Obesity diminishes synaptic markers, alters microglial morphology, and impairs cognitive function

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Obesity is a major public health problem affecting overall physical and emotional well-being. Despite compelling data suggesting an association between obesity and cognitive dysfunction, this phenomenon has received relatively little attention. Neuroimaging studies in obese humans report reduced size of brain regions involved in cognition, but few studies have investigated the cellular processes underlying cognitive decline in obesity or the influence of obesity on cognition in the absence of obesity-related illnesses. Here, a rat model of diet-induced obesity was used to explore changes in brain regions important for cognition. Obese rats showed deficits on cognitive tasks requiring the prefrontal and perirhinal cortex. Cognitive deficits were accompanied by decreased dendritic spine density and synaptic marker expression in both brain regions. Microglial morphology was also changed in the prefrontal cortex. Detrimental changes in the prefrontal cortex and perirhinal cortex occurred before metabolic syndrome or diabetes, suggesting that these brain regions may be particularly vulnerable to early stage obesity.

obesity | prefrontal cortex | cognition | dendritic spine | microglia

Obesity is a growing public health concern, affecting more than one-third of the United States adult population (1). Obesity increases the incidence of diabetes, vascular disease, and mental illness, all of which reduce quality of life and place a heavy financial burden on patients and society. Less recognized consequences of obesity are its deleterious effects on cognitive function. Individual cognitive performance, both rudimentary and scholastic, declines with increases in body mass and energy consumption (2–5). These deficits can be observed throughout life, from childhood to late adulthood. Obesity is also associated with an increased incidence of mild cognitive impairment and dementia in the elderly (6).

Some evidence suggests that obese individuals have decreased overall brain volume (7), with further studies indicating more specific reductions in the volume of brain regions important for cognition, including the hippocampus, prefrontal cortex (PFC), and anterior cingulate (5, 8, 9). Few studies, however, have investigated the cellular and biochemical mechanisms that might underlie obesity-induced changes in brain volume and cognitive function.

Although clinical studies strongly suggest that obesity produces cognitive impairment and brain atrophy, it is difficult to determine whether these changes arise from obesity itself or from conditions often associated with obesity, like metabolic syndrome, diabetes, and vascular disease. Such conditions, even in the absence of obesity, have been linked to cognitive dysfunction and decreases in brain volume (10–13). Little research has been done exploring the effects of obesity on brain structure and cognition in obese, but otherwise healthy, individuals. To investigate whether obesity in the absence of obvious illness alters brain structure and function, we used a rat model of diet-induced obesity to assess the PFC, the perirhinal cortex (PRC), and the hippocampus (HIP), brain areas linked to cognitive function. Here, we report that early stage obesity, defined as 25% greater body weight than age-matched controls, before the onset of overt metabolic syndrome or diabetes, is associated with a decrease in performance on cognitive tasks that require the PFC and PRC, but not the HIP. Furthermore, early stage obesity produced a decrease in dendritic spine density on pyramidal neurons, as well as decreases in synaptic protein levels in the PFC and PRC, but not the HIP. Obesity was also associated with altered morphology of microglia in area of synapse loss in the PFC. These brain changes were associated with reduced levels of circulating corticosterone and elevated levels of circulating leptin and suggest that certain brain regions may be especially susceptible to the negative consequences of obesity.

Results

Metabolic Characteristics. For these studies, we used diet to induce obesity because the United States obesity epidemic is driven primarily by consumption of calorically dense palatable foods, rather than by genetic mutations (14, 15). Adult male rats fed a high-fat, palatable, nutritionally complete diet showed increased body weight over the 8-wk access period compared with controls fed standard rodent chow, with an effect of week ($F_{8,112}$ = 1,244.80, $P < 0.0001$) and diet group ($F_{1,14} = 32.34$, $P < 0.0001$), and an interaction effect between week and diet group ($F_{8,112}$ = 33.84, $P < 0.0001$) (*SI Appendix*, Fig. S1). Differences in body weight between groups, with high-fat diet rats weighing significantly more than controls, were apparent as early as week 2 (t_{14} = 3.50, $P = 0.004$). At the end of the study (week 8), rats in the obese group were >25% heavier and had increased abdominal ($t_{14} = 4.84$, $P < 0.001$) and gonadal fat deposition ($t_{14} = 6.01$,

Significance

In humans, obesity impairs cognition and produces atrophy of brain regions associated with learning and memory, but few studies have investigated the underlying cellular mechanisms. We used a diet-induced model of obesity in rats to study excessive weight gain and found that early stage obesity, before the onset of diabetes or metabolic syndrome, produced deficits on cognitive tasks that require the prefrontal cortex. Impaired cognition was associated with synapse loss, including reduced numbers of dendritic spines and expression of synaptic proteins, as well as structural alterations in the brain's immune cells, the microglia. These results strongly suggest that obesity must be considered as a contributing factor to brain dysfunction, with implications for its increasing frequency in contemporary western society.

