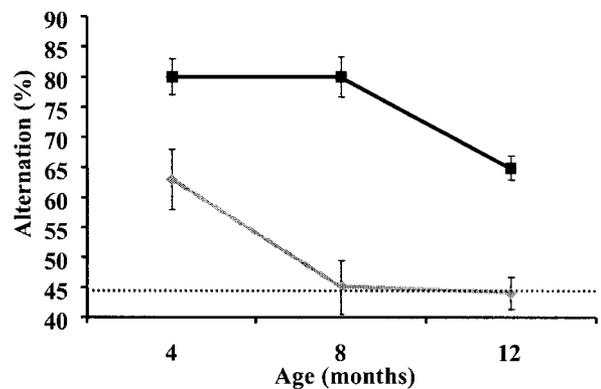


FIG. 3. Maze alternation performance at euglycemia across age-groups. The black line denotes recurrently hypoglycemic animals; the gray line denotes control animals. Recurrent hypoglycemia produces an enhancement of performance at all ages, with control animals' performance dropping to chance levels by 8 months of age. Data are means  $\pm$  SE. Chance level performance, indicated by dotted line, is 44%.

12 months, recurrently hypoglycemic animals were performing at the same level as 4-month-old control animals (62 vs. 64% for 4-month-old controls, NS).

**ECF glucose and lactate.** Consistent with previous work (18–21), 4-month-old control animals showed a maze-associated dip in ECF glucose (to 81% of baseline) when studied at euglycemia (Fig. 4). This dip during maze testing was reversed in recurrently hypoglycemic animals ( $P < 0.02$ ). In contrast, during hypoglycemia, ECF glucose levels fell in all animals, but more rapidly in recurrently hypoglycemic animals, with a significant reduction seen in the first sample after insulin administration to  $66 \pm 4\%$  of baseline [vs.  $98 \pm 2\%$  of baseline in control-acutely hypoglycemic animals;  $t(11) = 6.00$ ,  $P < 0.0001$ ]. However, the two groups' ECF glucose levels plateaued at a similar level after maze testing.

As shown in Fig. 5, at 12 months, recurrently hypoglycemic animals, who showed similar maze performance to 4-month-old control animals, also resembled 4-month-old control animals in having a significant task-associated dip in hippocampal ECF glucose during the first maze-testing period ( $P < 0.0002$ ), whereas 12-month-old control-euglycemic animals, who performed the maze task at chance level, showed no dip in ECF glucose ( $P < 0.05$  vs. recurrently hypoglycemic group).

Throughout the study, recurrent hypoglycemia resulted in a greater rise in ECF lactate in response to cognitive testing at euglycemia. At 4 months, average hippocampal ECF lactate during the first maze sample rose by 78% in recurrently hypoglycemic animals versus 35% in controls ( $P < 0.01$ ), and at 12 months, ECF lactate was unchanged in control animals but rose 42% in recurrent hypoglycemia ( $P < 0.03$ ).

**Electrophysiological measures.** We hypothesized that the changes in task performance and hippocampal ECF produced by recurrent hypoglycemia might be accompanied by alterations in hippocampal neuronal electrophysiology. Slices were taken from 12-month-old control and recurrently hypoglycemic animals 1 week after maze testing. No changes in the input-output (stimulus intensity versus population spike amplitude) relationship were seen between the two groups. However, in 10 mmol/l glucose aCSF, recurrently hypoglycemic animals showed enhanced paired pulse inhibition (PPI) (control:  $-30.4 \pm 5.05$ ; recurrent hypoglycemia:  $-70.2 \pm 6.26$ ,  $n = 5$  each;

