HHS Public Access

Reversal of dopamine system dysfunction in response to high fat diet

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Abstract

Objective—To test whether high fat diet (HFD) decreases dopaminergic tone in reward regions of the brain and evaluate whether these changes reverse after removal of the HFD.

Design and Methods—Male and female mice were fed a 60% HFD for 12 weeks. An additional group was evaluated 4 weeks after removal of the HFD. These groups were compared to control fed, age-matched controls. Sucrose and saccharin preference was measured along with mRNA expression of dopamine related genes by RT-qPCR. Dopamine and DOPAC were measured using high performance liquid chromatography. DNA methylation of the DAT promoter was measured by methylated DNA immmunoprecipitation and RT-qPCR.

Results—After chronic HFD, sucrose preference was reduced, and then normalized after removal of the HFD. Decreased expression of dopamine genes, decreased dopamine content and alterations in DAT promoter methylation was observed. Importantly, response to HFD and the persistence of changes depended on sex and brain region.

Conclusions—These data identify diminished dopamine tone after early-life chronic HFD with a complex pattern of reversal and persistence that varies by both sex and brain region. CNS changes that did not reverse after HFD withdrawal may contribute to the difficulty in maintaining weight-loss after diet intervention.

Keywords

Dopamine; High-Fat diet; DAT; sex differences; obesity; withdrawal; DNA methylation

Introduction

Overconsumption of widely available, calorie-dense palatable food is considered a major factor contributing to the high rates of obesity in the U.S (1). Because palatable foods are

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often consumed after energy requirements have been met, the rewarding properties of palatable foods may override homeostatic satiety signals. Many neurotransmitters play a role in feeding behavior (e.g. opioids, dopamine, GABA, serotonin) as well as the integration of peripheral nutrient signals (e.g., leptin, insulin, ghrelin). Dopamine signaling is a key mediator in both food reward and reward-seeking behavior, as dopamine in the mesolimbic/mesocortical region is associated with the rewarding properties of food, sex, and drugs of addiction (2). Acutely, palatable food causes a burst of dopamine in the central reward system (3,4). With chronic consumption of rewarding food, the increased dopamine release over time may lead to adaptations that are associated with reward hypo-function.

Several lines of evidence support the hypothesis of altered dopamine function in obesity. Human imaging studies revealed blunted activation in reward regions of obese patients while drinking a highly palatable solution (milkshake) (5). The blunted reward response was associated with less brain dopamine receptor D2 availability. In fact, mutations in the human dopamine D2 receptor have been linked with both obesity and addiction (6). Dopamine content in the synapse is largely controlled by dopamine transporter (DAT) uptake. Dopamine transporter levels are negatively correlated with body mass index and genetic variants of DAT are also associated with obesity (7,8). Animal models of obesity have demonstrated decreases in basal extracellular dopamine and reduced dopamine neurotransmission in the nucleus accumbens and ventral tegmental area (9,10,11). Decreases in dopamine-related genes after chronic high fat (HF) diet suggest decreased signaling in reward regions (12, 13,14,15). This decrease in dopamine activity after chronic high-fat diet may reduce the sensitivity to natural rewards and facilitate continued overconsumption and further weight gain.

Early life is a critical period in brain development, and the early nutritional environment can influence brain pathways controlling food intake and energy metabolism. Early exposure of mice to a high fat diet for as little as one week altered adult caloric intake and expression of dopamine-related signaling molecules (16). Further, early postnatal over nutrition in mice, driven by a small litter number throughout lactation, predisposes the offspring to adulthood obesity by altering hypothalamic development (17). While it is clear that early life nutrition can affect brain development and obesity risk, little is known about the relative permanence of these changes across the lifespan. Additionally, previous studies have been done in male animals but females have been rarely studied in this context. To these ends, both male and female mice were studied for changes in gene expression and dopamine metabolism after they were made obese in early life through chronic consumption of a HF diet from birth through 8 weeks of age. The dopamine system was also evaluated 4 weeks after removal of the HF diet, to examine whether the changes persisted or reversed.

Methods and Procedures

Animals and experimental model

C57BL/6J females were bred with DBA/2J males (The Jackson Laboratory, Bar Harbor, ME). All dams were fed standard control diet (#5755; 18.5% protein, 12% fat, 69.5% carbohydrate) up until parturition when half the dams/litters were placed on high fat diet (Test Diet, Richmond, IN #58G9; 18% protein, 60% fat, and 20.5% carbohydrate).

Offspring were weaned at 3 weeks of age and remained on either the control diet or the high fat diet until 12 weeks of age. Body weights were recorded weekly, and both male (n=5–10) and female (n=5–10) mice were used. The Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania approved all procedures.