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Table 1. High-fat diet for 8 wk produces obesity without definitive signs of metabolic syndrome or diabetes

Metabolic indicator	Control	Obese
Triglycerides, mg/dL	103.7 ± 12.0	164.12 ± 15.8*
Glucose, mg/dL	275.2 ± 6.1	261.3 ± 7.5
Insulin, ng/mL	2.0 ± 0.3	2.7 ± 0.4
Corticosterone, μg/dL	124.0 ± 34.0	46.5 ± 11.7*
Leptin, ng/mL	6.9 ± 0.9	23.1 ± 2.3*
Testosterone, ng/mL	2.3 ± 0.5	1.7 ± 0.3

*$P < 0.05$ (unpaired two-tailed Student's t test) compared with control. Mean ± SEM, $n = 8$ per group.

$P < 0.0001$) (*SI Appendix*, Fig. S2), as well as elevated serum triglyceride levels compared with controls ($t_{14} = 3.70$, $P = 0.002$) (Table 1). Together, these traits were used to characterize the rats fed a high-fat diet as obese.

Metabolic syndrome, a frequent precursor to diabetes that commonly occurs with obesity, is associated with elevated glucose levels, as well as elevated baseline corticosterone levels (12, 16). Our obese rats did not show these signs of metabolic syndrome nor did they show signs of diabetes. First, both glucose and insulin levels did not differ between groups (Table 1). These measures were used to determine the quantitative insulin sensitivity check index (QUICKI). QUICKI is a mathematical model based on log-transformed plasma glucose and insulin values, as follows: 1/(log [glucose] + log [insulin]), which predicts insulin sensitivity, an important indicator of diabetes, with lower values representing more insulin resistance. In the current study, there was no difference in QUICKI values between groups ($t_{14} = 0.70$, $P = 0.50$; obese, 0.36 ± 0.01; control, 0.37 ± 0.01). Second, corticosterone levels were reduced, not increased, in our obese rats ($t_{14} = 2.38$, $P = 0.03$) (Table 1).

Taken together, these findings indicate that we assessed our rats when they were clearly obese but still relatively healthy. It should be noted, however, that our obese rats had elevated peripheral leptin levels (Table 1), which, together with increased triglyceride levels and increased abdominal fat (*SI Appendix*, Fig. S2), are considered to be predictors of impending metabolic syndrome (12, 16). Testosterone levels in males have been shown to be reduced in cases of long-term and extreme obesity (17), but we found no statistically significant difference in testosterone levels between control and obese groups (Table 1), again suggesting that our obese rats were in the early stages of obesity.

Obesity Produces Cognitive Deficits. We used cognitive tasks that do not require much locomotion to avoid the potential confounds of obesity-related decreases in physical performance. We used three object memory tasks: novel object recognition, object location, and object-in-place (*SI Appendix*, Fig. S3), each of which requires different brain regions for optimal performance (18). Obese rats performed poorly on the novel object recognition task, which requires the PRC, compared with normal weight controls ($t_{12} = 2.343$, $P = 0.04$) (Fig. 1*A*). By contrast, no statistical difference was observed between the obese and control groups on the object location task, which requires the HIP ($t_{14} = 1.03$, $P = 0.32$) (Fig. 1*B*). Obese rats also performed poorly on the object-in-place task, which requires the PFC and PRC or HIP ($t_{13} = 2.38$, $P = 0.03$) (Fig. 1*C*). However, the obese rats did not display a lack of object-in-place memory, but rather a reversed preference compared with controls: that is, an apparent preference for the objects that remained in place. Whether these results indicate some other type of performance change remains unknown, but clearly the difference reflects atypical behavior in the obese rats. The differences observed with novel object recognition and object-in-place in obese rats do not reflect differences in motivation to explore objects because on all three object memory tasks, there were no differences in overall time spent exploring objects during either the familiarization or test between control and obese rats (*SI Appendix*, Table S1).