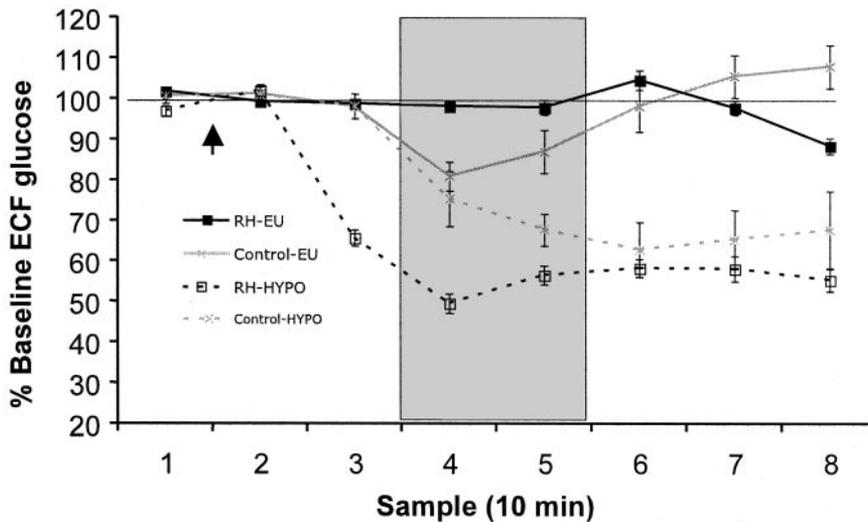


FIG. 4. ECF glucose levels in 4-month-old animals. The black line denotes recurrently hypoglycemic animals; the gray line denotes control animals, with dashed lines indicating acutely hypoglycemic animals and solid lines showing data from euglycemic rats. The thin solid line marks 100% of baseline. The shaded rectangle represents the maze-testing period. The arrow indicates the time of injection: insulin for acutely hypoglycemic animals, saline for euglycemic animals. Data are means  $\pm$  SE. For details of significant group differences, see RESULTS.

$P < 0.001$ ; Fig. 6). PPI measures feedback  $\gamma$ -aminobutyric acid (GABA)ergic synaptic inhibition; a conditioning stimulus is followed by a test stimulus at a 10-ms delay, shown in Fig. 6A (35). The effect of feedback inhibition (produced by the first stimulus) is to reduce the amplitude of response to the second test pulse, so that greater (more negative) PPI indicates an increased inhibitory effect. This can be considered as producing a reduction in "noise" and hence an increased signal-to-noise ratio within the hippocampus of the recurrently hypoglycemic animals.

When bath glucose was reduced to 2 mmol/l, PPI was reduced in both groups of animals, confirming our expectation that inhibitory synaptic function would be sensitive to low bath glucose. However, the magnitude of the reduction in PPI was much greater in slices from recurrently hypoglycemic animals (Fig. 6B and C).

To verify that altered polysynaptic inhibition had occurred, we measured the fast (A-type GABA channel-mediated) IPSP conductance using intracellular recordings. This method allows for a direct (versus inferential) measurement of inhibitory synaptic strength. Consistent with the field potential studies, we found a significant difference in IPSP conductance at 10 mmol/l glucose (control:  $65.4 \pm 14.4$  mS,  $n = 4$ ; recurrent hypoglycemia:  $135.2 \pm 23.3$  mS,  $n = 5$ ,  $P < 0.05$ ). In 2 mmol/l glucose, IPSP conductance did not change in controls

relative to that seen at 10 mmol/l ( $60.45 \pm 16.5$  mS,  $n = 5$ ) but decreased in recurrently hypoglycemic animals ( $66.1 \pm 44.7$  mS,  $n = 4$ ,  $P < 0.05$ ; Fig. 6D). Thus, the two measures gave a consistent picture of recurrently hypoglycemic animals having increased GABAergic inhibitory function at euglycemia, but being more susceptible to hypoglycemia, paralleling the behavioral findings.

Finally, we examined slices following repetitive stimulation, aimed at mimicking the effects of cognitive testing. We used 10-Hz, 10-s stimulus trains, a protocol known to activate neural oxidative metabolism (36). Immediately following such a train, both PPI and the amplitude of the population spike to a single test stimulus were reduced in all slices, and the time needed for PPI and spike amplitude to return to baseline was greater at 2 mmol/l bath glucose versus 10 mmol/l. In light of the behavioral data, we predicted that recurrently hypoglycemic animals might show impaired responsiveness under low bath glucose: indeed, recurrent hypoglycemia caused a markedly increased time for both population spike and PPI to recover to steady state (population spike,  $P < 0.02$ ; PPI,  $P < 0.05$ ,  $n = 5$  each), shown in Fig. 7. In other words, recurrently hypoglycemic slices were less able to maintain function at hypoglycemia when stimulated. Overall, the electrophysiology results confirmed our hypothesis that alterations of hippocampal synaptic function (and in particular inhibitory function) may contribute to the observed cognitive impact of recurrent hypoglycemia.