Sucrose and Saccharin Preference

In separate experiments, mice were individually housed (n=8–10/group) in standard cages for 3 days with one bottle of 200 ml of the test solution (4% sucrose or 1% saccharin solution (w/v)) and another bottle with 200 ml of tap water. House chow was available *ad libitum*. Sucrose (ml), water (ml), and food consumption (g), were measured and the placement of the bottles was reversed daily. Preference was calculated using the average of the measurements from the last 2 days as follows: preference % = [(sucrose consumption/ sucrose + water consumption) × 100].

Genomic DNA and Total RNA isolation from the brain

Animals (n=5/group) were euthanized with an overdose of carbon dioxide, followed by cervical dislocation; a method recommended by the Panel on Euthanasia of the American Veterinary Medical Association. Brains were then rapidly removed and placed in RNAlater (Ambion, Austin, TX) for 4–6 hours before dissection. Brain dissections to isolate the prefrontal cortex, the nucleus accumbens and the ventral tegmental area were preformed as previously described (18,19, 20). Genomic DNA and total RNA were isolated simultaneously using AllPrep DNA/RNA Mini Kit (Qiagen).

Gene expression analysis by quantitative Real-Time PCR

For each individual sample, 500ng of total RNA was used in reverse transcription using High Capacity Reverse Transcription Kit (ABI, Foster City, CA). Expression of target genes was determined by quantitative RT-PCR using gene specific Taqman Probes with Taqman gene expression Master Mix (ABI) on the ABI7900HT Real-Time PCR Cycler. Gene probes are listed in supplemental material. Relative amount of each transcript was determined using delta CT values as previously described in (21). Changes in gene expression were calculated against an unchanged GAPDH standard.

Ex vivo Dopamine and Dopamine Metabolites

High performance liquid chromatography (HPLC) was used to measure the content of dopamine and its metabolites in the mesolimbic reward areas of the brain (n=8–12), as described previously (22,18). Brains were collected from animals and bisected into right and left hemispheres. The NAc and PFC were dissected out and quickly frozen by dry ice and stored at −80°C. The tissue was prepared for analysis by homogenization in 0.1 N perchloric acid, centrifuged at 15,000 rpm for 15 min at 2–8°C, and the supernatant filtered. Samples were analyzed by a Bioanalytical Systems HPLC (West Lafayette, IN, USA) using a LC-4C electrochemical detector. Samples (12 ul) were injected onto a reverse phase microbore column at a flow rate of 0.6 ml/min and electrodetection set at +0.6 V. Separation for dopamine and dopamine metabolites was accomplished by a mobile phase consisting of 90-mM sodium acetate, 35-mM citric acid, 0.34-mM ethylenediamine tetraacetic acid, 1.2-mM

sodium octyl sulfate, and 15% methanol v/v at a pH of 4.2. Peak heights of samples were measured and compared with standards for dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC).

Methylated DNA Immunoprecipitation (MeDIP) Assay

MeDIP assay was preformed using MagMeDIP kit (Diagenode, Denville, NJ). Methylated DNA was immunoprecipitated using 0.15ul of magnetic beads coated with anti-5methylcytidine antibody (Diagenode) or mouse pre-immune serum. Enrichment in MeDIP fraction was determined by quantitative RT-PCR using ChIP-qPCR Assay Master Mix (SuperArray) on the ABI7900HT Real-Time Cycler. For all genes examined, primers were obtained from SuperArray (ChIP-qPCR Assays (−01) kb tile, SuperArray) for the amplification of genomic regions spanning the CpG sites located approximately 300–500 bp upstream of the transcription start sites. MeDIP results were expressed as fold enrichment of immunoprecipitated DNA for each site. To calculate differential occupancy fold change (% enrichment), the MeDIP DNA fraction CT values were normalized to Input DNA fraction CT values.

Statistics

Gene expression analysis was performed using Student T-test comparing aged matched controls to HF and HF + recovery groups. The alpha level was adjusted for the multiple brain regions surveyed. Significance of a gene used in one brain region was p=.05; for two regions, p=0.025, for 3 brain regions p=.016. Sucrose preference, saccharin preference, HPLC and MEDIP, body weights and corticosterone assay analyzed using one-way ANOVA to compare control, HF, and HF + recovery groups. Post-hoc Bonferonni Multiple Comparison Tests were used to compare pair-wise differences between groups. Significance for these tests was set at an alpha level of p=.05.

Results

Mice had continuous access to control diet (control) or 60% high fat diet (HFD) until 12 weeks of age. At 12 weeks of age, half of the HF-fed animals were placed on the house chow for 4 weeks (HF +recovery). In both males and females, HFD animals (circles) were heavier than controls beginning at 9 weeks of age (p<.05) and remained heavier than controls throughout the recovery period (Supplemental Figure 1).