To further investigate the potential impairment of prefrontal function in obesity, we examined performance on an attentional set-shifting task (ASST), a test of behavioral flexibility (*SI Appendix*, Table S2), components of which depend on the rat cingulate, prelimbic/infralimbic, and orbitofrontal cortex but not the HIP (19–21). Obese rats performed poorly on the ASST compared with normal weight controls ($F_{1,12} = 20.45$, $P < 0.001$) (Fig. 1 *D* and *E*). Robust differences between obese and control rats

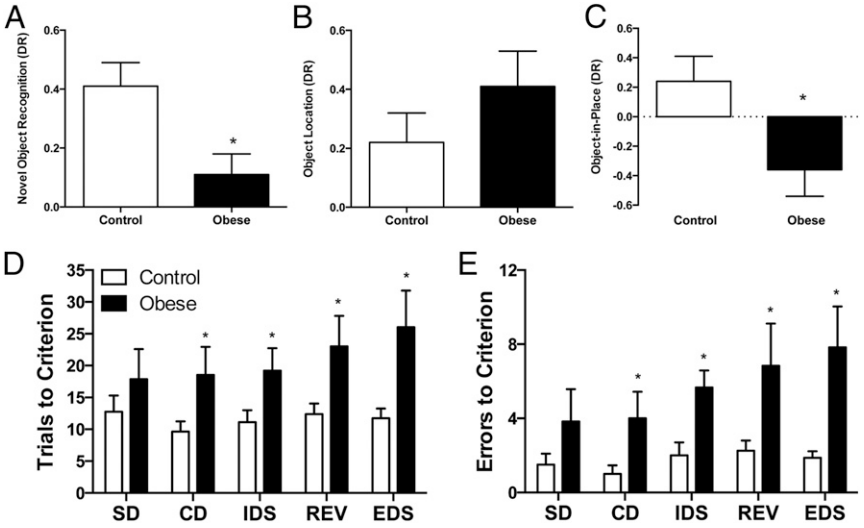

Fig. 1. Obese rats were impaired on some but not all cognitive tasks. Obese and control rats were tested on object memory tasks and attentional set-shifting (ASST). Obese rats had lower discrimination ratios for tests of novel object recognition (*A*) and object-in-place (*C*), but not object location (*B*). *$P < 0.05$ (unpaired two-tailed Student's t test); bars represent mean + SEM ($n = 7$ rats per group for object recognition; $n = 8$ per group for object location; $n = 7$ obese, $n = 8$ control for object-in-place). On ASST, obese rats reached criterion with more trials on the compound discrimination (CD), intradimensional shift (IDS), reversal learning (REV), and extradimensional shift (EDS) than control rats (*D*). Only the initial simple discrimination (SD) was not statistically different between groups. Obese rats also made more errors before reaching criterion (*E*) on all phases, with the exception of the SD. *$P < 0.05$ [ANOVA followed by Tukey's honestly significant difference (HSD) post hoc comparisons]; bars represent mean + SEM ($n = 6$ obese, $n = 8$ control for ASST).

were observed on the complex discrimination, intradimensional shift, reversal, and extradimensional shift components of the task (Fig. 1 *D* and *E*), suggesting impairments in cingulate, prelimbic/infralimbic, and orbitofrontal function (19, 20). The differences between obese and control rats on this task cannot be explained by lower motivation in the obese rats because the majority of animals in this group reached criterion, with seemingly more, not less, effort. That is, the obese rats made more attempts to find the food rewards than the control rats, but were less successful at obtaining them.

Obesity Does Not Alter Basic Locomotion, Sensory Function, or Anxiety-Like Behavior. The deficits observed in cognitive testing might occur as a result of differences in processes related to movement, sensation, or anxiety regulation, instead of cognitive dysfunction. To explore possible confounds of motor or sensory impairment, rats were tested for overall ambulatory activity in an open field, response to tactile and vibrissae stimulation, and response to two odorants (vanilla and female rat) (22, 23). There were no differences between control and obese rats on any of these tests (locomotion, $t_{14} = 0.52$, $P = 0.62$; tactile, $\chi^2_1 = 0.20$, $P = 0.65$; vibrissae, χ^2_1 = n/a because 100% response from both groups; $\text{olfactory}_{\text{vanilla}}$, $t_{14} = 0.23$, $P = 0.82$; $\text{olfactory}_{\text{female}}$, $t_{14} = 0.56$, $P = 0.58$) (*SI Appendix*, Table S3). Behavioral tests of anxiety, including novelty suppressed feeding and time spent exploring the center of an open field, also showed no differences between groups (novelty suppressed feeding, $t_{14} = 0.19$, $P = 0.85$; open field, $t_{14} = 0.71$, $P = 0.49$) (*SI Appendix*, Table S3). Together, these data indicate no gross deficits in basic locomotion, sensory processing, or anxiety regulation in our obese rats although we cannot rule out the possibility that subtler differences may have existed.

