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# High-fat diet and aging interact to produce neuroinflammation and impair hippocampal- and amygdalar-dependent memory

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ABSTRACT

More Americans are consuming diets higher in saturated fats and refined sugars than ever before, and based on increasing obesity rates, this is a growing trend among older adults as well. While high saturated fat diet (HFD) consumption has been shown to sensitize the inflammatory response to a subsequent immune challenge in young adult rats, the inflammatory effect of HFD in the already-vulnerable aging brain has not yet been assessed. Here, we explored whether short-term (3 days) consumption of HFD would serve as a neuroinflammatory trigger in aging animals, leading to cognitive deficits. HFD impaired long-term contextual (hippocampal dependent) and auditory-cued fear (amygdalar dependent) memory in aged, but not young adult rats. Short-term memory performance for both tasks was intact, suggesting that HFD impairs memory consolidation processes. Microglial markers of activation Iba1 and cd11b were only increased in the aged rats, while MHCII was further amplified by HFD. Furthermore, these HFD-induced long-term memory impairments were accompanied by IL-1β protein increases in both the hippocampus and amygdala in aged rats. Central administration of IL-1RA in aged rats following conditioning mitigated both contextual and auditory-cued fear memory impairments caused by HFD, strongly suggesting that IL-1β plays a critical role in these effects. Voluntary wheel running, known to have anti-inflammatory effects in the hippocampus, rescued hippocampal-dependent but not amygdalar-dependent memory impairments caused by HFD. Together, these data suggest that short-term consumption of HFD can lead to memory deficits and significant brain inflammation in the aged animal, and strongly suggest that appropriate diet is crucial for cognitive health.



## 1. Introduction

In the last 100 years, diet in developed countries has seen astounding increases in the amounts of fats and sugar consumed (Guyenet and Carlson, 2015; Putnam and Allshouse, 1999). Not surprisingly, these increases are correlated with rising obesity rates. Approximately, 40% of the adult U.S. population is currently obese, a dramatic increase from the 13% incidence of 1960 (Fryar et al., 2016; Ogden et al., 2015). Importantly, the prevalence among older individuals (aged 60–74) has nearly doubled since just 1980 (Fakhouri et al., 2012), and with the population of Americans over the age of 65 expected to reach 25% by the year 2030 (Alzheimer's Association, 2016; Wimo et al., 2013), any health risks associated with aging and unhealthy diets are likely to become even greater in the future.

Aging is a major risk factor for inflammation-induced mild cognitive impairments, as demonstrated in both the clinical and preclinical literature (Alzheimer's Association, 2016; Barrientos et al., 2015; Corona et al., 2012; Holmes et al., 2003; Moller et al., 1998; Murray et al., 2012). Importantly, mild cognitive impairments among older individuals increase the probability for developing Alzheimer's disease later in life (Alzheimer's Association, 2016; Miller and Spencer, 2014). As many have shown, microglia, the brain's resident immune cells, become sensitized with age, lowering their threshold for activation (Frank et al., 2006, 2010b; Perry et al., 1993; Rogers et al., 1988; Rozovsky et al., 1998). When either a peripheral or central inflammatory challenge is experienced, these sensitized microglia become hyperactivated and produce pathologic levels of proinflammatory cytokines, thereby interfering with synaptic plasticity processes, potentially resulting in precipitous memory declines (Chapman et al., 2010, 2012;

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Combrinck et al., 2002; Cunningham et al., 2009). Previous research has demonstrated that this sequence occurs in aged rodents in response to bacterial and viral infections, and surgical insults (Abraham et al., 2008; Barrientos et al., 2006, 2012; Chen et al., 2008; Rosczyk et al., 2008). We hypothesize that acute high-fat diet (HFD), such as occurs with binge-eating and fast-food meals, may be an important pathologic trigger, similar to infection or surgery, to induce overt brain inflammation and memory loss in the already-sensitized aging brain.

While HFD in young adults produces low-grade inflammation in the circulation and peripheral tissues (Cano et al., 2009; Coppack, 2001; Xu et al., 2002), its ability to directly induce inflammation within the brain is limited to the hypothalamus (De Luca et al., 2016; Maric et al., 2014; Milanski et al., 2009), except after extremely long-term HFD during which diabetes-like symptoms are starting to occur (Jeon et al., 2012). In the hippocampus, HFD in the young adult is not directly inflammatory and only sensitizes cells to over-respond to future inflammatory stimuli (Sobesky et al., 2014, 2016). In the absence of such stimuli, no inflammatory response is detected nor memory impairments observed. Interestingly, even as little as 3 days, HFD is sufficient to sensitize the hippocampus in this way (Sobesky et al., 2014, 2016). Despite these pronounced effects of short-term HFD on extra-hypothalamic regions of the brain in the young adult, the inflammatory effect of HFD in the already-vulnerable aging brain has not yet been assessed. Here, we hypothesized that consumption of a HFD would serve as a neuroinflammatory trigger in aging animals, leading to cognitive deficits, without having an overt proinflammatory or cognitive effect in young adults.

