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Dietary obesity reversibly induces synaptic stripping by microglia and impairs hippocampal plasticity

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ABSTRACT

Obesity increases risk of age-related cognitive decline and is accompanied by peripheral inflammation. Studies in rodent models of obesity have demonstrated that impaired hippocampal function correlates with microglial activation, but the possibility that neuron/microglia interactions might be perturbed in obesity has never been directly examined. The goal of this study was to determine whether high fat diet-induced obesity promotes synaptic stripping by microglia, and whether any potential changes might be reversible by a return to low-fat diet (LFD). Time course experiments revealed that hippocampal inflammatory cytokine induction and loss of synaptic protein expression were detectable after three months of HFD, therefore subsequent groups of mice were maintained on HFD for three months before being switched to LFD for an additional two months on LFD (HFD/LFD). Additional HFD mice continued to receive HFD during this period (HFD/HFD), while another group of mice were maintained on LFD throughout the experiment (LFD/LFD). Dietary obesity impaired hippocampus-dependent memory, reduced long-term potentiation (LTP), and induced expression of the activation marker major histocompatibility complex II (MHCII) in hippocampal microglia. Diet reversal only partially attenuated increases in adiposity in HFD/LFD mice, but plasticity deficits and MHCII induction were normalized to within the range of LFD/LFD mice. Microglial activation and deficits in hippocampal function were accompanied by perturbation of spatial relationships between microglial processes and synaptic puncta. Analysis of primary microglia isolated from HFD/HFD mice revealed selective increases in internalization of synaptosomes labeled with a pH-sensitive fluorophore. Taken together, these findings indicate that dietary obesity reversibly impairs hippocampal function, and that deficits may be attributable to synaptic stripping by microglia.

1. Introduction

Obesity is associated with chronic systemic inflammation and has also been shown to increase risk of age-related cognitive decline (Kanneganti and Dixit, 2012; Whitmer et al., 2008; Xu et al., 2011). While the pathogenesis of metabolic comorbidities in obesity is well-characterized, cellular mechanisms for cognitive impairment in obesity remain less so. Multiple reports indicate that that high-fat diet (HFD)-induced obesity impairs cognitive function in rodent models (Molteni et al., 2002; Stranahan et al., 2008; McNay et al., 2010). Because hippocampal neurons exhibit early vulnerability during age-related cognitive impairment, studies of cognitive dysfunction in obesity have primarily focused on differences in hippocampal synaptic plasticity. Parallel observations by multiple groups now support the idea that obesity-induced systemic inflammation is accompanied by inflammation in multiple brain regions, including the hippocampus (Erion et al., 2014; Buckman et al., 2014; Sobesky et al., 2014).

Microglia are brain-resident phagocytes that continuously monitor the neuropil with motile processes for detection and clearance of cellular debris. In addition to removing dead cells and extracellular aggregates, microglia also internalize synaptic terminals (Kettenmann et al., 2013). Synaptic internalization by microglia is critical for developmental synaptic pruning (Paolicelli et al., 2011) and evidence from the visual cortex indicates that this process is activity-dependent (Tremblay et al., 2010). Microglia/neuron interactions are not limited to internalization or 'stripping,' as a number of signaling pathways that attract microglia to inactive synapses have now been identified (Stevens et al., 2007; Schafer et al., 2012). Microglia also influence plasticity through local release of neurotrophins at dendritic spines (Parkhurst

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et al., 2013), but the idea that synapse loss in obesity might be mediated by microglia has never been directly examined.

These studies were designed to determine whether interactions between microglia and neurons might be altered in dietary obesity. To answer this question, we used mice exposed to high-fat diet (HFD/HFD), low-fat diet (LFD/LFD), or HFD followed by LFD (HFD/LFD). The duration of HFD applied prior to diet reversal was determined in time course experiments, which identified 3 months of HFD as the onset of inflammatory cytokine accumulation and loss of hippocampal synaptic proteins. Concurrent measures of adiposity and insulin resistance revealed that hippocampal inflammation and synaptic loss occurred after the development of obesity, but before the onset of diabetes. The diet reversal experiments revealed that a return to LFD normalizes hippocampus-dependent memory, long-term potentiation (LTP), and spatial relationships between microglia and hippocampal synapses. Experiments in primary microglia from LFD/LFD, HFD/HFD, and HFD/LFD mice identified selective, obesity-induced increases in microglial internalization of synaptosomes labeled with a pH-sensitive fluorophore. Taken together, these results indicate that obesity reversibly increases synaptic internalization by microglia, and implicate this process as a mechanism for impaired hippocampal function.