Obesity Reduces Dendritic Spine Density and Synaptic Proteins in PFC and PRC. Dendritic spine density on the apical dendrites of Golgi-impregnated pyramidal neurons in layer II/III of the PFC ($t_{14} = 2.63$, $P = 0.02$) (Fig. 2) and layer II/III of the PRC ($t_{14} = 2.24$, $P = 0.04$) (Fig. 2*D*) decreased compared with controls whereas dendritic spine density on the apical dendrites of pyramidal neurons in the CA1 region of the HIP in obese rats was no different from controls ($t_{14} = 1.21$, $P = 0.25$) (Fig. 2*D*). On all three neuronal populations, no changes in dendritic spine density or complexity were observed in the basal dendritic tree of Golgi-impregnated cells (spine density, PFC $t_{14} = 0.93$, $P = 0.37$, PRC $t_{14} = 0.96$, $P = 0.35$, HIPCA1, $t_{14} = 0.20$, $P = 0.85$; branch points, PFC $t_{14} = 1.17$, $P = 0.26$, PRC $t_{14} = 0.21$, $P = 0.84$, HIPCA1 $t_{14} = 1.12$, $P = 0.28$) (*SI Appendix*, Tables S4 and S5). Likewise, no differences in apical dendritic tree complexity were seen in layer II/III of the PFC ($t_{14} = 0.41$, $P = 0.69$), layer II/III of the PRC ($t_{14} = 0.04$, $P = 0.97$), and CA1 ($t_{14} = 0.43$, $P = 0.68$) of the HIP (*SI Appendix*, Table S5). Because chronic stress decreases the complexity of the apical dendritic tree of CA3 pyramidal neurons (24), we examined dendritic branch points on the CA3 apical tree and found no differences between control and obese rats ($t_{14} = 0.72$, $P = 0.48$) (*SI Appendix*, Table S5).

Because dendritic spines are primary sites of excitatory synapses, we sought to corroborate our findings by examining the levels of synaptic proteins, including a dendritic spine marker, spinophilin (25), and the presynaptic markers synaptophysin (26), vesicular GABA transporter (vGAT) (27, 28), and vesicular glutamate transporter (vGLUT) (28, 29). We also examined microtubule associated protein 2 (MAP-2), a general marker of dendritic structure (30). Confocal optical intensity analyses of immunolabeling for these proteins revealed that obesity produced region-specific decreases in spinophilin, synaptophysin, vGAT, and vGLUT, but not MAP-2, in the PFC and PRC (Figs. 3 and 4 and *SI Appendix*, Fig. S4*A*). No changes were seen for any of the markers in the HIP (spinophilin, PFC, $t_{14} = 2.87$, $P = 0.01$, PRC, $t_{14} = 3.75$, $P = 0.002$, HIP, $t_{14} = 1.37$, $P = 0.19$; synaptophysin, PFC, $t_{14} = 2.20$, $P = 0.04$, PRC, $t_{14} = 4.74$, $P < 0.001$, HIP, $t_{14} = 1.08$, $P = 0.29$; vGAT, PFC, $t_{14} = 4.94$, $P < 0.001$, PRC, $t_{14} = 2.62$, $P = 0.02$, HIP, $t_{14} = 0.03$, $P = 0.98$; vGLUT, PFC, $t_{14} = 2.98$, $P = 0.01$, PRC, $t_{14} = 2.52$, $P = 0.02$, HIP, $t_{14} = 0.40$, $P = 0.69$; MAP-2, PFC, $t_{14} = 0.26$, $P = 0.78$, PRC, $t_{14} = 0.83$, $P = 0.42$, HIP, $t_{14} = 0.97$, $P = 0.35$) (Figs. 3 *A* and *C* and 4 *A* and *C* and *SI Appendix*, Fig. S4*A*). Western blots were used to confirm the optical intensity immunolabeling data and yielded similar results in the PFC, PRC, and HIP for spinophilin, synaptophysin, vGAT, vGLUT, and MAP-2 (spinophilin, PFC, $t_{14} = 2.34$, $P = 0.03$, PRC, $t_{10} = 2.61$, $P = 0.03$, HIP, $t_{14} = 0.11$, $P = 0.91$; synaptophysin, PFC, $t_{14} = 2.41$, $P = 0.03$, PRC, $t_{11} = 2.70$, $P = 0.02$, HIP, $t_{14} = 0.92$, $P = 0.38$; vGAT, PFC, $t_{14} = 3.10$, $P = 0.008$, PRC, $t_{11} = 6.12$, $P < 0.001$, HIP, $t_{14} = 0.20$; $P = 0.84$; vGLUT, PFC, $t_{14} = 4.88$, $P < 0.001$, PRC, $t_{11} = 3.05$, $P = 0.011$, HIP, $t_{14} = 1.75$; $P = 0.10$; MAP-2, PFC, $t_{14} = 1.75$, $P = 0.10$, PRC, $t_{11} = 1.05$, $P = 0.32$, HIP, $t_{14} = 0.23$, $P = 0.82$.) (Figs. 3 *B* and *D* and 4 *B* and *D* and *SI Appendix*, Fig. S4*B*).