Sucrose and saccharin preference tests were administered to assess animals' response to natural and nonnutritive rewarding stimuli. Sucrose preference but not saccharin preference was altered after HF diet exposure and returned to normal levels after HFD recovery in males and females. One-way ANOVA revealed sucrose preference was significantly decreased in males (Fig. 1A) and trended toward a decrease in females (Fig. 1B) after HFD exposure (F(2,16)=4.82, p<.05; F(2,16)=5.41, p<.06, respectively). After removal of the HFD, this behavior normalized and sucrose preference no longer differed from controls. Saccharin preference was not altered in either males (Fig. 1C) or females (Fig. 1D) as a result of HFD exposure.

Because dopamine is a key regulator of reward behavior, dopamine-related gene expression was examined within the reward circuitry of a separate cohort of males and females after 12 weeks on the HFD, and in an additional group, after 4 weeks recovery from the HFD. Table 1 summarizes the gene expression patterns and statistical analysis in the VTA, PFC and NAc. In the VTA, three genes important in regulating dopamine levels at synaptic terminals were measured: catecholamine methyl transferase (COMT) involved in inactivation of catecholamine neurotransmitters; dopamine transporter (DAT), membrane spanning pump that clears dopamine from the synapse, and tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine synthesis. Fold change values for each group were determined using aged matched controls (e.g. both control time points are set to 1, and for clarity only the control for HFD is depicted on the graph). Student's t-test (n=5/group) revealed in the male VTA that COMT, DAT, and TH mRNA were significantly decreased by HFD exposure (Fig 2A) and returned to or exceeded the control levels after a recovery period off the diet (HF +recovery).

In the PFC and NAC, genes important for dopamine signaling and dopamine turnover were examined (n=5/group): COMT; protein phosphatase 1 regulatory subunit 1B (DARPP-32), a down stream signaling protein regulated by receptor stimulation; dopamine receptor D1 (DRD1), a postsynaptic G-protein coupled receptor that stimulates adenylyl cyclase; and dopamine receptor D2 (DRD2), a postsynaptic G-protein coupled receptor that inhibits adenylyl cyclase. In the male PFC (Fig. 2B), DARPP-32 was increased, while DRD1, and DRD2 were decreased after HFD exposure, and these changes persisted after the removal of the HFD (although the increase in DARPP-32 mRNA was not statistically reliable). In the male NAC (Fig. 2C), COMT, DRD1, and DRD2 were decreased by HFD exposure, and remained below control levels after removal of the HFD. DARPP-32 levels were increased by HFD, but significantly decreased from controls after 4 weeks off the HFD.

The same brain regions and genes were examined in female mice (n=5/group). As shown in Table 2, there were significant differences observed in the pattern of gene expression in response to the HFD, as well as to the recovery off the diet. Similar to males, in the VTA, mRNA levels of COMT, and TH were significantly decreased after HFD exposure (Fig 2D). However, unlike the males, these changes persisted after the removal of the HFD. Further, in direct opposition to the pattern observed in males, HFD exposure increased DAT mRNA expression in the VTA in females, and after removal of the HFD levels were even lower than age matched controls. In the PFC, only DARPP-32 was affected by chronic HFD, with a significant increase in mRNA levels after 12 week HFD, and a return to control levels after removal of the HFD. Both COMT and D1R mRNA were significantly decreased after 4 weeks off the HFD. In the female NAC, COMT, DRD1, and DRD2 were all decreased after HFD exposure (Fig. 2F). DRD1 and DRD2 recovered to control levels after diet removal, while COMT remained levels remained significantly decreased after 4wk recovery.

Given the consistent decrease in gene expression for dopamine regulating genes in the VTA, dopamine and dopamine metabolites were quantified in regions that receive projections from the VTA, the PFC and NAC. Figure 3 shows dopamine (DA) and the dopamine metabolite (DOPAC) from the PFC and NAC in males (Fig. 3A, 3C) and females (Fig. 3B, 3D). In males, exposure to the HFD produced a decrease in dopamine levels in PFC (Fig. 3A) and

NAC (Fig 3C) (F(2,13)=3.95; F(2,18)=3.536, p<.05), which recovered after HFD removal only in the NAC. Dopamine turnover (DOPAC:DA ratio) increased in male PFC (F(2,12)=3.85, p<.05) and NAC (F(2,17)=4.69, p<.05). In contrast, the effect of HFD on DA and DOPAC in females was qualitatively different than in males. In the PFC, HFD did not affect DA or DOPAC levels. In the NAc, DA levels were decreased in HFD-fed animals and remained decreased even after removal of the HFD (Fig. 3D, F(2,23)=4.79, p<.05). DOPAC levels were unchanged in the NAc of females, which resulted in an increase in DA turnover (DOPAC:DA ratio) (F(2,23)=7.00, p<.01).

Given that DAT transcription can be regulated by differential DNA methylation and the observation of a notable sex difference in the expression of DAT in the VTA, DNA methylation in the promoter region of DAT was examined. In Figure 4A, 4C DAT gene expression in the VTA is presented again for clarity (taken from Fig 2A and 2D). DAT promoter methylation was significantly increased in males (Fig. 4B) after HFD and returned to control levels in HFD + recovery males (F(2,11)=23.64, p<.01). In females, DAT promoter methylation trended toward decrease in HFD animals (D) and was significantly decreased in HFD + recovery females (Fig 5D, F(2,12)=5.70, p<.05).

