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Memory Impairment in Obese Zucker Rats: An Investigation of Cognitive Function in an Animal Model of Insulin Resistance and Obesity

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The genetically obese Zucker rat is a widely investigated model of pathological changes associated with type 2 diabetes. To assess cognitive function, obese and lean Zucker rats were tested on a variable-interval delayed alternation test of learning and memory. There were no group differences in learning the alternation rule or at short intervals, but obese rats were impaired at longer intervals where performance is hippocampus dependent. Plasma membrane association of the insulin sensitive glucose transporter, GLUT4, was reduced in the hippocampus of obese rats in the absence of changes in total GLUT4 and insulin receptor expression. These results parallel those of human studies in pointing to the susceptibility of the hippocampus and related structures to the adverse environment of diabetes mellitus.

Keywords: memory, cognition, obesity, type 2 diabetes mellitus, Zucker rats

The long-term complications of insulin resistance (IR) and type 2 diabetes mellitus (DM2), resulting from impaired insulin receptor signaling (Shepherd & Kahn, 1999) and altered glucose metabolism, include peripheral neuropathy, retinopathy, cardiovascular disease, and renal failure (American Diabetes Association, 2004). Memory loss and impaired executive function also accompany DM2 (Ryan & Geckle, 2000; Strachan, Deary, Ewing, & Frier, 1997), especially in older adults (Awad, Gagnon, & Messier, 2004). Even in the absence of DM2, IR associates with accelerated age-related cognitive decline (Messier, Awad, & Gagnon, 2004; Watson & Craft, 2004). Physiologically, hyperinsulinemia is linked to disturbances in glucose metabolism and insulin signaling that affect several brain regions (Craft & Watson, 2004), including those involved in cognition (e.g., frontal lobes, hippocampus), which points to plausible biologic mediators of the cognitive deficits.

Investigation of the relationship between impaired glucoregulation and cognition in animal models is extremely limited. Streptozotocin (STZ)-induced diabetic rats are impaired on tests of spatial (Gispen & Biessels, 2000) and nonspatial (Flood, Mooradian, & Morley, 1990) learning and memory. However, though this model is "useful for studying the effects of chronic hyperglycemia, its endocrinological features do not adequately reflect either type 1 or type 2 diabetes" (Gispen & Biessels, 2000). By contrast, rodent models of DM2, including the Zucker fa/fa rat, which presents with IR, glucose intolerance, obesity, as well as endocrinological characteristics of DM2 (Zucker & Antoniades, 1972), have not been subjected to rigorous cognitive testing, with conflicting results observed in measures of spatial memory (Bélanger, Lavoie, Trudeau, Massicotte, & Gagnon, 2004; Li et al., 2002). Nevertheless, obese Zucker rats exhibit electrophysiological deficits in the hippocampus (Gerges, Aleisa, & Alkadhi, 2003), a

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critical center for learning and memory (McEwen & Sapolsky, 1995; Squire, 1992), which suggests that this model may prove useful for understanding the origins of cognitive deficits in humans with similar abnormalities.

To more fully explore cognitive function in an animal model of IR, we compared obese and lean Zucker rats on a go/no-go delayed alternation task with a variable interval between trials (VIDA). Successful performance involves several functionally dissociable, component processes involving different brain regions. Rats with frontal-lobe lesions are selectively impaired in learning the basic response-alternation rule. By comparison, rats with hippocampal lesions perform normally in learning the rule and at short intertrial intervals (ITIs). However, their performance deteriorates at longer ITIs that challenge the ability to recall specific information (Winocur, 1991). This task is also sensitive to other effects, including dietary fat manipulation (Greenwood & Winocur, 1996), normal aging (Winocur, 1986), and glucose treatment (Greenwood & Winocur, 2001). Thus, the VIDA task is an appropriate instrument for assessing neurocognitive function in the Zucker rat and for determining whether this strain can effectively model cognitive changes associated with IR and obesity in humans. Following the evidence that links insulin and glucose to cognitive function (Messier, 2004; Park, 2001), and as a first step in identifying physiologic correlates of altered cognition, we examined hippocampal insulin receptor expression and the translocation of the insulin sensitive glucose transporter, GLUT4, as a measure of insulin receptor signaling.