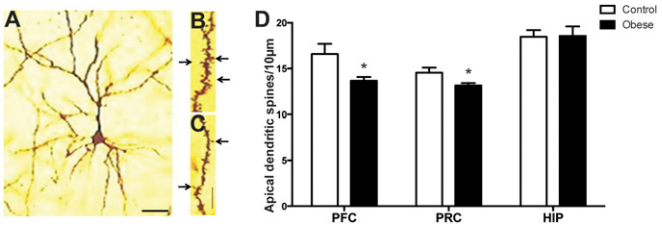

Fig. 2. Photomicrograph of a Golgi-impregnated layer II/III pyramidal cell in the PFC (*A*), with enlarged views of representative apical dendritic segments, from control (*B*) and obese (*C*) rats. Obese rats had decreased dendritic spine density on the apical dendritic tree in layer II/III pyramidal neurons in the PFC and layer II/III pyramidal neurons of the PRC, but not pyramidal neurons of the HIP CA1 region (*D*). $*P < 0.05$ (unpaired two-tailed Student's *t* tests within brain region); bars represent mean + SEM, $n = 8$ rats per group (for each rat, for each cell type, six cells were analyzed; for each cell, five apical and five basal dendritic segments were analyzed). (Scale bar: *A*, 30 μm; dendritic segments, 5 μm.) Arrows point to dendritic spines.

Obesity Increases Length of Microglial Processes in the PFC. Because microglial activation has been associated with synapse loss (31–34), we examined the morphology of microglia stained for the ionized calcium-binding adaptor molecule 1 (iba-1) (a calcium-binding protein specific for microglia/macrophages) in the PFC, PRC, and HIP. In the PFC, microglia in layers I and II/III were examined because these layers contain the apical dendrites of layer II/III pyramidal neurons, cells that exhibit evidence of dendritic spine loss in obesity. Obesity was associated with small but significant increases in the length of secondary and tertiary microglial processes in layer I (secondary process length, $t_{14} = 2.44$, $P = 0.03$; tertiary process length, $t_{14} = 3.23$, $P = 0.006$) (Fig. 5). Similar increases were observed in secondary processes of layer II/III microglia ($t_{14} = 2.55$, $P = 0.02$, control 7.13 ±0.38; obese, 8.95 ± 0.63). By contrast, no changes were observed in microglial process length in PRC or HIP ($P > 0.05$ for all comparisons) (Fig. 5 *B* and *C*).

Obesity Reduces PFC but Not HIP Volume. Estimates of PFC volume showed a slight but statistically significant reduction in obese rats compared with control rats ($t_{14} = 2.34$, $P = 0.03$; control, 1.3 ± 0.02 mm^3 vs. obese 1.2 ± 0.02 mm^3) whereas no differences were seen in HIP volume between groups ($t_{14} = 0.83$, $P = 0.42$; control, 2.4 ± 0.02 mm^3 vs. obese, 2.3 ± 0.02 mm^3). Volume of the PRC was not determined given the difficulty in delineating firm boundaries for this area. No differences in total brain weight were observed between the obese rats and the control rats ($t_{14} = 0.82$, $P = 0.43$; control, 1.99 ± 0.03g vs. obese, 2.06 ± 0.08g).

Discussion

Here, we have shown that early stage obesity influences cognitive function, as well as the structure of the adult brain. After just 8 wks on a high-fat diet, rats exhibited impaired performance on