2. Materials and methods

2.1. Animals and diets

Male C57Bl6J mice were purchased from Jackson Laboratories (Bar Harbor, Maine) at 5 weeks of age. After one week acclimation, mice were housed one per cage, with high-fat diet chow (HFD; Research Diets 12492) or low-fat diet chow (LFD; Research Diets 12450J) and water available ad libitum (see Supplementary Table 1 for diet composition). For the time course experiments, (n = 6–7) mice were maintained on HFD or LFD for one, two, or three months, with food intake measured on two successive days per week and body weights collected weekly. One week before euthanasia, mice were fasted overnight for analysis of glucose and insulin levels, as described (Dey et al., 2014). At sacrifice, the weights of the epididymal fat pads were collected as a measure of visceral adipose tissue and the inguinal depot was weighed as a measure of subcutaneous fat. Hippocampal protein extracts from mice exposed to different durations of HFD or LFD were collected to measure interleukin 1β (IL1 β) protein and synaptic marker expression.

For diet reversal experiments, mice were maintained on HFD for three months before being switched to LFD chow for two months (HFD/LFD) or continued availability of HFD chow (HFD/HFD). Additional groups consumed LFD chow throughout all five months of the experiment (LFD/LFD). For immunofluorescence and stereology, (n = 8) mice from each condition were transcardially perfused with 4% paraformaldehyde in phosphate buffer (PFA). For analysis of long-term potentiation and dendritic spine density, (n = 8–11) from each condition were rapidly decapitated under light Isoflurane anesthesia for preparation of acute slices (described below). Additional groups of (n = 12) mice per condition were transcardially perfused with sterile saline for isolation of primary microglia by density gradient centrifugation, as described (Erion et al., 2014; Dey et al., 2014). All cohorts of mice generated for these experiments were used for behavioral tests of hippocampus-dependent memory.

2.2. Behavioral testing

Groups of mice from each cohort were tested in the Y-maze and novel object preference tests. Testing in both paradigms was carried out between 1800 and 2200 h (lights-off at 1800) under red light illumination, as described (Erion et al., 2014; Wosiski-Kuhn et al., 2014). For Y-maze testing, mice were transferred from the home cage into a randomly selected start arm (length x width x height, cm: 20 × 6 × 10). Each alternation trial consisted of free exploration until a complete arm entry. After entering the choice arm, mice were confined to that arm by lowering a guillotine door during a 1-min inter-trial interval before beginning the next trial. Each of the (6) alternation trials was captured on digital video and video clips were coded for offline analysis in a blinded manner. Correct choices were expressed relative to the total number of potential alternations and used for subsequent statistical analysis.

Novel object recognition testing involved 10 min exposure to two identical objects, followed by 30 min in the home cage, after which the mouse was returned to the arena in the presence of one novel and one familiar object. After 5 min object exploration, the mouse returned to the home cage for 1.5 h before re-exposure to the familiar object and a different novel object. Test sessions were captured on video and analyzed offline at half speed by an observer blinded to the treatment conditions. The duration of each exploratory interval was recorded and expressed relative to the total amount of object exploration (novel + familiar) for each trial.

2.3. Hippocampal slice preparation and electrophysiology

Hippocampal slice preparation and extracellular recording followed previously published methods (Wosiski-Kuhn and Stranahan, 2012). In brief, 300 μm slices were cut on a Vibratome (Leica, Buffalo Grove, IL, USA) into a bath of oxygenated artificial cerebrospinal fluid (ACSF). After 1 h recovery at 37 °C, extracellular recordings were performed in ACSF supplemented with 50 μM picrotoxin (Sigma–Aldrich, St. Louis, MO, USA). Electrodes were positioned in the middle molecular layer superficial to the dentate gyrus and medial perforant path inputs were functionally identified by the presence of paired-pulse depression (PPD; Asztely et al., 2000). PPD magnitude was determined using interpulse intervals of 50, 200, 500, and 1000 ms, and quantified by expressing the slope of the second (S2) field excitatory postsynaptic potential (fEPSP) as a percent of the first fEPSP (S1). The baseline stimulation intensity was set at 50% of maximal fEPSP slope generated from the input/output curve. Stimuli were delivered at 0.05 Hz for baseline and post-tetanus recording and LTP was induced with a single train delivered at 100 Hz for 1 s. Data were collected using pClamp version 10.3.4 and analyzed in Clampfit (Molecular Devices, Sunnyvale, CA, USA).

2.4. DiI labeling, dendrite imaging, and quantification of dendritic spine density

Juxtacellular labeling with the lipophilic membrane tracer 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI; Invitrogen, Carlsbad, CA) followed previously published methods (Wosiski-Kuhn et al., 2014). In brief, hippocampal slices were generated as described above, but instead of recording, slices were fixed in PFA for 1 h and DiI crystals were placed in the dentate hilus inferior to the dentate granule cell layer. Following 48 h incubation at 37 °C, slices were counterstained with DAPI (1:1,000, Sigma–Aldrich) and z-stack images of dendritic segments were acquired through an oil immersion objective (NA 1.4) on a Zeiss LSM510 Meta confocal microscope.

Dendritic segments were sampled from the secondary and tertiary dendrites of dentate gyrus granule neurons, as described (Erion et al., 2014; Wosiski-Kuhn et al., 2014). Full-resolution images of each plane were imported into Reconstruct (http://synapses.clm.utexas.edu) for spine counting and three-dimensional