To assess if removal of the HFD in the recovery period was a stressor, baseline plasma corticosterone levels (ug/dl) were taken in control, HFD exposed (12 weeks), HFD +1wk recovery and HFD + 4wk recovery groups (n=5/group, Supplemental Fig. 2). One-way ANOVA revealed no significant differences between groups in male animals (F(3,16)=3.21, n.s.).

Discussion

Chronic consumption of a high fat diet (HFD) beginning in early life was used to establish diet-induced obesity in mice. Mice displayed decreased sucrose preference and evidence of reduced dopaminergic tone in reward regions of the brain. After 4 weeks off the HFD, sucrose preference normalized in both males and females, however, some dopamine gene expression changes persisted. These experiments provide important new data describing the effect of chronic HFD on the brain reward system, highlighting the capacity for recovery and key sex differences between male and female mice.

In the HFD fed animals, a decreased sucrose preference was observed, which reversed after a recovery period. These findings extend our previous report of HFD intake driving a reduced sucrose preference (23) by demonstrating that this can occur with a shorter duration of HFD exposure (12 weeks versus 22 weeks), and importantly, that the response recovers in the absence of HFD. Female mice demonstrated the same response patterns as males. These findings are consistent with others in the literature which have shown through the inclusion of a pair fed group that chronic HFD, and not obesity per se, attenuates the response for sucrose in an operant task (24). Similarly, in the current study, sucrose preference recovered after 4 weeks off the HFD, while body weight remained significantly elevated, supporting the conclusion that decreased sucrose preference was driven by the HFD exposure and not the accompanying body weight gain. It was particularly interesting that there was no change in saccharin preference. This may indicate that chronic HFD differentially affects the

response to caloric and non-caloric sweet rewards. Post ingestive effects have been shown to influence preference independent of palatability, as sucrose intake has been shown to induce dopamine release in "sweet-blind" taste receptor knockout mice (25), nutritional value is required for reward and reinforcement (26) and taste-independent metabolic sensing pathways have been defined in drosophila (27). Saccharin is significantly sweeter than sucrose, so an effort was made to establish equivalency in sweetness (typically 4–10x higher concentration of sucrose (26)) however the overall preference for saccharin was lower than that for sucrose in these animals. Therefore, an alternative explanation may be that HFD differentially affected the sucrose preference because it was relatively more rewarding than saccharin (high vs. low value reward), although animals still evinced a strong preference for saccharin (~75–80% prefence for saccharin compared to ~85–90% preference for sucrose).

Overall, dopaminergic gene expression within the VTA, NAc and PFC was decreased in male mice following chronic HFD. These findings are consistent with other studies that observed decreases in dopamine related genes in response to HFD (12,13,15). Decreases in dopamine D2 receptor expression and function have been observed in human imaging studies (5, 28) and rodent obesity models (14, 29). Decreased dopamine signaling reduces the sensitivity to natural rewards and may therefore facilitate continued overconsumption of palatable foods and further weight gain (30,31). Further, disrupted dopamine homeostasis driven through decreased DAT surface expression is known to drive increased intake of high fat diet (32). An exception to this pattern was seen with DARPP-32, a dopamine- and cyclic AMP-regulated phosphoprotein, which increased after HFD in NAc and PFC. DARPP-32 plays a key role in integrating a variety of biochemical and behavioral responses controlled by dopamine. It may be that DARPP-32 upregulation was compensatory in response to the chronic down regulation of D1R. In a similar model (12 wk HFD in mice), it has been shown that D1R down regulation was matched by an increase in phosphorylation of DARPP-32 in NAc (33).

Few studies have examined the capacity for recovery of these changes after removal of the HFD. However, in two recent reports, gene expression changes and reward system defects persisted after a short withdrawal period (14–18d) (13, 14). In contrast, studies in obese patients before and after gastric bypass surgery have shown a reversal of dopaminergic changes after a longer period of weight loss (34). In males, the pattern of recovery varied by brain region. In the VTA, the observed decreases in COMT, DAT, and TH were all normalized with the removal of the HFD. In contrast, all gene expression changes observed in the NAc and PFC did not normalize. In the current study, chronic HFD led to significant weight gain and after 4 weeks off the diet, animals were still significantly heavier than controls. Therefore, the subsequent metabolic and hormonal changes that accompany obesity (e.g., increased leptin, elevated adipokines) were likely still present at 4 weeks off the diet. Therefore, gene expression changes that normalized (e.g., in the VTA) may have been primarily driven by the HFD, while those that were maintained (in NAc and PFC) may be more tightly coupled to obesity. Maintenance of weight loss by dieting is characteristically low (with 67% (35) to 80% (36) of patients regaining the lost weight). This persistence of gene expression changes in reward regions could be important in partly explaining this common occurrence. It is also important to note that the observed behavioral and gene expression changes are not likely to be due to stress associated with changing

diets, as there were no significant changes in basal plasma corticosterone levels on the HFD or after 1wk or 4wk recovery.