Method

Subjects

Subjects were obese (fa/fa) Zucker rats and their lean (FA/?) controls (n = 20/genotype; Charles River, MA). Following protocol approval by Trent University's animal ethics committee, animals arrived at approximately 4 months of age (body weight-lean: 227 ± 18g and obese: 277 ± 19g) and were housed for an additional 8 weeks prior to behavioral testing. Throughout the experiment, rats were housed individually in wire mesh cages with water available at all times. Standard lab chow was available in accordance with experimental conditions.

Apparatus and Procedure

The VIDA test has been described in detail elsewhere (Greenwood & Winocur, 2001; Winocur, 1986). All testing was conducted in computer-controlled Skinner boxes, outfitted with a single retractable lever to the right of a central feeder, housed in a sound-proof chamber and illuminated by a 3-watt light centrally installed in the roof of the chamber.

Two weeks before training, rats were handled regularly and restricted to 70% of their ad libitum food intake. Rats were then trained to press the lever for food according to a continuous reinforcement (CRF) schedule. CRF training, which consisted of one 30-min session per day, continued until a rate of 80 responses per session was achieved over 2 consecutive days. During training, each leverpress was rewarded with a single 45-mg Noyes food pellet. After each session, rats were returned to their cages and received their daily allotment of standard chow.

VIDA testing was initiated the day after criterion was reached in CRF training. Each test session consisted of 12 reinforced (go) trials alternating with 12 nonreinforced (no-go) trials. The lever was always present during the go and no-go trials, which were 20 s long. During the go trials, each leverpress produced a 45-mg Noyes food pellet, whereas leverpresses during the no-go trials were not rewarded. The go and no-go trials were separated by a variable ITI, during which the lever was retracted. Each session provided 12 go and 12 no-go trials. ITIs were 0, 5, 10, 20, 40, or 80 s long with each interval occurring twice after go trials and twice after no-go trials, so that each ITI occurred four times per session. The ITI sequence varied for each session, which always began with a go trial. Testing sessions continued daily for 15 consecutive days.

Biochemical Measurements

One week after testing sessions were completed, trunk blood was collected from overnight fasted rats maintained on their food restriction schedule. Plasma glucose analysis was performed by using the glucose oxidase method (glucose [trinder] kit, Sigma Chemical Co., St. Louis, MO) as described previously (Piroli et al., 2002). Radioimmunoassays (RIAs) were performed according to manufacturers' instructions for plasma levels of insulin, leptin, adiponectin (Linco Research Inc., St. Charles, MO), and corticosterone (Diagnostics Products Corp., Los Angeles, CA). Plasma triacylglyceride analysis was performed by using a glycerol phosphate oxidase trinder reaction according to the manufacturer's instructions (Raichem, San Diego, CA).

Immunoblot Analysis

At sacrifice, the hippocampus was quickly removed and hippocampal membrane-containing fractions were isolated (Piroli et al., 2002). Proteins were separated by SDS/PAGE (10%), transferred to nitrocellulose (NC) membranes and blocked in TBS plus 10% nonfat dry milk for 60 min. NC membranes were incubated with IR-β primary antisera (Santa Cruz Laboratories, Santa Cruz, CA; 1:1000) or previously characterized GLUT4 primary antisera (1:1000; Charron, Brosius, Alper, & Lodish, 1989) in TBS/5% non fat dry milk and developed by using enhanced chemiluminescence reagents as described by the manufacturer. Computer-assisted microdensitometry of autoradiographic images were determined on the MCID image analysis system (Imaging Research Inc., St. Catherines, Ontario, Canada; see Piroli et al., 2002).

Data Handling and Statistical Analysis

For the VIDA task, data are expressed as latency to first leverpress and as go/no-go latency ratios. Ratios were calculated at each ITI by dividing the mean latency to first leverpress in the go trials by the mean latency to first leverpress in the no-go trials, with lower latency ratios indicating better performance.

Data were collapsed across days and ITIs prior to statistical analyses. To assess rats' ability to learn the basic alternation rule, data from ITI-0 were averaged across five blocks of 3 days for each animal. To examine the impact of increasing the delay between the go and no-go trials, and consequently the memory demands of the task, analyses were limited to the last 3 days of testing (Block 5). Data for ITI-5 and ITI-10 were averaged and referred to as *short intervals*, whereas data for ITI-40 and ITI-80 were collapsed and referred to as *long intervals*.