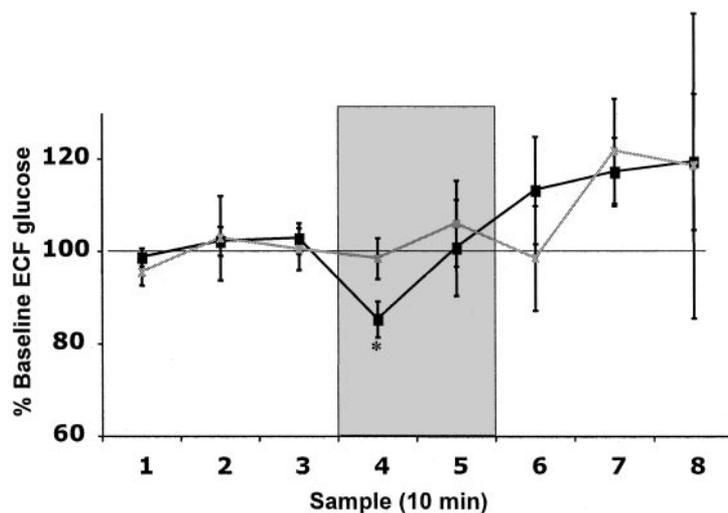
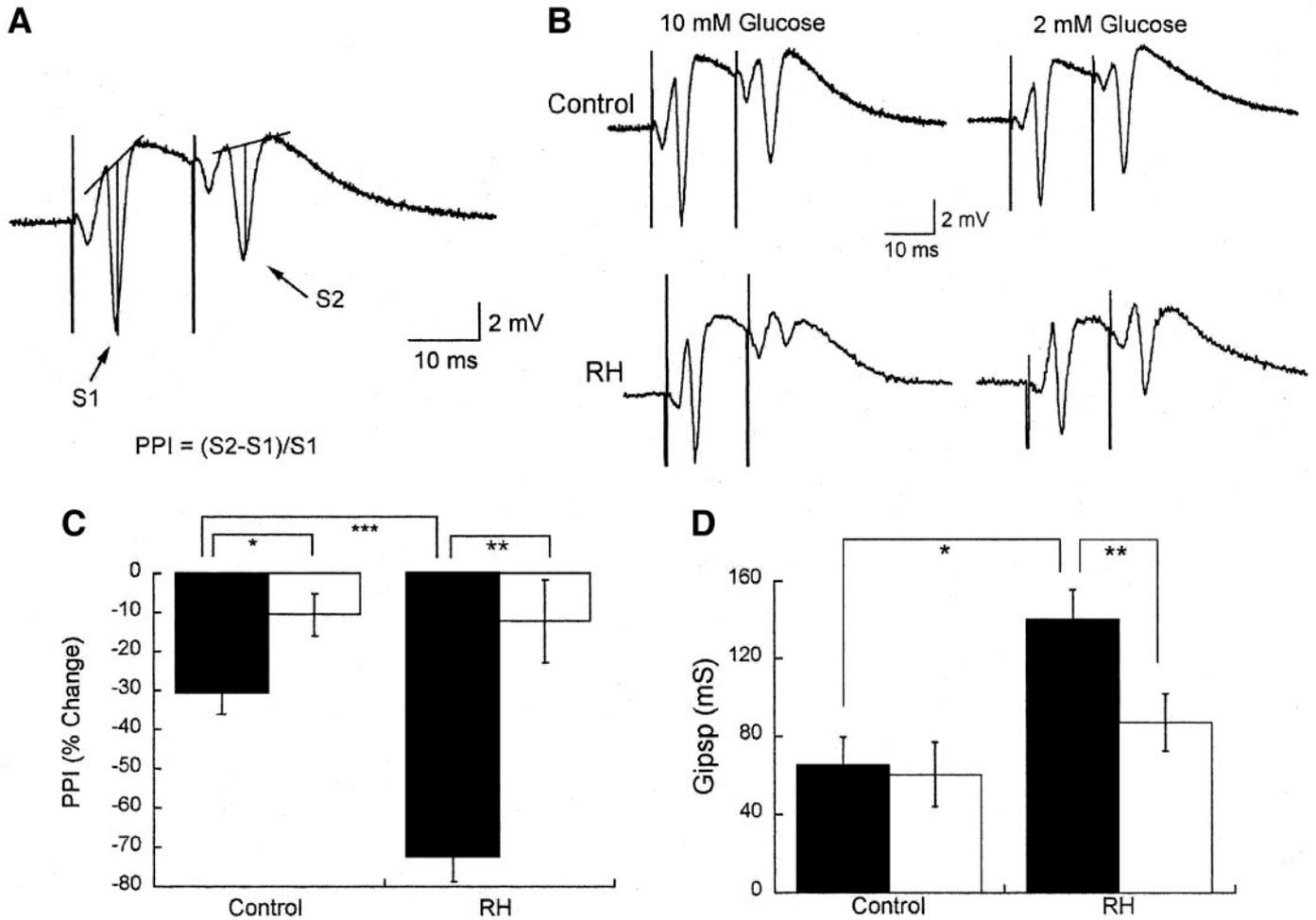


