High-Fat Diet Modulates Dopaminergic Network Activity: An Analysis of Functional Connectivity

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Introduction

Dopamine (DA) signaling modulates brain circuits mediating complex motivated behaviors [1], and its dysregulation underlies compulsive substance seeking and use that are hallmarks of addiction [2]. Neuroimaging studies suggest similar DAergic dysfunction in obese individuals [3], but the degree to which this predisposes to, or is a consequence of, obesity remains unclear. Recent studies have demonstrated that a high-fat (HF) diet induces insulin resistance in the central nervous system [4,5], and that insulin signaling regulates synaptic DA clearance by maintaining the dopamine transporter (DAT) in the plasma membrane [6-8], suggesting a novel mechanism by which HF diets may promote hyperphagia and obesity: diet-induced insulin resistance leads to impaired DA clearance in brain areas mediating reward (nucleus accumbens) and motivated behavior (dorsal striatum) with a consequent disruption in functionally connected circuits. In this study, we examine effects of HF diet on striatal insulin signaling and DAT activity, and modifications of functional connectivity between key DAergic targets and downstream areas.

Methods

MR studies were performed on a 4.7T scanner (Varian Inc., Palo Alto, CA) using a 20-mm surface coil with a multi-slice multi-echo gradient echo (R2*-weighted) sequence (TR = 210 ms; TE = 3, 8, 13, 18, 23, 28 ms; $\theta = 27°$; FOV = 35 x 35 mm^2; in-plane resolution = 273 x 273 μm^2; slice thickness = 2 mm (x 7 slices, coronal); NEX = 2; acquisition time = 1 min). Each scan consisted of a reference $R2^*_0$ map and 40 R2* maps post-MION (monocrystalline iron oxide nanoparticles; 7 mg iron/kg IV; BioPal, Worcester, MA) with AMPH (3 mg/kg IV; Sigma, St. Louis, MO) or saline (1 ml/kg IV) injected after a 10-map baseline ($R2^*_{0,\,post}$). Male Sprague-Dawley rats were scanned after 14 days of a low-fat (LF; 10% fat) or HF (60% fat) diet in LF-AMPH (N=5), HF-AMPH (N=5), LF-saline (N=3), and HF-saline (N=5) groups. Rats were tracheotomized, mechanically ventilated (30% O_2 : 70% N_2O; 0.88% isoflurane), and paralyzed (pancuronium bromide 2 mg/kg IP; Sigma, St. Louis, MO). Respiration, temperature, end-tidal CO_2, and heart rate were monitored.

R2* maps were generated by fitting the multi-gradient echo data to a mono-exponential decay on a voxel-wise basis. The R2* time series was used to calculate the time course of fractional CBV change following AMPH or saline. Fractional CBV changes to AMPH/saline were calculated as $\Delta CBV(t) / CBV_0 = (R2^*(t) - R2^*_{0,post}) / (R2^*_{0,post} - R2^*_0)$ [9]. The ΔCBV time courses were filtered using principal component analysis to exclude extraneous sources of variance (physiological noise, drift, motion) and retain the response to AMPH/saline injection. Group mean %CBV changes for the first 20 min following AMPH/saline vehicle were calculated for anatomically defined regions of interest (ROI) and analyzed by one-way ANOVA and Newman-Keuls post-hoc tests.

Inter-regional correlations in the amplitude of response to AMPH across animals were investigated [10] by computing the Pearson linear correlation coefficient between mean %CBV response for each ROI pair in the LF and HF groups. The correlation coefficients, r, were converted to z-scores ($z = \ln[(1+r) / (1-r)] / [2*(1 / (N-3))^{0.5}]$) and thresholded at a 2-sided 99% confidence interval ($|z| > 2.576$). The resulting correlation matrices are shown in Figs. 1A (LF) and 1B (HF). Permutation analysis [11] was used to identify the correlations that were different (Δz, $p < 0.05$) between LF and HF groups (Fig. 1C).

