High-fat diet impairs spatial memory after short-term but not long-term exposure: sex-differences, receptor expression, hippocampal plasticity, and peripheral metabolism

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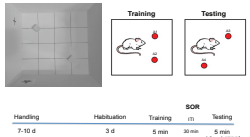
Introduction

Global prevalence of dementia in 2010 was 35.6 million people, with numbers expected to double every 20 years, reaching 115.4 million by 2050 (Alz. Disease Int'l, 2009). Simultaneously, obesity is increasing worldwide, with 1.46 billion adults overweight (BMI ≥ 25 kg/m2) and 502 million adults obese (BMI ≥ 30; Finucane et al., 2009). While obesity may contribute to risk of dementia, the association is complex and far from clear. Here we investigate the short-term (ST) and long-term (LT) effects of a high-fat diet (HFD) in both male and female rats on performance in a spatial object recognition (SOR) task, on CA1 hippocampal neuron intrinsic excitability, on peripheral metabolic markers relevant in type-2 diabetes, and on CA1 protein expression.

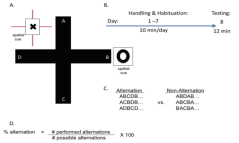
Methods

Subjects: Experiments were performed using male & female Long-Evans rats. Rats were bred use and maintained in our animal facility under conditions approved by the UT Dallas ACUC on a 12hr/12hr light/dark schedule with ad libitum access to food and water. Littermates were distributed across all treatment conditions to reduce variance, and males and females were socially housed.
Diet: Subjects in the CD cohort received a standard rat pellet diet (20.7% protein, 35.8% fat, and 35% carbohydrate). Subjects in the HFD cohort received a diet of standard rat pellet augmented to achieve an energy contribution of: 15.6% protein, 57.6% fat, and 26.8% carbohydrate. Subjects received only their assigned diet from weaning (21 d). Subjects were maintained on the diet for either short-term (ST; 12 weeks) or long-term (LT; > 52 weeks) prior to experimentation.

Spatial Object Recognition (SOR):



Spontaneous Alternation Task (SAT):



Systemic Measures: Blood samples were collected during decapitation and plasma was frozen until use. Insulin was assessed using rat insulin ELISA kits (Abnova) and cortisol was assessed using CSCI ELISA kits (Abcam), using a ELx800 plate reader (BioTek) abd Gen5 software.
Western Blotting: Coronal cryosections of 400 um thickness were taken and the vCA1, vCA3, and LHA dissected using a tissue punch kit 0.2-0.9mm in diameter. Western blotting was used to quantify protein expression of insulin receptor (IR) protein (mouse; 1:1000; Enzo), SK2 channel protein (rabbit; 1:1000; Alamone), and pAKT Ser473 (rabbit; 1:2500; Abcam) in the vCA1, vCA3, and LHA of niave CD^M, HFD^M, CD^F, and HFD^F animals. Data shown has not yet been normalized to B-tubulin. Analyses between dietary groups (but within each sex group) were done using t-tests.
Slice preparation: Rats were anesthetized with isoflurane and decapitated. The brain was quickly hemisected and immersed in cooled sucrose-aCSF [in mM: 124 sucrose; 3 KCl; 1.3 $MgSO_4$; 1.24 NaH_2PO_4; 2.4 $CaCl_2$; 26 $NaHCO_3$; 10 d-glucose]. After the brain chilled for 3-4 min, it was blocked and 400 µm slices cut using vibratomes then placed in room temperature (25°C) aCSF [in mM, 124 NaCl; 3 KCl; 1.3 $MgSO_4$; 1.24 NaH_2PO_4; 2.4 $CaCl_2$; 26 $NaHCO_3$; 10 d-glucose]. Both aCSFs were continuously oxygenated (95% O_2; 5% CO_2; pH 7.4).
Current-clamp recordings: Sharp electrodes were prepared from borosilicate glass filled with 3 M KCl (30-80 MΩ), and intracellular recordings made (using an AxoClamp-2B amplifier and National Instrument LabView interface) from submerged slices (31°C) . Measures of excitability included AHP peak amplitude, and duration, as well as measurements of AHP amplitude at varying intervals post-burst (to assess mAHP and sAHP components).
Insulin Perfusion: After baseline recordings, brain slices were perfused with the most effective dose of insulin (12.5 nM), as previously determined via dose response curve and recordings were repeated.