FIG. 5. ECF glucose levels in 12-month-old animals tested at euglycemia. The black line denotes recurrently hypoglycemic animals; the gray line denotes control animals. The thin solid line marks 100% of baseline. The shaded rectangle represents the maze-testing period. Data are means  $\pm$  SE. \*Intergroup significant difference during first maze-testing period,  $P < 0.05$ .

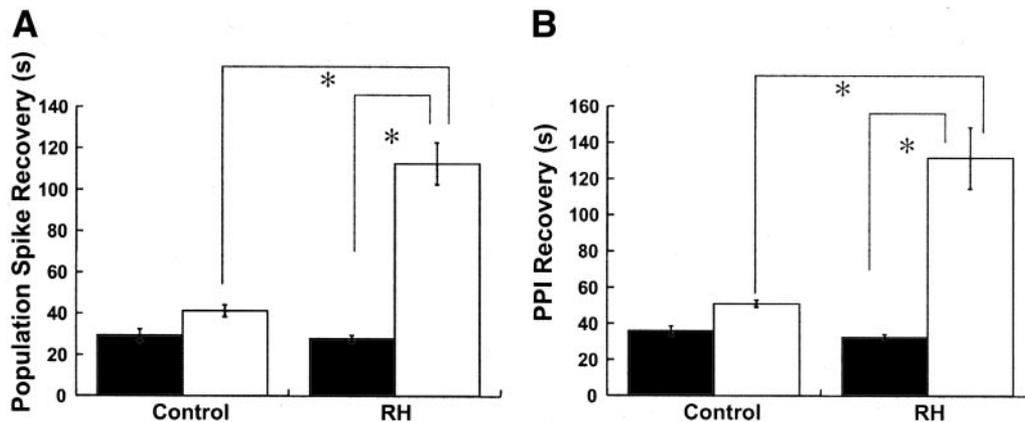


**FIG. 6.** Changes in synaptic inhibition in recurrently hypoglycemic rats. *A*: An example of PPI obtained in a slice from a control rat in 10 mmol/l glucose aCSF. S1 and S2 refer to stimulus 1 and stimulus 2, each of which generate a synaptic response from the many neurons stimulated, producing a downward deflection in field potential known as the “population spike.” The population spike amplitude is shown by the vertical lines marked by arrows. The percent change between the amplitudes of the two evoked population spikes [i.e., (S2-S1)/S1] is a measure of GABA-mediated inhibition. *B*: Examples of representative extracellular field potential recordings from individual slices showing that PPI is 1) enhanced in the recurrently hypoglycemic animals in 10 mmol/l glucose but 2) similar to controls in low glucose (2 mmol/l). Group data for PPI at both 10 mmol/l (■) and 2 mmol/l (□) bath glucose are shown in *C*, *n* = 5 control; 6 recurrent hypoglycemia. The greater loss of PPI when moving from 10 to 2 mmol/l glucose seen in recurrently hypoglycemic animals indicates greater dependence of synaptic inhibition on ambient glucose versus controls. *D*: Grouped data for the intracellularly measured fast IPSP conductance (Gipsp) recorded in 10 and 2 mmol/l glucose aCSF (*n* = 5 controls; 4 recurrent hypoglycemia; raw data not shown). Note that, as for the PPI results, recurrent hypoglycemia slices showed enhanced synaptic inhibition in 10 mmol/l glucose aCSF, which was depressed to a greater degree in low glucose aCSF. \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.005.