Repeated measures analyses of variance (ANOVAs), to compare the fixed effects of genotype, time (block), ITI, and their interactions, were conducted with SAS for Windows (Version 8.0, SAS Institute Inc., Cary, NC) through the use of PROC MIXED and by using an autoregressive covariance structure to account for heteroscedastic data distribution. When significant interactions were apparent, the model was subdivided by delay, and the main effect of genotype was further examined by ANOVAs. Data from RIA and Western blot analyses were compared by using a one-way ANOVA.

Results

Subjects

Obese Zucker rats were heavier (344 ± 6 vs. 520 ± 4g) and had marked elevations in fasting plasma glucose (127 ± 4 vs. 188 ±

20 mg/dl), insulin (1.52 ± 0.13 vs. 21.7 ± 4.7 ng/μl), leptin (4.71 ± 0.84 vs. 37.12 ± 3.51 ng/μl), adiponectin (4.3 ± 0.2 vs. 7.3 ± 0.4 μg/ml), corticosterone (17.6 ± 2.5 vs. 31.6 ± 3.4 μg/dl), and triacylglycerides (72 ± 4 vs. 627 ± 106 mg/dl) levels relative to the lean controls (all *p*s < .001; data are *M* ± *SEM*; *n* = 8/genotype for blood measures and *n* = 20 for weight).

Behavioral Training

During training, obese and lean rats did not differ in acquiring the leverpress response on a CRF schedule. Both groups reached criterion within 3–5 days of training, with obese rats requiring an average of 3.9 days, as compared with 4.3 days for the lean rats, ($t < 1$). These scores are typical of those seen in normal adult rats trained in the same way (Winocur, 1986, 1991).

Alternation Rule Learning

The rats' ability to learn the basic alternation rule was determined by examining performance at ITI-0, where there was no delay between the go and no-go trials (Figure 1 A). Both lean and obese rats improved performance over time (blocks of 3 days), with asymptotic performance being observed by about Block 4, evidenced as decreasing latency to first leverpress in the go trials, increasing latencies in the no-go trials, and an overall lowering of the go/no-go latency ratios (all *p*s < .001). Both genotypes showed similar rates of learning (Block × Genotype interactions for go, $F(4, 152) = 1.64$, $p = .20$; no-go, $F(4, 152) = 0.31$, $p = .87$; and ratio, $F(4, 152) = 1.59$, $p = .18$, data).

In addition, obese and lean rats were compared on latency to first leverpress on the go and no-go trials, following the reasoning that overall longer latencies could reflect reduced physical ability secondary to obesity and/or motivation to perform the task. There was no significant difference between genotypes on either the go, $F(1, 152) = 2.32$, $p = .13$, or the no-go, $F(1, 152) = 1.11$, $p = .29$, trials. Taken together, the data suggest similar motivational-performance properties and comparable rates of alternation rule learning in the lean and obese rats.

Memory

The impact of imposing a memory demand, by increasing ITIs, was examined at Block 5 when animals had reached asymptotic performance at ITI-0 (Figure 1B). Independent of genotype, increasing the ITI adversely affected performance, resulting overall in higher go/no-go ratios, $F(2, 78) = 112.30$, $p < .0001$. This effect was associated primarily with a reduced latency to first leverpress in the no-go trials as ITI increased (from 9.24 ± 0.64 s [ITI-0] to 6.27 ± 0.37 s [for short delay] and 2.40 ± 0.23 s [for long delay], *M* ± *SEM*, $F[2, 78] = 73.56$, $p < .0001$). There was also a nonsignificant trend for latency to first leverpress to increase in the go trials with increasing ITIs (from 1.11 ± 0.14 s [ITI-0] to 1.26 ± 0.18 s [for short delay] to 1.53 ± 0.13 s [for long delay], *M* ± *SEM* for lean and obese rats, respectively, $F[2, 78] = 2.51$, $p = .09$). Although there was no main effect of genotype on performance, $F(1, 38) = 2.77$, $p = .10$, there was a significant Genotype × Delay interaction, $F(2, 76) = 5.50$, $p = .006$. Go/no-go latency ratios at ITI-0, $F(1, 38) = 0.34$, $p = .56$, and the short delay, $F(1, 38) = .08$, $p = .78$, were comparable in lean and obese rats. By contrast, a higher go/no-go latency ratio, indicating impaired performance, was observed in the obese relative to the lean rats at the long delay, $F(1, 38) = 16.47$, $p = .0002$. Further analysis revealed that this effect was due to faster responding by the obese rats during the no-go trials. At the long delays, latency to first leverpress was indistinguishable between genotypes during