## 2. Materials and methods

### 2.1. Subjects

Subjects were male F344×BN F1 rats obtained from the National Institute on Aging Rodent Colony maintained by Envigo (Indianapolis, IN, USA). On arrival at our facility, aged rats were 24 months old and weighed approximately 500 g. Young adult rats were 3 months old and weighed approximately 300 g. Following arrival, animals were allowed to acclimate to the facility for at least 7 days before diet modifications. With the exception of the exercise experiments, where they were individually housed, rats were pair-housed in standard cages (46 cm × 26 cm × 21 cm; L × W × H) with food and water available ad libitum. The colony room was maintained at 22 °C on a 12-hour light/dark cycle (lights on at 07:00h). All experiments were performed during the light phase. All experiments were conducted in accordance with the protocols approved by the University of Colorado Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

### 2.2. Diet

Animals were assigned to either continue consuming their regular chow (Teklad Diets, TD. 8640, energy density of 3.0 kcal/g; 29% calories from protein, 54% from carbohydrates (no sweetener added), and 17% from fat [0.9% saturated, 1.2% monounsaturated, and 2.7% polyunsaturated]), or the HFD, which is an adjusted calorie HFD (TD.06414, Envigo, energy density of 5.1 kcal/g; 18.4% calories from protein, 21.3% from carbohydrates (90 g/kg sucrose, 160 g/kg maltodextrin), and 60.3% from fat [37% saturated, 47% monounsaturated, and 16% polyunsaturated]). Body mass and food and water consumption were weighed (in g) daily at the same time of day (9:00–9:30 am). Energy intake was calculated by multiplying the kcal/g by the total grams consumed per cage, divided by the starting average body mass per cage.

### 2.3. Contextual fear conditioning

After 3 days of consuming their respective diets, rats were taken 2 at a time from their home cage and each was placed in a conditioning chamber (26 L × 21 W × 24 H, cm) made of clear plastic and topped with a wire mesh top. Each chamber was housed inside an ice chest (54 L × 30 W × 27 H, cm). A speaker, a fan, and 2 24-V DC lightbulbs (1 white and 1 red) were mounted on the ceiling of each ice chest. Rats were allowed to explore the chamber for 2 minutes before the onset of a 15-second tone (76 dB), followed immediately by a 2-second footshock (1.5 mA) delivered through a removable floor of stainless steel rods. Each rod was wired to a shock generator and scrambler (Coulbourn Instruments, Allentown, PA, USA). To assess obvious signs of lethargy or sickness, locomotion was scored during conditioning. Immediately after the termination of the shock, rats were removed from the chamber and returned to their home cage. At this point, HFD-fed rats were switched back to chow. After 4 days, all rats were tested for fear of the conditioning context, a hippocampal-dependent task (Matus-Amat et al., 2004; Rudy et al., 2002), and then for fear of the tone, an amygdala-dependent task (Kim and Fanselow, 1992; Phillips and LeDoux, 1992). Chambers were cleaned with water and a mild detergent before each animal was conditioned or tested. For the context fear test, rats were placed in the exact context in which they were conditioned and were observed for 6 minutes and scored for freezing behavior. For the auditory-cued fear test, rats were placed in an altered context (e.g., differently shaped and sized chamber, red light, no grid floor) and scored for freezing behavior for 3 minutes. Following the 3 minutes, the tone was activated and freezing behavior was scored for an additional 3 minutes. All scoring was done in real time by researchers who were blind to the experimental conditions. Freezing is the rat's dominant defensive fear response and it is a common measure of conditioned fear (Kim and Fanselow, 1992). Freezing was defined as the absence of all visible movement, except for respiration. Using a time-sampling procedure, every 10 seconds each rat was judged as either freezing or active at the instant the sample was taken. Inter-rater reliability exceeded 97% for all experiments. For the long-term memory experiments, memory was assessed 4 days after conditioning; for short-term memory experiments, memory was assessed 1–2 hours after conditioning. Separate cohorts of rats were used for the long-term and the short-term memory tests. An additional experiment was conducted whereby rats were maintained on the HFD throughout the acquisition and retention phases of the experiment (8 days), to rule out the possibility that any impairments observed with the 3-day protocol was due to stress related to switching diets mid-way through the behavioral paradigm. Conditioning coincided with day 3 on the respective diets, as with the other studies presented here. Memory testing occurred on day 7.

### 2.4. Morris water maze

Spatial learning and memory was assessed using the Morris water maze. Rats received acquisition training on day 4 of the diet regimen and were tested for long-term spatial memory 4 days later. Diet conditions were maintained through day 8. The water maze consisted of a circular galvanized steel pool approximately 110 cm in diameter and 58 cm deep. A movable escape platform constructed of a Plexiglass base column having a height of 43 cm and topped by a round platform 15 cm in diameter, was placed in a quadrant of the pool and was maintained there throughout the acquisition of the task. The water was filled to a height of 47 cm and