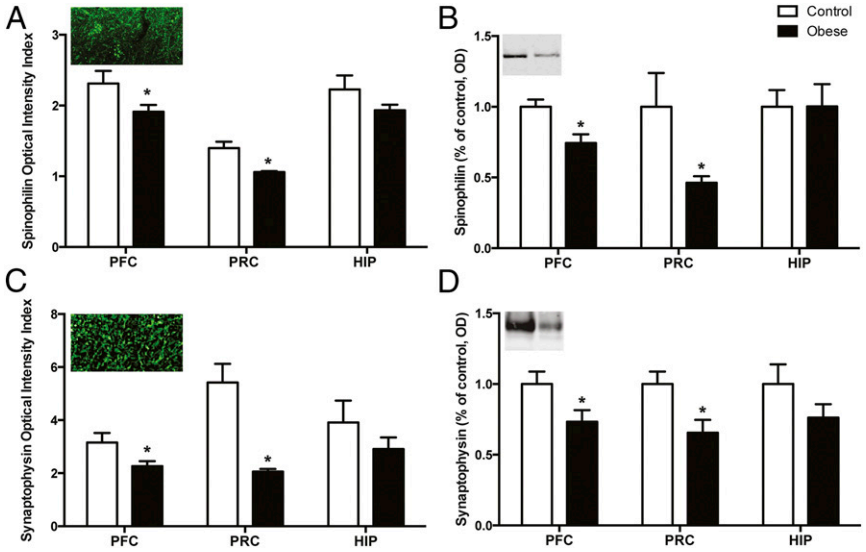

Fig. 3. Obesity lowered levels of the synaptic proteins spinophilin and synaptophysin in the PFC and PRC, but not HIP. Immunofluorescence (*A*) and Western blotting (*B*) for spinophilin and immunofluorescence (*C*) and Western blotting (*D*) for synaptophysin showed decreases in both proteins in the PFC and PRC. No statistical differences were observed in HIP. *Insets* are representative samples of immunofluorescence and Western blots (control on left; obese on right) in the PFC. $*P < 0.05$ (unpaired two-tailed Student's *t* tests within brain region); bars represent mean + SEM. For immunofluorescence, $n = 8$ rats per group for PFC, PRC, and HIP. For Western blots, $n = 8$ rats per group for PFC and HIP, but, due to inadequate sample size in PRC, some rats were excluded ($n = 5$ rats for control, $n = 7$ for obese).

tasks requiring the PRC or PFC: novel object recognition, object-in-place, and attentional set-shifting. Along with these cognitive deficits, obese rats showed decreased spine density on the apical dendritic trees of pyramidal cells in the PRC and PFC, which coincided with decreased expression of several proteins associated with synapses, including spinophilin, synaptophysin, vGLUT, and vGAT. By contrast, early stage obesity was not associated with a decline in an object location task that requires the hippocampus. Consistently, no changes in dendritic spine density or synaptic markers were observed in the hippocampus. Taken together, these findings suggest that early stage obesity in rats impairs cognitive function and reduces the number of synapses in the PRC and PFC, but not the HIP.

Our observations that early stage obesity is associated with reduced density of dendritic spines, as well as reduced expression of presynaptic and postsynaptic markers in PRC and PFC, suggest an underlying mechanism for cognitive dysfunction. Each of the synaptic proteins we examined (spinophilin, synaptophysin, vGAT, and vGLUT) has been linked to synaptic plasticity and cognition. The combined decrease in all of these markers, along with a decrease in the number of dendritic spines, strongly suggests a global loss of synapses in both of these brain regions. Although it remains possible that reduced neuronal activity and impaired function might precede synapse loss, a more plausible scenario is that synapse loss with obesity leads to cognitive dysfunction. A previous study showing circadian disruption-induced cognitive dysfunction, along with overall decreased spine number on apical dendrites of PFC pyramidal neurons, is consistent with our findings, but the extent to which cognitive impairment is directly tied to the associated weight gain remains to be determined (35).

Our results also suggest small but significant changes in the morphology of microglia located in some areas of synapse loss, namely, layers I and II/III of PFC. Microglia, the resident immune cells of the brain, are known to alter their morphology in response to damage (31, 32). A known function of microglia is to clear neuronal debris by phagocytosis. Developmental studies indicate that microglia actively prune synapses in the brain (32–34), raising the possibility that such a process may be occurring in areas of synapse loss with obesity. The link between microglia and synapse loss in obesity is unknown. As well, the extent to which microglial changes are beneficial (i.e., serving to eliminate synaptic material that is no longer useful) or detrimental (i.e., serving to prematurely eliminate synapses and/or releasing cytokines that produce synapse loss) deserves further study. It is somewhat surprising that we did not observe a similar change in microglia located in the PRC, an additional brain region showing decreased dendritic spine density and synaptic marker expression with obesity. Although additional studies are needed to draw definitive conclusions, one possibility is that microglia in PFC are more sensitive to cues associated with synapse loss than those in PRC. However, examining microglia at later time points in the development of obesity, including the emergence of diabetes, may reveal a more global, as opposed to region-specific, pattern of microglial change.