Interesting sex differences were revealed, both in the response to chronic HFD, as well as in response to diet removal. Females were similar to males in showing an overall decrease in dopamine related genes that would predict a decrease in DA activity, particularly in the VTA and the NAc. One noteworthy sex difference was the increase in DAT mRNA expression in the female VTA after HFD. This difference in gene expression, coupled with similar decreases in TH gene expression in both sexes, would suggest significant differences in dopamine neurotransmission within the NAc, both at the end of the HFD exposure as well as after the recovery period. A greater appreciation for the functional significance of these differences is an important focus of future research.

Additionally, while COMT and TH decreases recovered in the male VTA, these decreases persisted in the females after 4-week off the HFD. It is yet to be determined whether these differences would reverse with a longer time off the diet, however, it supports the conclusion that females are, at the very least slower to recover, if they recover at all. Further, gene expression changes of D1R and D2R in NAc and PFC were quite different between males and females. In males, there was a general decrease in gene expression in both regions that largely persisted after diet removal. In females, D1R and D2R were decreased in the NAc and then recovered, but there was no effect of HFD on dopamine receptors in PFC. In the current studies, female animals were sacrificed without accounting for estrus stage. While some of the observed endpoints are known to vary across the estrus cycle, female animals in this study did not demonstrate increased variance across the endpoints, particularly when compared to the effect of the diet manipulations.

To complement the gene expression findings, dopamine was measured in the primary projection regions of the VTA, namely the PFC and the NAc. Dopamine levels tended to parallel changes seen in TH mRNA in the VTA. In the NAc of both males and females, levels of DA decreased in response to the HFD diet; a response which recovered in males, but not females. In the PFC, dopamine levels were also decreased by HFD, however, there was no recovery off the diet in the PFC. Additionally, females had lower levels of dopamine in the prefrontal cortex than males. Sex differences in DAT expression and function are well known in the literature, with females demonstrating increased DAT expression (37) and function (38), and these differences may contribute to the different baseline levels of dopamine between males and females. Examination of the DOPAC:DA ratio is informative as well. An increase in this ratio may have reflected a compensatory response driven by decreases in DA. The long-term functional significance of these changes in dopamine metabolism would be illuminated by measuring changes in dopamine release using *in vivo* microdialysis.

Moreover, these data identify dynamic regulation of DNA methylation within the promoter of the DAT gene, particularly in the males. Recently, we have demonstrated that DAT expression can be dynamically regulated by differential DNA methylation in response to HFD (12), and that increased DAT promoter methylation correlates with a decrease in gene expression. Here we identify the plasticity of this response, as the increased DNA

methylation (and decreased expression of the mRNA) seen in males reverses upon removal of the HFD. Epigenetic gene regulation, for example through changes in DNA methylation, presents a pathway whereby organisms can readily adapt to environmental challenges. Epigenetic marks can be maintained across the lifespan (39), and in cultured embryonic stem cells, both reversible and persistent patterns of differential DNA methylation were observed in response to changing environmental conditions (40). These data are the first to demonstrate *in vivo* a dynamic methylation pattern that changes with the presence or absence of an environmental challenge. It was notable that this same pattern was not observed in females. While the initial response to the HFD was as predicted (decreased DNA methylation driving increased gene expression), this pattern was not maintained throughout the recovery period. This suggests that DNA methylation and gene expression may become uncoupled during the four weeks off the HFD or it may suggest that DAT mRNA is regulated by other means in females.

In males, sucrose preference, DA-related gene expression in the VTA, and dopamine in the NAc follow a consistent pattern, of suppression in response to the chronic HFD that recovers after removal of the diet. Interestingly, while the behavioral responses to sucrose are similar in the females, both the gene expression pattern and NAc dopamine levels show a lack of recovery upon removal of the HFD. Reward-related behaviors are clearly influenced by additional neurotransmitter systems such as the opioids, and perhaps in females, the behavioral response to sucrose is more strongly associated with changes in opioids. Overall, the present data suggest that sex differences in the both the initial response to HFD, as well as to recovery after removal of the HFD, with regard to dopamine-related gene expression represent an important direction for future research directed at how chronic consumption of a HFD impacts the brain reward system. Most notably, these data identify significant plasticity in the dopaminergic response to HFD, suggesting that while the adverse effects of chronic HFD consumption and/or obesity are significant, the potential for recovery exists.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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What is already known about this subject

- Dopamine receptor expression and function are decreased in obese patients

- Chronic exposure to high-fat diet causes changes in dopamine related genes and reward behavior

- Dopamine neurotransmission is altered in obese rodents.