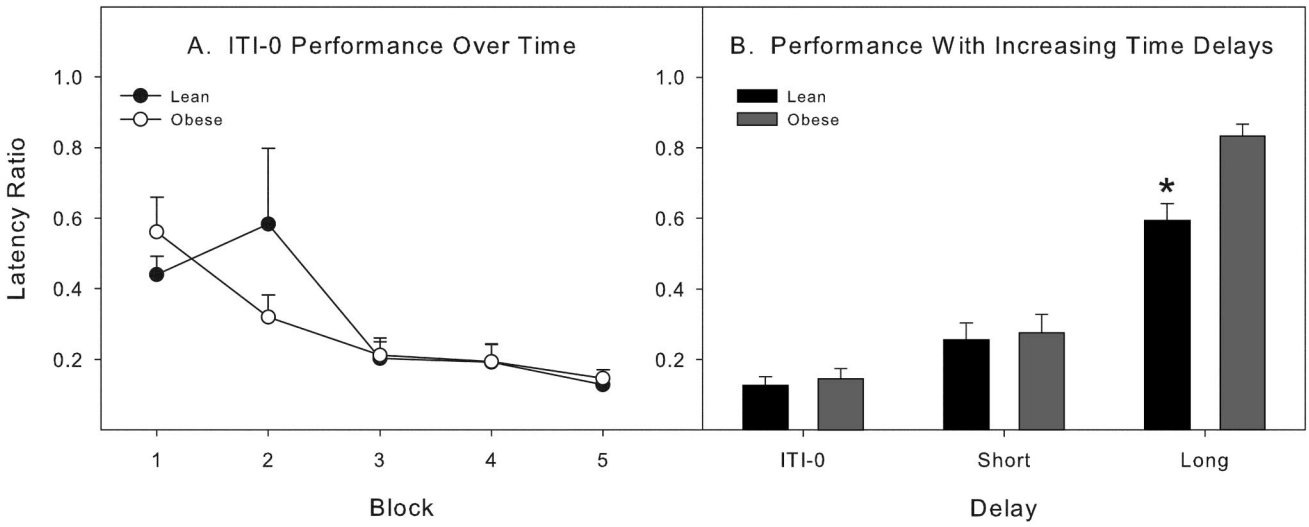

Figure 1. A: Delayed alternation task with a variable interval between trials (VIDA) performance of lean and obese Zucker rats at intertrial interval (ITI)-0 over five blocks of 3 days each, representing the rats' ability to learn the basic alternation rule. Data (*M* ± *SEM*, *n* = 20/genotype) represent go/no-go latency ratios. B: VIDA performance of lean and obese Zucker rats under immediate (ITI-0), short (5 and 10 s), and long (40 and 80 s) delays in the memory test. Data (*M* ± *SEM*, *n* = 20/genotype) represent go/no-go latency ratios. The asterisk indicates significant difference ($p < .001$) between genotypes at the long delay only.

the go trials, $F(1, 38) = 0.01$, $p = .93$ (1.54 ± 0.19 vs. 1.52 ± 0.19 s; $M \pm SEM$ for lean vs. obese), but was significantly shorter in the obese rats relative to the lean rats, $F(1, 38) = 6.68$, $p = .01$ (2.96 ± 0.37 vs. 1.85 ± 0.23 s; $M \pm SEM$ for lean vs. obese) during the no-go trials.

Western Blot Analysis for the Insulin Receptor and GLUT4

Western blotting analysis with primary antisera selective for the β subunit of the IR revealed comparable expression of IR-β in the hippocampus of lean and obese rats (Figure 2A and 2B). Similarly, total protein expression of the insulin sensitive glucose transporter, GLUT4, was similar in the hippocampus of lean or obese rats (Figure 2C and 2D). Conversely, plasma membrane association of GLUT4 was significantly reduced in the hippocampus of obese Zucker rats compared with their lean littermates (Figure 2E and 2F). Because deficits in insulin receptor signaling and GLUT trafficking are proposed to contribute to the deleterious consequences of diabetes in peripheral tissues (Shepherd & Kahn, 1999), these results suggest that similar impairments in CNS insulin receptor signaling may contribute to the deficits in hippocampal-dependent cognitive function observed in obese rats.