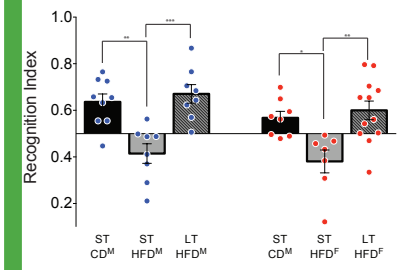


Figure 1: ST but not LT HFD Impairs Spatial Object Recognition Performance in both Males and Females. No sex- or diet-dependent differences were observed in total object exploration, total line crosses, centerline crosses, or time spent in the center (data not shown). However, HFD effects were reported in recognition index, the comparison of time exploring object in novel location to total time exploring objects, after ST exposure to diet in both males (CD^M vs. HFD^M; p = 0.0029) and females (CD^F vs. HFD^F; p = 0.050) Interestingly, after LT exposure to the HFD, spatial object recognition was fully recovered in both sexes (ST vs. LT HFD^M, p = 0.0008; ST vs. LT HFD^F, p = 0.0083).

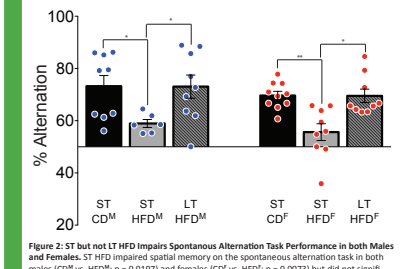


Figure 2: ST but not LT HFD Impairs Spontanous Alternation Task Performance in both Males and Females. ST HFD impaired spatial memory on the spontaneous alternation task in both males (CD^M vs. HFD^M; p = 0.0197) and females (CD^F vs. HFD^F; p = 0.0073) but did not significantly alter total exploration of the plus-maze (F(3, 30) = 1.319, p = 0.2866). Interestingly, after LT exposure to the HFD, spontaneous alternation task performance was fully recovered in both sexes (ST vs. LT HFD^M, p = 0.0455; ST vs. LT HFD^F, p = 0.0240).

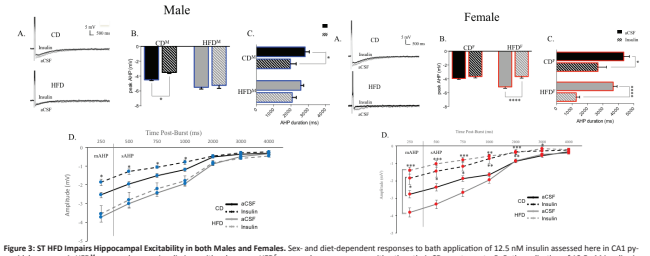


Figure 3: ST HFD Impairs Hippocampal Excitability in both Males and Females. Sex- and diet-dependent responses to bath application of 12.5 nM insulin assessed here in CA1 pyramidal neurons. A. HFD^M neurons became insulin insensitive, however HFD^F neurons became more sensitive than their CD counterparts. B. Bath application of 12.5 nM insulin significantly reduced peak AHP amplitudes in CD^M but not HFD^M neurons. C. AHP durations were significantly reduced in CD^M neurons, but not in HFD^M neurons. Again, HFD^F neurons retained insulin sensitivity and were, in fact, more insulin sensitive than CD^F neurons. D. While mAHP and sAHP measures were significantly reduced by 12.5 nM insulin in neurons from CD^M rats, both mAHPs and sAHPs from HFD^M rats were insulin-insensitive. The mAHPs and sAHPs from both CD^F and HFD^F rats were insulin sensitive and reduced by bath application of insulin* p < 0.05, ** p < 0.01, ***p < 0.001, ****p < 0.0001.