What this manuscript adds to subject

- Identification of sex differences in the CNS response to high fat diet.

- Evaluation of the plasticity of dopaminergic changes upon removal of the high fat diet.

- Identification of dynamic DNA methylation changes in response to high fat diet
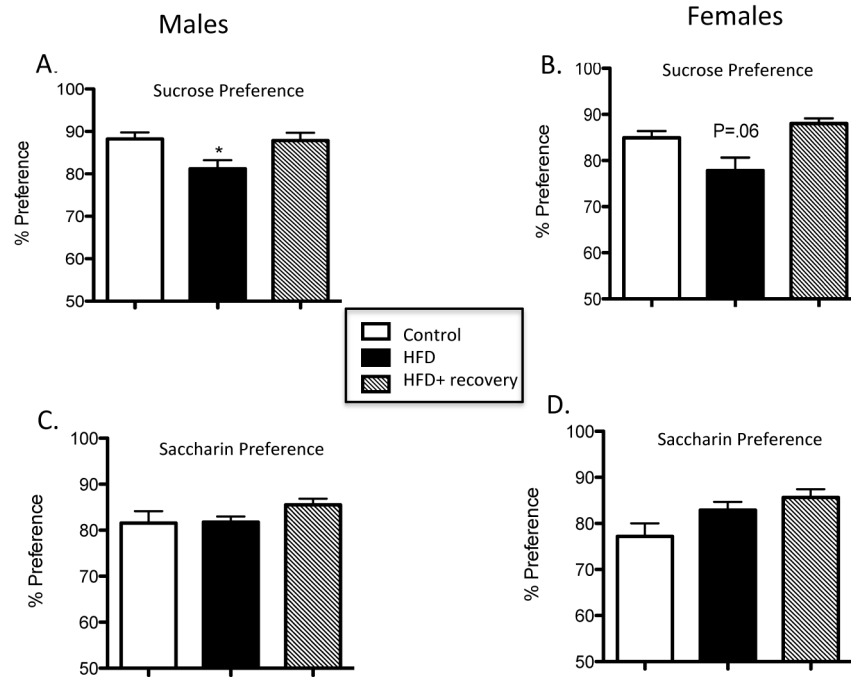
Figure 1. Sucrose preference but not saccharin preference is altered after high-fat diet (HFD) exposure and returns to control levels after HFD recovery in males and females

Sucrose preference (A,B) and saccharin preference (C,D) were evaluated in counter balanced order of control (white bars) HFD (black bars) and HFD + recovery (patterned bars) males and females. (n=8/group) *p<.05 significantly different from control group.
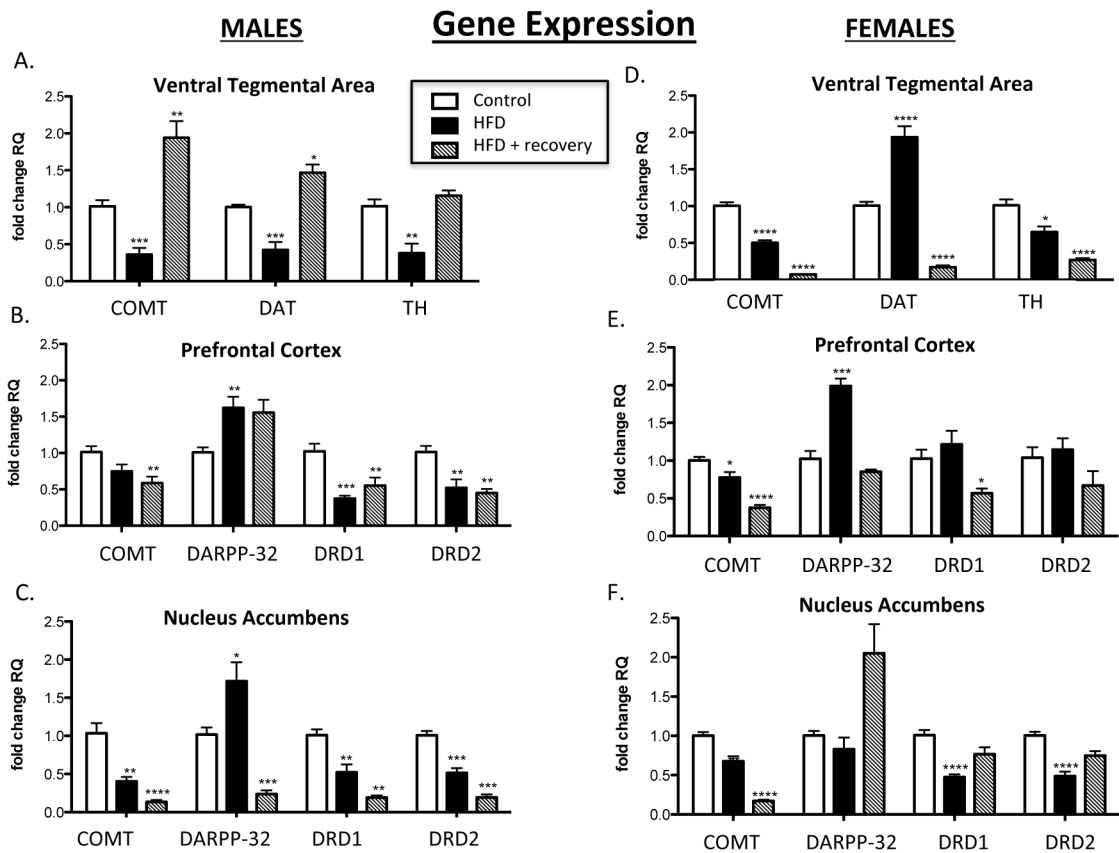
Figure 2. Chronic high-fat diet (HFD) and recovery after HFD alters dopamine related gene expression in males and females