Discussion

This study assessed cognitive performance in Zucker rats in a behavioral task that provided dissociable measures of various learning and memory processes. The results demonstrate selective memory impairment in obese Zucker rats that did not extend to other aspects of task performance. Obese rats also exhibited increases in plasma insulin, leptin, and corticosterone and significant decreases in hippocampal plasma membrane levels of the GLUT4, suggesting that neuroendocrine and neurochemical changes contribute to the deficits in hippocampal-dependent behavior.

The behavioral profile of the obese rats on the VIDA task, with differences in performance confined to the longer ITIs, suggests that strategic and working memory functions that are controlled by the prefrontal cortex and required to learn the basic response alternation rule were not affected. By contrast, the selective impairment at longer ITIs signifies a form of memory impairment that is associated with hippocampal dysfunction. In previous work with brain damaged rats (Winocur, 1991), rule learning on the VIDA task and performance at relatively short ITIs were linked to a neural network in which the prefrontal cortex appears to be the principal region. The hippocampus is not part of this network but damage to this structure selectively impairs VIDA performance at ITIs 20–80, precisely those intervals at which the obese rats were impaired in the present experiment.

It is unlikely that factors such as fatigue or reduced motivation can account for the obese rats' impairment. Over the course of behavioral testing, both lean and obese Zucker rats acquired the basic leverpress response at the same rate and, in the VIDA task, performed equally well at the 0 and short ITIs. Thus, the obese rats appear to have been motivated and capable of performing the task. Moreover, analysis of the obese rats' behavior at the longer ITIs, where they were selectively impaired, showed that their latencies to first leverpress, though not different from those of the lean rats on the go trials, were significantly lower on the no-go trials. If the obese rats were fatigued or less motivated to perform for food reward, one might expect, if anything, an increase in response latencies. It should be noted that, in all testing sessions, ITIs were randomly distributed so that rats could not anticipate longer ITIs in a way that could affect their motivation to perform at those intervals.

It could be argued conversely that the obese rats' shorter latencies on the no-go trials at the longer ITIs reflect heightened motivational levels in these animals. However, following this argument, one would also expect them to exhibit shorter latencies than the lean rats in the no-go trials at 0 and short ITIs, and there was no evidence that was the case. The obese rats' quick responding on no-go trials at the longer ITIs could also be seen as a failure to suppress the leverpress response, which was appropriate for the go trials where it was reliably associated with food reward. Although response perseveration may have factored into the obese rats' poor performance, this cannot be attributed to a fundamental deficiency in inhibitory control mechanisms. A loss of response control would have been expressed at all intervals and not limited to the long ITIs. Taken together, the evidence points to the conclusion that the obese rats' behavior at long ITIs reflects impaired recall of response-related events of the previous trial, a failure to discriminate between go and no-go trials and, consequently, a generalized bias to perform the well-learned and rewarded leverpress response.

The Zucker rat is presented as a model of obesity with IR, a condition associated with accelerated age-related cognitive decline (Messier et al., 2004; Watson & Craft, 2004). Normal old rats are typically impaired, relative to young adult rats, on measures of both strategic learning and memory, when tested on the VIDA (Winocur, 1986) and related tasks (Winocur, 1992). In the present study, the obese Zucker rats exhibited impaired memory but preserved strategic functions, indicating that the hippocampus is particularly susceptible to the effects of IR. However, it is not clear how the effects of IR interact with the aging process with respect to cognitive function. The rats in this experiment were relatively young adults (approximately 6 months old), and it is conceivable that older obese Zucker rats would exhibit a wider range of cognitive deficits. As well, the generality of the present results needs to be examined by testing Zucker rats on other tests (e.g., conditional associative learning, complex maze learning) that challenge different brain systems in ways that may reveal other forms of cognitive impairment.