**DISCUSSION**

We show here that intermittent moderate hypoglycemia, a common side effect of intensive insulin therapy for type 1 diabetes, markedly affects subsequent performance on a

hippocampally dependent spatial working memory task. This is accompanied by alterations within the hippocampus, including both ECF glucose and lactate levels during cognitive testing and electrophysiological function. In-



**FIG. 7.** Recovery times for the population spike (*A*) and for PPI (*B*) after a 10-Hz, 10-s stimulus train. The amplitude of the population spike and the extent of PPI was measured before (i.e., baseline) a 10-Hz, 10-s stimulus train and then every 7 s posttrain. There was a significant prolongation of the time needed for both the population spike amplitude and for PPI to recover to baseline levels in 2 mmol/l glucose compared with 10 mmol/l; this effect was much more pronounced in the recurrently hypoglycemic animals than in controls. \**P* < 0.02. ■, 10 mmol/l bath glucose; □, 2 mmol/l bath glucose.

triguingly, the effects of recurrent hypoglycemia depended on the ambient glycemic state at testing.

At euglycemia, alternation was enhanced in recurrently hypoglycemic animals at all ages. This difference became striking as the animals aged; recurrent hypoglycemia acted to preserve and improve task performance. In 4-month-old animals, recurrent hypoglycemia reversed the task-associated dip in hippocampal glucose. The impact of recurrent hypoglycemia on both cognitive performance and hippocampal ECF glucose at euglycemia in the 4-month-old animals is similar to the effect of exogenous glucose administration (19), in line with data suggesting that prolonged hypoglycemia leads to increased brain glucose transport (6,37) as well as data showing that just 3 days of recurrent hypoglycemia results in greater increases in ECF glucose after intraperitoneal glucose (18). This possibility is also consistent with results in the older age-groups, as provision of additional glucose has been consistently shown to have greater cognitive impact in older animals, including humans (21, rev. in 34). Increased hippocampal glucose transport may not be the only possible explanation. For instance, the brain might adapt to recurrent hypoglycemia by making greater use of alternate fuels or through a reduction in glucose demand (that is, more efficient use of glucose). However, the increase in ECF lactate seen in recurrently hypoglycemic animals during testing suggests that, if anything, more glucose is being used glycolytically during the cognitive demand. Regardless, it is clear that recurrent hypoglycemia affects local hippocampal cognitive metabolism while enhancing euglycemic function.

In older animals, we encounter an apparent paradox in the ECF glucose data. The 12-month-old recurrently hypoglycemic animals, who behaviorally resemble 4-month-old control rats, also show a similar task-associated dip in hippocampal ECF glucose. However, their 12-month-old control counterparts—who perform the maze task only at chance levels—show no dip during task performance, much like 4-month-old recurrently hypoglycemic animals, who show enhanced rather than reduced task performance. The two ECF glucose profiles can be distinguished, however, by lactate measurements. At 4 months, recurrently hypoglycemic-euglycemic animals showed a greater hippocampal ECF lactate increase during the first maze sample, suggesting that increased glucose metabolism within the hippocampus after recurrent hypoglycemia accompanied improved task performance. The effect of recurrent hypoglycemia is in the same direction in the 12-month-old animals: control-euglycemic animals show no significant increase in ECF lactate in the first maze sample, likely reflecting no hippocampal activation. In short, the ECF glucose “flatline” seen in 4-month-old recurrently hypoglycemic animals is accompanied by elevated ECF lactate (and enhanced task performance); the similar ECF glucose of 12-month-old controls is accompanied by flat ECF lactate and markedly impaired performance.