Gene expression was measured in the ventral tegmental area (VTA; A,D.), prefrontal cortex (PFC; B,E), and nucleus accumbens (NAc; C,F) of control (white bars), HFD (black bars) and HFD + recovery (patterned bars) males and female mice (n=5/group). HFD and HF + recovery groups are fold change from their own aged matched controls. Only one control group (12wk) is shown and set to 1. *p<.05, **p<.01, ***p<.001, ****p<.0001 significantly different from own control group.
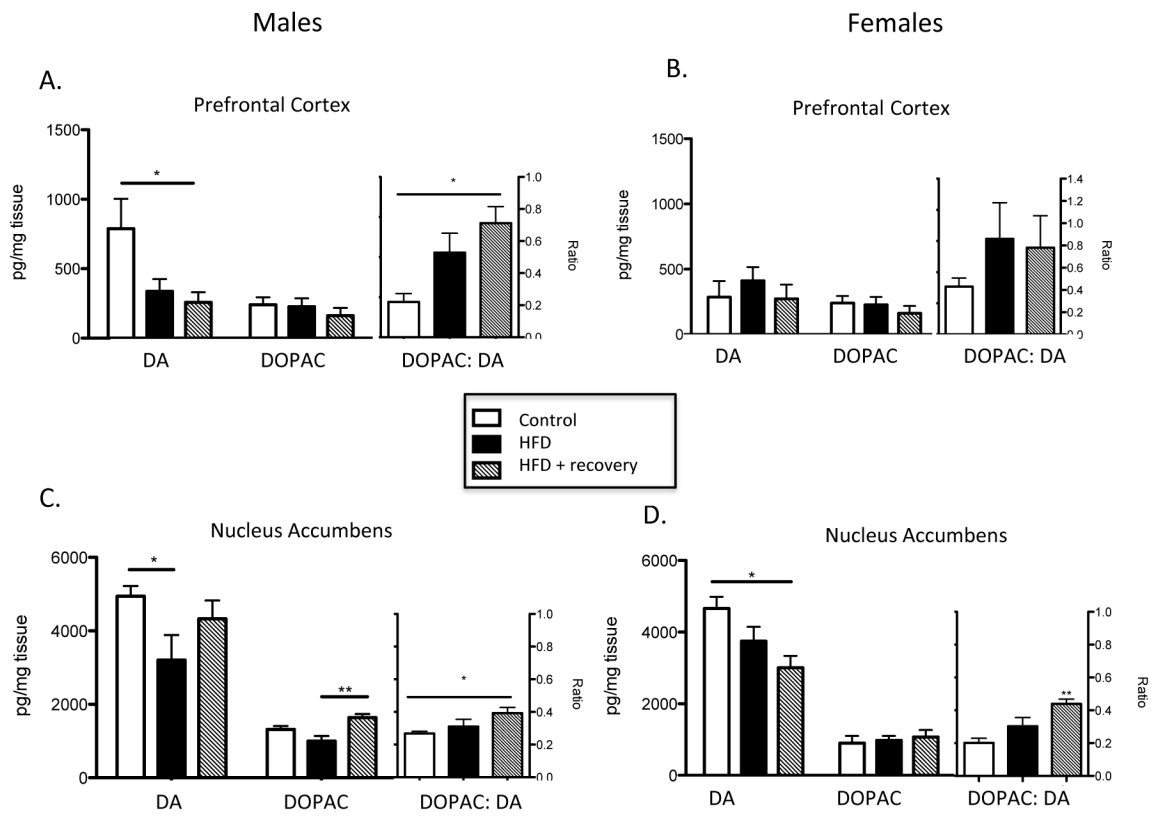
Figure 3. Decrease in Dopamine levels in PFC and NAC after HFD from birth and mixed recovery after HFD removal