The present results are consistent with impaired spatial memory in the Morris Water maze observed in obese Zucker rats and db/db mice (Li et al., 2002), but they are at variance with a report of normal Morris Water maze performance in Zucker ZDF rats (Bélanger et al., 2004). Although there is no a priori reason to anticipate differences between Zucker and Zucker ZDF rats, it is noteworthy that our Zucker rats likely were somewhat older, were considerably more obese, and had higher blood glucose levels than the ZDF animals in the Bélanger et al. (2004) study. Significantly, Bélanger et al. reported normal long-term potentiation (LTP) in hippocampal slices of their ZDF rats, indicating that the structure was functionally intact, whereas Li and colleagues (2002) observed alterations in LTP performance in both strains of rodents. In addition, we report several biochemical changes in the hippocampus that would predict the type of memory loss observed in the present study. Thus, the current balance of evidence supports

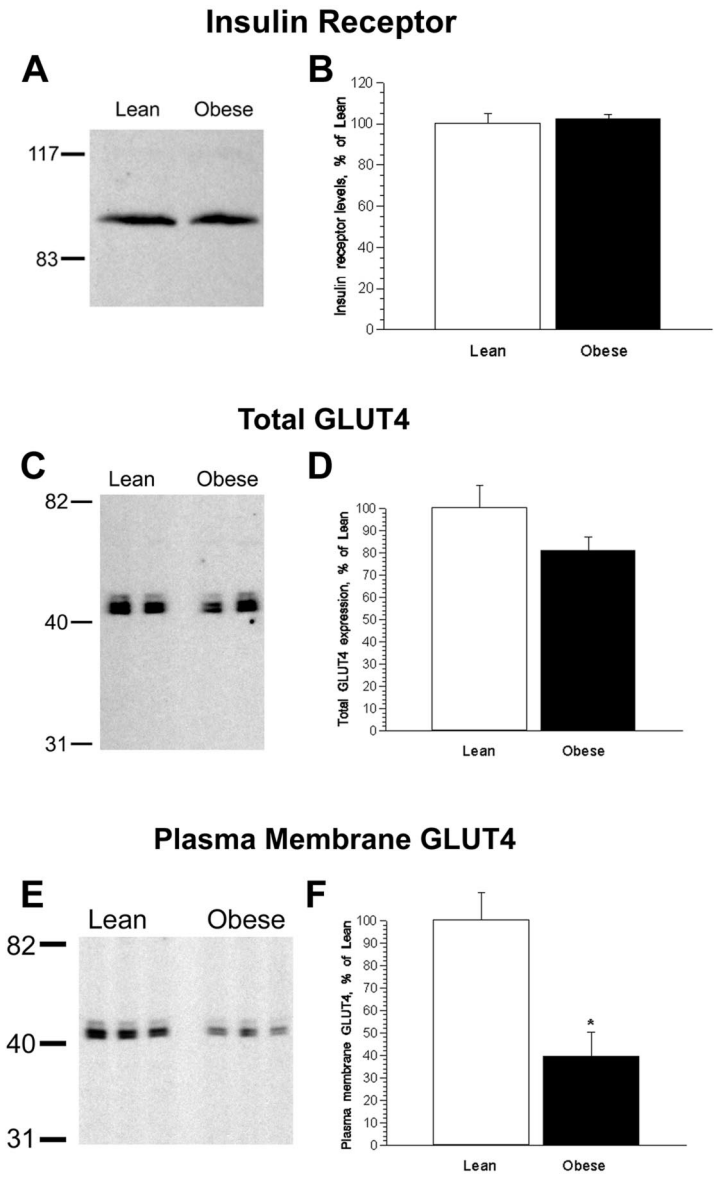

Figure 2. Immunoblot analysis of insulin receptor and GLUT4 protein expression in hippocampal plasma membrane fractions. A: Antisera selective for the β subunit of the insulin receptor (1:1000; Santa Cruz Biotechnology) immunodetects a single protein at approximately 95 kDa in plasma membranes isolated from the hippocampus of lean and obese Zucker rats (20 μg protein/lane). B: Insulin receptor immunoreactive levels were similar in the hippocampus of obese and lean Zucker rats. (Molecular weight standards in kDa are shown on left. Data expressed as percentage of insulin receptor immunoreactivity detected in lean control rats.) C and E: GLUT4 antisera (1:1000) immunodetects a single protein at approximately 45 kDa in total membrane (C: [50 μg protein/lane]) and plasma membrane (E: [40 μg protein/lane]) fractions isolated from the hippocampus of lean and obese Zucker rats. D: Total GLUT4 immunoreactive levels were comparable in the hippocampus of obese and lean Zucker rats. E: GLUT4 immunoreactive levels were decreased in plasma membrane fractions isolated from the hippocampus of obese Zucker rats compared with lean controls. (*$p \leq .02$; molecular weight standards in kDa are shown on left. Data, expressed as $M \pm SEM$, are percentage of GLUT4 immunoreactivity detected in lean control rats.)