In contrast to the results at euglycemia, we saw a paradoxical change in performance in recurrently hypoglycemic animals when rendered acutely hypoglycemic. Performance at hypoglycemia was significantly worse in the recurrently hypoglycemic rats, suggesting that recurrent hypoglycemia leads to impaired cognitive function during subsequent moderate hypoglycemia. This impairment may contribute to the impaired hypoglycemia awareness seen in patients with type 1 diabetes (25), and the

finding highlights the importance of designing therapies for type 1 diabetes that minimize the risk of hypoglycemia. The more rapid fall in hippocampal ECF glucose seen in recurrently hypoglycemic—acutely hypoglycemic animals is consistent with the reduced ability to support complex cognitive processes when hypoglycemic.

The present experiments examined for the first time the possibility of altered hippocampal synaptic transmission following recurrent hypoglycemia. Remarkably, the pattern of changes seen closely paralleled the behavioral findings. First, at euglycemia, synaptic inhibition in slices from recurrently hypoglycemic animals, measured both as PPI and as the IPSP conductance, was enhanced. Functionally, we hypothesize that this may allow for more precise point-to-point transmission, i.e., essentially, an increase in the hippocampal signal-to-noise ratio. Behavioral studies show that in some cases, small amounts of glucose given via direct brain injection act to enhance GABAergic function (38–40), and GABAergic interneurons are among the most energetically demanding cells in the hippocampus, so that enhanced hippocampal inhibitory function would be consistent with the apparent increase in recurrently hypoglycemic animals’ hippocampal glucose supply shown in our microdialysis data. A possible underlying mechanism is suggested by the fact that hippocampal GABAergic cells appear to express ATP-gated potassium channels ( $K_{ATP}$  channels) in greater numbers than do the pyramidal cells (41).  $K_{ATP}$  channels are implicated in sensing of ambient glucose levels by brain cells (42) and have also been suggested to be a mechanism by which glucose modulates hippocampal cognitive performance (43); elevated glucose supply leads to channel closing and hence potentially increased GABAergic activity.

Second, recurrently hypoglycemic slice function was much more sensitive to reduction in glucose than was control function, with a much greater reduction in PPI measured in recurrent hypoglycemia slices (versus controls) when bath glucose was lowered. Moreover, following a metabolic challenge in the form of a stimulus train, the time needed for both the population spike amplitude and PPI to reach a steady-state level was prolonged in slices from recurrently hypoglycemic animals under conditions of low bath glucose. The underlying mechanism(s) responsible for these changes remain to be determined, but there are some intriguing possibilities. For one example, if astrocytes within recurrently hypoglycemic tissue are unable to adequately regulate the extracellular environment (for instance, extracellular potassium and glutamate) when glucose availability is reduced, control of the extracellular environment could fail, contributing to the delayed recovery times for the population spike amplitude and PPI seen here. The functional consequence of these data are that when hypoglycemic, recurrently hypoglycemic tissue is impaired in maintaining normal synaptic function under a cognitive load.

The key message is that our results show that the impact of recurrent hypoglycemia on cognitive function is likely to involve both metabolic and synaptic effects, with a close correspondence between changes observed in cognitive performance and hippocampal function.

Overall, the present data show that a clinically relevant model of recurrent hypoglycemia, with moderate and widely spaced episodes of hyperinsulinemic hypoglycemia such as those experienced by type 1 diabetic patients on intensive insulin therapy, has a marked impact on many

aspects of brain function, including alteration of both metabolism and cellular electrophysiology. At least with regard to the spatial memory task used in this study, contrary to many patients' worries, recurrent hypoglycemia does not appear to degrade subsequent cognitive performance but in fact may preserve it, at least at euglycemia.

#### ACKNOWLEDGMENTS

This work was funded by the Juvenile Diabetes Research Foundation (JDRF) Center for the Study of Hypoglycemia at Yale and by grants from the National Institutes of Diabetes and Digestive and Kidney Diseases (DK 20495 and the Diabetes Endocrinology Research Center) and the National Institute of Neurological Disorders and Stroke (NS 45792). E.C.M. was the recipient of a JDRF Postdoctoral Fellowship and is now funded by an American Diabetes Association Career Development Award.

We thank Catherine Kotz, Charlie Billington, Barry Levin, and Charles Mobbs for helpful discussion and Ralph Jacob for analysis of microdialysis; Yuyan Ding for superb surgical assistance in implanting vascular catheters; and Wanling Zhu for essential help in animal care and preparation.

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