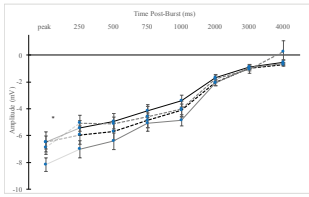


Figure 4: LT Diet Exposure Impairs Hippocampal Excitability and Insulin Sensitivityin Males. In general, AHPs were enhanced by LT exposure to both diets, likely due to the advancing age of the subjects. However, peak AHP was further enhanced by LT HFD exposure (p = 0.0192). Neither CD^M or HFD^M neurons retained neuronal insulin sensitivity.

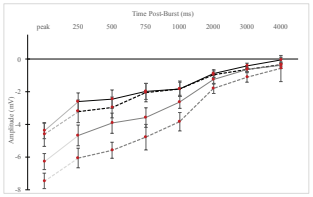


Figure 5: LT HFD Impairs Hippocampal Excitability in Females. Unlike in males, AHPs were not enhanced in LT cohorts, both ST CD^F and LT CD^F had comparable excitability measures. Additionally while CD neurons becale insulin insensitive, HFD neurons showed a reversal of insulin sensitivity compared to their ST counterparts, with insulin now reducing excitability rather than enhancing it. Showing enhancement in the HFD^F neurons was peak AHP (p = 0.0407) and the sAHP (500ms, p = 0.0189; 750ms, p = 0.0403; 100ms, p = 0.0250; and 3000ms, p = 0.0275).

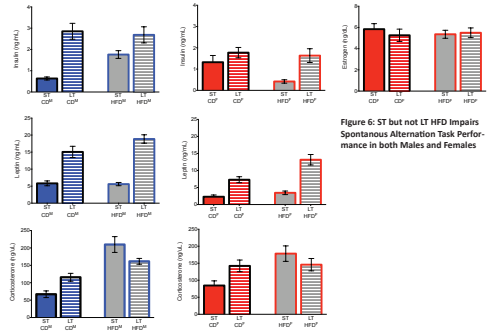


Figure 6: ST but not LT HFD Impairs Spontanous Alternation Task Performance in both Males and Females

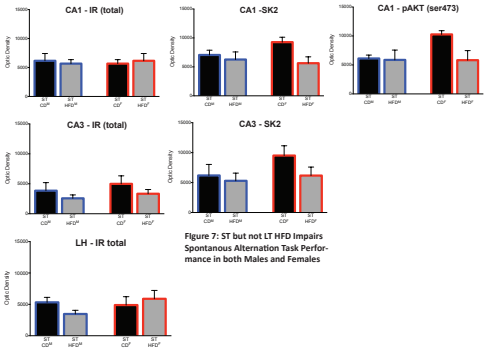


Figure 7: ST but not LT HFD Impairs Spontanous Alternation Task Performance in both Males and Females

Discussion

- **ST HFD impairs spatial memory** in both males & females, but this impairment is recovered after LT HFD exposure.
- After ST exposure, **circulating insulin** was significantly **increased** in HFD males BUT was significantly **decreased** in HFD fed females.
- **Cortisol** was significantly **elevated** in ST HFD males and females.
- After ST HFD, **intrinsic excitability** of male and female CA1 neurons was significantly **reduced**.
- **Insulin** perfusion **enhanced CA1 hippocampal excitability in** male and female **ST CD** fed rats.
- **Male HFD neurons** became **insulin-insensitive**
- **Female HFD neurons** NOT ONLY remained **insulin-sensitive**, they became **MORE insulin-sensitive** than female control neurons

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