deficits in hippocampally dependent behaviors in obese Zucker rats that occur in tandem with indicators of altered hippocampal physiology.

The impaired hippocampal LTP in obese Zucker rats (Gerges et al., 2003; Li et al., 2002; but see Bélanger et al., 2004) may underlie the behavioral deficits as LTP is a widely accepted cellular model of learning and memory. An important question is whether the LTP impairments can be solely attributed to the leptin receptor (Ob-Rb) mutation in the obese Zucker rat (Iida et al., 1996). The presence of leptin receptors in the dentate gyrus and

CA1/CA3 hippocampal fields and reports that leptin receptor signaling can facilitate hippocampal N-methyl-D-aspartate receptor-mediated synaptic transmission (reviewed in Harvey, 2003) argue for a pivotal role of the dysfunctional Ob-Rb receptor. Nevertheless, other endocrine and neuroendocrine abnormalities associated with cognitive deficit and apparent in this rat model should not be overlooked.

There is reason to hypothesize that IR and/or abnormal insulin receptor signaling contributed to the memory deficits observed in the obese Zucker rats. A potential role of the insulin receptor in cognition (Park, 2001) is supported by numerous lines of evidence, including (a) the ability of insulin to improve cognitive performance in humans and animals (Zhao et al., 1999), (b) the presence of insulin receptors in the hippocampus (Doré, Kar, Rowe, & Quirion, 1997), and (c) the animals' increase in insulin receptor expression and signaling following spatial learning (Zhao et al., 1999). A common indicator of insulin receptor signaling is insulin-stimulated translocation of the GLUT4 transporter to the plasma membrane—a feature seen in hippocampal neurons following increases in plasma insulin levels elicited by peripheral glucose administration to normal animals (McEwen & Reagan, 2004). Thus, the present results, demonstrating a failure to enhance hippocampal plasma membrane GLUT4 translocation despite chronic elevations in plasma glucose, combined with no change in the density of hippocampal insulin receptors, are consistent with an earlier report of impaired hippocampal insulin receptor signaling in obese Zucker rats (Figlewicz, Szot, & Greenwood, 1990). Whether these changes in GLUT4 transporter translocation, because of their potential impact on neuronal glucose uptake, are directly involved in the memory deficit of obese Zucker rats or simply an indicator of impaired insulin receptor signaling, which could impact on cognition through other events downstream to the insulin receptor, cannot be addressed in this study. It is interesting to note that disturbances in insulin receptor signaling, neuronal glucose transporter trafficking, glucose uptake and utilization (for review, see McEwen & Reagan, 2004) are also seen in insulin-deficient STZ-diabetic rats. Because cognitive deficits are apparent in both the STZ-diabetic (Gispen & Biessels, 2000) and obese Zucker rat, it is interesting to speculate that decreased insulin receptor activation is a common feature that contributes to cognitive impairment in these two models of diabetes. However, other metabolic abnormalities, including the elevations in plasma cortisol and triacylglycerides apparent in our obese animals are also implicated in cognitive decline with aging (McEwen & Sapolsky, 1995; Messier et al., 2004) and may also contribute to the memory deficits observed.

Collectively, the results of this study point to the value of the fa/fa Zucker rat in understanding the origins and pathologic events associated with cognitive deficits in adults with IR and/or DM2. The greater impairment in memory-demanding components of the VIDA task observed in the fa/fa Zucker rat parallel human studies, indicating greater susceptibility of the hippocampus and related structures to the adverse environment of diabetes mellitus. Furthermore, leptin resistance and other endocrine and neuroendocrine abnormalities are not uncommon in human DM2, making the Zucker rat a suitable model to understand the complexities of multiple disturbances to neuronal networks involved in cognitive processing.

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