Each of the brain regions examined in this study—the PFC, PRC, and HIP—has reciprocal connections with the others (36, 37). Connections among these brain regions raise the possibility that detrimental changes in one area may produce negative effects in another. For example, because PFC axons synapse on dendritic spines of PRC pyramidal neurons (36), obesity-induced changes in PFC neurons may lead to reduced activity in the PFC–PRC projection, which, in turn, reduces spine and synapse number in PRC. Because the connections between these areas are reciprocal, however, it is difficult to predict which, if any, of the examined neuronal populations might initiate a cascade of events leading to synapse loss in a downstream area. Whether a change in PFC produces one in PRC or vice versa, it is clear that synapse loss in either of these areas is not sufficient to measurably impact synapses in the HIP, at least at the time point we examined. In addition to changes in behavior, brain structure, and synaptic markers, we noted hormonal changes in our early stage obese rats, namely, reduced corticosterone levels and increased circulating leptin levels. Although the extent to which these hormonal changes are responsible for the structural and functional changes we observed in our obese rats remains unknown, some evidence suggests a causal link. For example, reduced glucocorticoid levels have been associated with diminished dendritic spine density in the PFC (38) and impaired performance on PFC-dependent cognitive tasks (39). The relative protection of the hippocampus during early stage obesity may be due to the temporary ability of elevated circulating leptin levels to compensate for reduced glucocorticoid levels. Indeed, leptin has been shown to enhance hippocampus-dependent cognition,

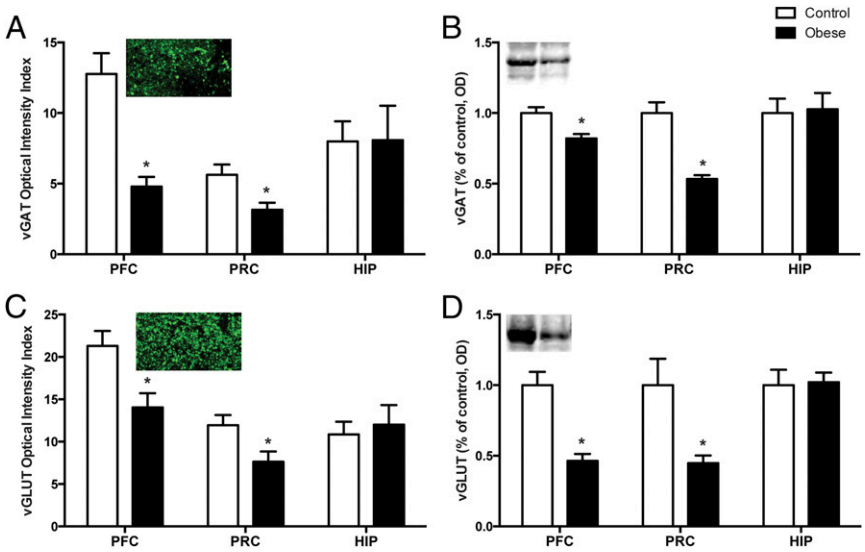

Fig. 4. Obesity lowered levels of the synaptic proteins vGAT and vGLUT in the PFC and PRC, but not HIP. Immunofluorescence (*A*) and Western blotting (*B*) for vGAT and immunofluorescence (*C*) and Western blotting (*D*) for vGLUT showed decreases in both proteins in the PFC and PRC, but not HIP. *Insets* are representative samples of immunofluorescence and Western blots (control on left; obese on right) in the PFC. $*P < 0.05$ (unpaired two-tailed Student's *t* tests within brain region); bars represent mean + SEM. For immunofluorescence, $n = 8$ rats per group for PFC, PRC, and HIP. For Western blots, $n = 8$ rats per group for PFC and HIP, but, due to inadequate sample size in PRC, some rats were excluded ($n = 6$ rats for control, $n = 7$ for obese).

as well as synaptic plasticity, dendritic spines/synapses and adult neurogenesis in the hippocampus (40–42). The cognitive- and plasticity-enhancing characteristics of leptin may thus be capable, at least during a short time frame, of preventing hippocampal dysfunction in the face of lower glucocorticoid signaling. A recent study has shown that longer time periods of high fat diet feeding impair cognitive tasks associated with the hippocampus, as well as reduce synaptic markers and increase microglia activation in the hippocampus (43), but another recent study has reported no effect of long term high fat diet feeding on cognitive behaviors associated with the hippocampus (44). It remains additionally possible that, although obesity alone is not sufficient to compromise hippocampal structure and function at this early time point, it may produce vulnerability that is evident only when obesity occurs in conjunction with additional perturbations. Indeed, a previous study showed that, whereas neither high-fat diet nor chronic stress alone were sufficient to impact hippocampal plasticity, the combination of both manipulations had a detrimental impact (45).