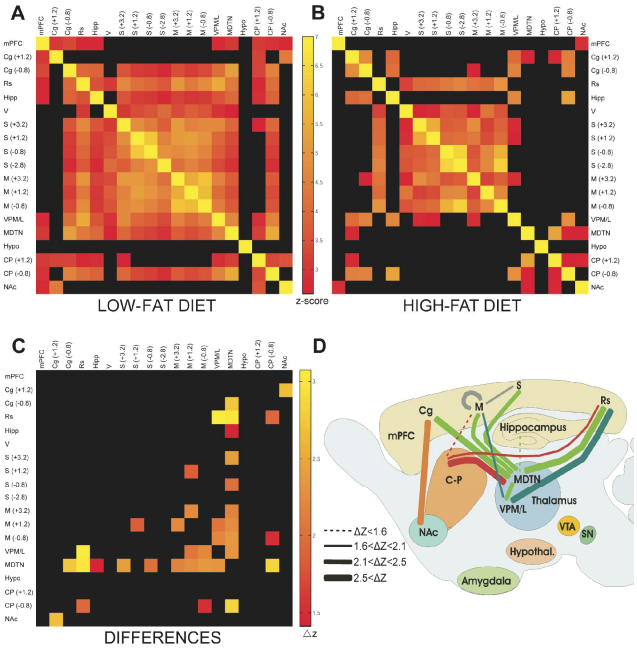

Fig. 1: Correlation analyses (as described in Methods) for the following regions in the rat brain (with Bregma in mm): medial prefrontal cortex (mPFC, includes PrL/IL, +3.2); cingulated cortex (Cg, +1.2 and -0.8); retrosplenial cortex (Rs, -2.8); visual cortex (V, -4.8); somatosensory cortex (S, +3.2, +1.2, -0.8, -2.8); motor cortex (M, +3.2, +1.2, -0.8); caudate-putamen (CP, +1.2, -0.8); hypothalamus (Hypo, -2.8); nucleus accumbens (NAc, Bregma +1.2); hippocampus (Hipp, -2.8); ventral posterior medial and lateral thalamus (VPM/L, -2.8, including ventral lateral, ventral medial, and posterior thalamic nuclear group); and medial dorsal thalamic nuclei (MDTN, -2.8, including lateral, medial, and central parts plus centromedial thalamic and paraventricular thalamic nuclei).

Results

In HF rats, AMPH-evoked CBV increases were significantly blunted in the caudate-putamen (11.5 ± 3.8% vs. 22.1 ± 3.4%, $p < 0.05$), somatosensory (70.4 ± 12.3% vs. 33.0 ± 5.1%, $p < 0.01$) and motor cortices (41.8 ± 6.1% vs. 21.0 ± 4.7%, $p < 0.05$), and ventral thalamus (VPM/L, 28.3 ± 6.0% vs. 13.3 ± 4.1%, $p < 0.05$). There was no significant CBV response to saline in either group. HF striatal synaptosomes showed a 44 ± 13% reduction in Akt activity compared to the LF group, indicating reduced insulin signaling.

Functional connectivity analyses revealed multiple inter-regional correlations in LF rats (Fig. 1A), reflecting striato-cortical (CP – sensorimotor; NAc – cingulum), thalamo-cortical (VPM/L, mediodorsal – sensorimotor), and striato-thalamic (CP – VPM/L, mediodorsal) circuitry. Fewer inter-regional correlations were found in HF rats (Fig. 1B). Significant differences ($p < 0.05$) between the groups were identified (Fig. 1C), including CP with motor ($\Delta z = 1.52$), retrosplenium (Rs) ($\Delta z = 1.94$), and mediodorsal thalamus (MDT) ($\Delta z = 2.85$); and NAc with cingulum ($\Delta z = 2.68$). A rat brain schematic (Fig. 1D) depicts the ROI pairs with significant changes (as per Fig. 1C).

Discussion

Our findings of reduced striatal Akt activity (a reporter of insulin signaling) and blunted AMPH-evoked activation observed in HF rats suggest that diet can profoundly influence striatal DA transmission by modulating insulin signaling, and also demonstrate that a HF diet weakens functional connections (e.g., NAc-Cg and CP-motor, thalamus) with disruption of brain networks subserving reward, motivation, and cognitive inhibition. The weakened NAc-Cg coupling in HF rats predicts an uncoupling of the mesolimbic pathway directly involved in reward and novelty with craving. The CP ("motor striatum"), another region expressing insulin receptors and DAT, showed tightly coupled responses with motor cortices, Rs, and MDTN, predicting changes in motor output. Extrastriatal areas that were significantly less correlated in HF compared to LF rats (e.g., VPM/L with Rs and motor cortices) may reflect blunted functional connectivity with DAT-rich areas. Differences between LF and HF groups suggest that changes in DA signaling induced by a HF diet lead to network level changes mediated, at least in part, by altered DAergic functional connectivity. A complex interplay between DAT regulation and impaired Akt signaling may lead to chronically blunted DAT activity, decreased DA clearance, and, ultimately, reduced DA signaling that changes network activity. Early intervention for curbing obesity is necessary as striatal insulin resistance and corresponding changes in DAergic neurotransmission appear early in the path to chronic obesity. Whether this is reversible is the topic of future studies.

Acknowledgments

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References

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