Dopamine and dopamine metabolites were measured in the PFC (A,B) and NAC (C,D) of control (white bars), HF from birth (black bars) and HF + recovery (patterned bars). (n=8–10/group) *p<.05, **p<.01 significantly different from control group.
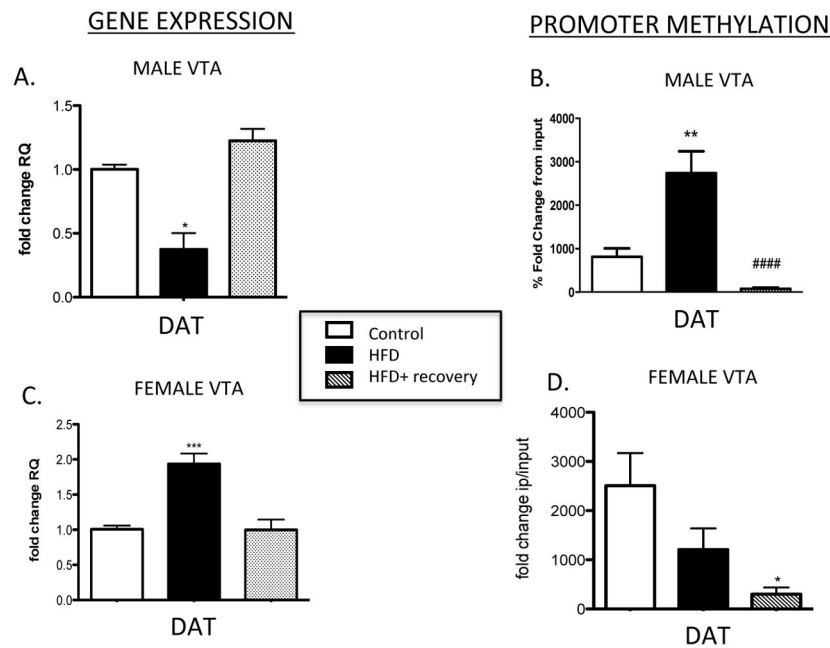
Figure 4. Changes in DNA methylation status of DAT promoter parallel changes in gene expression in the VTA

Genomic DNA was isolated from the dissected VTA of control (white bars), HF from birth (black bars) and HF + recovery (patterned bars). DNA was digested by restriction enzyme MSE1 and immunoprecipitated by a 5-methylcytosine antibody. The enrichment of DNA methylation relative to input DNA in the promoter region of DAT was quantified by qPCR (B, D). DAT mRNA levels, (A, C; previously presented in Fig 1) are shown for comparison (n=5/group). *p<.05, **p<.01, ***p<.001 significantly different from control group.

Table 1

Gene Expression Summary and Statistics in Males

Gene expression results for male ventral tegmental area (VTA), prefrontal cortex (PFC), and nucleus accumbens. Summary of fold change, significance and p values are presented for HF and HF +recovery groups as compared to their age matched control.

VTA	MALES			
GENE	**12wks HFD (Fold Change)**	**P value**	**4wks recovery (Fold Change)**	**P value**
COMT	< Control (.36)	.0007	>Control (1.93)	.0036
DAT	< Control (.42)	.0008	> Control (1.46)	.0132
TH	< Control (.38)	.0037	=Control (1.15)	.1454
PFC				
COMT	=Control (.74)	.066	< Control (.58)	.004
DARPP-32	> Control (1.62)	.0064	> Control (1.55)	.03
DRD1	< Control (.37)	.0004	< Control (.55)	.0052
DRD2	< Control (.52)	.0096	< Control (.45)	.0042
NAC				
COMT	< Control (.40)	.0026	< Control (.13)	<.0001
DARPP-32	> Control (2.03)	.023	< Control (.23)	.0005
DRD1	< Control (.52)	.0053	< Control (.18)	.0014
DRD2	< Control (.51)	.0004	< Control (.19)	.009

Table 2
Gene Expression Summary and Statistics in Females

Gene expression results for male ventral tegmental area (VTA), prefrontal cortex (PFC), and nucleus accumbens. Summary of fold change, significance and p values are presented for HF and HF +recovery groups as compared to their age matched control.

VTA	FEMALES			
GENE	**12wks HFD (Fold Change)**	**P value**	**4wks recovery**	**P value**
COMT	< Control (.50)	<.0001	< Control (.07)	< .0001
DAT	> Control (1.93)	.0004	< Control (.17)	< .0001
TH	< Control (.64)	.01	< Control (.27)	< .0001
PFC				
COMT	< Control (.77)	.028	< Control (.37)	< .0001
DARPP-32	>Control (1.98)	.0002	= Control (.85)	.2113
DRD1	= Control (1.21)	.398	< Control (.56)	.0214
DRD2	= Control (1.14)	.6225	= Control (.67)	.1566
NAC				
COMT	< Control (.67)	.0027	< Control (.17)	< .0001
DARPP-32	= Control (.83)	.296	> Control (2.05)	.052
DRD1	< Control (.47)	< .0001	= Control (.76)	.1861
DRD2	< Control (.48)	< .0001	= Control (.74)	.063