Metabolic syndrome and diabetes are associated with increased glucocorticoid levels (16), conditions that have been clearly linked to hippocampal impairment. Our obese rats were not yet displaying definitive signs of metabolic syndrome but were clearly in a pre-metabolic syndrome state, as evidenced by increased abdominal fat and increased circulating leptin levels. It is likely that a greater duration of eating a high-fat diet would have resulted in the emergence of metabolic syndrome, and eventually diabetes, including an increase, instead of a decrease, in corticosterone levels. Because elevated glucocorticoid levels have been associated with impaired structure and function in the PFC (46–49) and other cortical areas, the transition to metabolic syndrome may not substantially alter the cognitive, structural, or biochemical impairments in the PFC. However, elevated glucocorticoids with metabolic syndrome would likely produce impairments in hippocampal function, resulting in more global cognitive deficits than what we observed in our early stage obese rats. Taken together, these findings suggest that obesity has negative consequences on the brain even before metabolic syndrome or diabetes sets in. They also suggest an involvement of hormonal systems both in producing and preventing early deficits.

Previous studies have shown that obese humans have reduced volume of the prefrontal cortex (8, 9). Our findings that a smaller prefrontal cortex in obese rats is accompanied by fewer dendritic spines and considerably lower levels of presynaptic and postsynaptic proteins suggest that similar synaptic changes may occur in obese humans. These changes in the rat are associated with behavioral deficits in object memory and cognitive flexibility, consistent with impairment in the frontal mechanisms responsible for cognitive control. Similarly, reduced synapse numbers in the frontal cortex of humans in early stage obesity may produce an obesogenic endophenotype that contributes to further overeating. That is, obese individuals with lower numbers of dendritic spines and synapses in the frontal cortex may be incapable of sufficiently activating this brain region in response to cues that normally engage mechanisms necessary for self-control. Indeed, studies have shown that obese humans display hypoactivation of the anterior cingulate and dorsolateral prefrontal cortex in response to food cues (50–52). These changes may reflect a reduced synapse number in early obesity, leading to failure of mechanisms involving

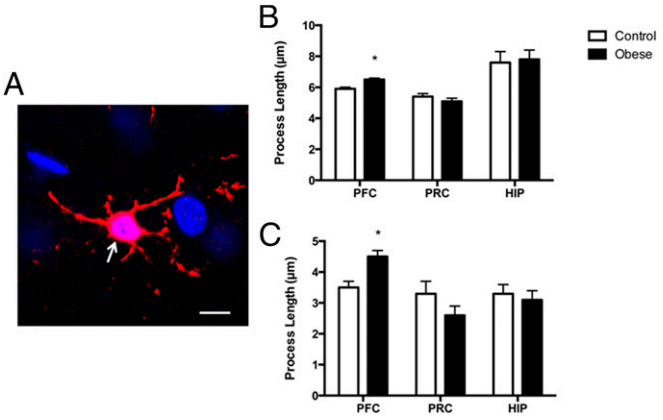

Fig. 5. Obesity increased the length of microglial processes in PFC. Confocal image shows iba-1–stained microglia (arrow) in obese PFC. Blue counterstain is a Hoechst DNA dye (*A*). Morphometric analyses of iba-1–stained microglia showed small but significant increases in the length of secondary (*B*) and tertiary processes (*C*) in the PFC (layer I) but not the PRC (layer II/III) or HIP (CA1). $*P < 0.05$ (unpaired two-tailed Student's *t* tests within brain region); bars represent mean + SEM ($n = 8$ rats per group). (Scale bar: 3 μm.)

cognitive control, including evaluative ones assigned to the anterior cingulate and regulative functions assigned to the lateral frontal cortex (53). This problem may make it increasingly difficult to engage in behaviors conducive to weight loss.

The findings of the present report suggest that certain brain regions, namely the prefrontal cortex and perirhinal cortex, become functionally compromised early in obesity. By contrast, the hippocampus, a structure known to be impacted by later stage obesity accompanied by metabolic syndrome, is relatively spared in early obesity. These results, along with the identification of substantial synapse loss in the functionally impaired areas, as well as changes in microglia and accompanying hormonal changes, suggest potential mechanisms that underlie brain shrinkage in obese humans and further suggest a sequence of brain changes that may serve as time points for different types of therapeutic intervention.

Materials and Methods

Adult male Sprague–Dawley rats were used for these studies. All animal procedures and protocols were approved by the Princeton University Institutional Animal Care and Use Committee and followed the National Research Council's *Guide for the Care and Use of Laboratory Animals* (54). Age-matched rats were given access to a high-fat diet or standard rodent chow. After 8 wks on the described diets, rats were tested on behavioral tasks and their brains were examined with Golgi-impregnation for dendritic spines, immunohistochemistry, and Western blots for synaptic proteins and immunohistochemistry for microglial morphology. Blood was also assayed for triglyceride, leptin, glucose, insulin, corticosterone, and testosterone levels (*SI Appendix, SI Materials and Methods*).

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