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ΔFosB-mediated alterations in dopamine signaling are normalized by a palatable high fat diet

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Abstract

Background—Sensitivity to reward has been implicated as a predisposing factor for behaviors related to drug abuse as well as overeating. However, the underlying mechanisms contributing to reward sensitivity are unknown. We hypothesized that a dysregulation in dopamine signaling might be an underlying cause of heightened reward sensitivity whereby rewarding stimuli could act to normalize the system.

Methods—We used a genetic mouse model of increased reward sensitivity, the ΔFosB-overexpressing mouse, to examine reward pathway changes in response to a palatable high fat diet. Markers of reward signaling in these mice were examined both basally and following six weeks of palatable diet exposure. Mice were examined in a behavioral test following high fat diet withdrawal to assess the vulnerability of this model to removal of rewarding stimuli.

Results—Our results demonstrate altered reward pathway activation along the nucleus accumbens-hypothalamic-ventral tegmental area circuitry resulting from overexpression of ΔFosB in the nucleus accumbens and striatal regions. Levels of phospho-CREB, BDNF, and DARPP-32 in the nucleus accumbens were reduced in ΔFosB mice, suggestive of reduced dopamine signaling. Six weeks of high fat diet exposure completely ameliorated these differences, revealing the potent rewarding capacity of a palatable diet. ΔFosB mice also showed a significant increase in locomotor activity and anxiety-related responses 24 hrs following high fat withdrawal.

Conclusions—These results establish an underlying sensitivity to changes in reward related to dysregulation of ΔFosB and dopamine signaling that can be normalized with palatable diets and may be a predisposing phenotype in some forms of obesity.

Keywords

obesity; overweight; diet; reward; dopamine; stress

Introduction

Despite our increasing knowledge of the neural systems that control appetite and satiety, rates of obesity continue to rise in the United States. Current drug treatments have limited efficacy, and behavioral modifications suffer from minimal long-term compliance (1). The consumption of calorically-dense, palatable foods has been linked to changes in stress and reward pathways

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in the brain, suggesting that the rewarding properties of such foods may override energy balance signals (2-4). Foods high in fat act as natural rewards, activating brain reward centers in a manner similar to drugs of abuse, and as such have been used in self-administration paradigms (5-8). Thus, it is likely that behaviors and motivation for overeating and drug abuse share common underlying mechanisms, potentially opening up new avenues of treatment for both conditions.

In studying the relationship between palatable foods and pathways regulating reward and stress in the brain, we have previously identified molecular and biochemical markers of reduced reward and increased stress following withdrawal from a palatable high fat diet (HF). Similar to drugs of abuse, exposure to a palatable diet in our studies resulted in increased levels of the transcription factor Δ FosB in the nucleus accumbens (NAc), a central brain reward structure (9,10). Mice that inducibly overexpress Δ FosB show increased instrumental responding for a food reward (11), making them a valuable tool for examining the role of reward sensitivity and long-term dysregulation of the reward system in the molecular and biochemical responses to a palatable diet.

In the present study, we utilized the Δ FosB-overexpressing mice to examine long-term alterations in markers of reward in the NAc-hypothalamus-ventral tegmental area (VTA) neurocircuitry in response to a palatable HF diet. Based upon previous studies in these reward-sensitive mice, we hypothesized that Δ FosB-induced changes in reward sensitivity involve a dysregulation in dopamine signaling resulting from NAc feedback to the VTA. Further, we hypothesized that exposure to a natural reward of an energy-dense HF diet would then normalize the dopaminergic system in these mice, resulting in an exaggerated response to the stress of withdrawal from this HF diet. The unique aspect of utilizing a palatable diet as a rewarding substance allows us to include the hypothalamic inputs to reward circuitry in a phenotype that may be predictive of a population predisposed to treatment-resistant obesity. To examine this hypothesis, we studied markers of dopamine neurotransmission, including pCREB, BDNF, and DARPP-32 in the NAc and tyrosine hydroxylase and the dopamine transporter in the VTA, following HF exposure. We also examined specific markers of energy balance known to influence dopamine output, including leptin and orexin receptors in the VTA and orexin expression within the lateral hypothalamus.

Materials and Methods

Animals

Male bitransgenic mice that inducibly overexpress Δ FosB in dynorphin-positive neurons in the NAc and dorsal striatum (Kelz et al., 1999) were generated on a mixed background (ICR:C57Bl6/SJL) at The University of Texas Southwestern Medical Center and maintained and tested at the University of Pennsylvania. All mice were maintained on doxycycline (100 μ g/ml in the drinking water) until arrival at the University of Pennsylvania. To induce overexpression, doxycycline was removed (n = 23)(12). Control mice (n = 26) continued to receive the drug. Mice were assigned to diet groups eight weeks following the removal of doxycycline at which time expression has been shown to reach maximal levels (13). Mice were maintained on a 12:12 light-dark cycle (lights on 0700) with food and water available ad libitum. All studies were performed according to experimental protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee, and all procedures were conducted in accordance with institutional guidelines.

Diet exposure

Mice were maintained on house chow (n=16) or placed on HF (n = 16-17) for six weeks. House chow (Purina Lab Diet, St. Louis, MO) contained 4.00 kcal/g, consisting of 28% protein, 12%

fat, and 60% carbohydrate. HF diet (Research Diets, New Brunswick, NJ) contained 4.73 kcal/g, consisting of 20% protein, 45% fat, and 35% carbohydrate.

Biochemistry and gene expression

Mice were analyzed after six weeks of diet exposure. Brains were removed from the skull and either frozen whole on dry ice or the NAc dissected (approximately 0.5 – 1.75 mm from bregma, at a depth of 3.5 – 5.5 mm) and frozen in liquid nitrogen. Tissue was stored at -80°C until assayed.

Biochemical analyses—Methods for Western blots are described in supplemental materials. The antibodies used were: Cdk5, CREB, and BDNF (1:500, Santa Cruz Biotechnology, Santa Cruz, CA) and phospho-CREB (pCREB) (Ser 133) (1:500, Cell Signaling Technology, Danvers, MA).

Receptor autoradiography—Detailed methods for autoradiography are described in supplemental materials. Ligands used were 2 nM H³ – SCH 23390 and 5 nM H³ – spiperone (PerkinElmer, Boston, MA).

In situ hybridization—Tissue processing and hybridization were performed as previously described (14). The DARPP-32 probe was kindly provided by P. Greengard (Rockefeller University), and the orexin probe by J. Elmquist (The University of Texas Southwestern Medical Center). Slides assayed for DARPP-32 were apposed to film for 3 days, and slides assayed for orexin were apposed to film for 4 days. Quantification of film images was conducted as previously described (10).

QRT-PCR—RNA was isolated from the VTA and expression of individual genes assessed using TaqMan gene expression assays (Applied Biosystems, Foster City, CA). Detailed methods and statistical analyses may be found in supplemental materials.

Behavioral analyses

In order to examine the effects of reward sensitivity on diet-induced behavioral changes, a subset of mice was withdrawn from HF following four weeks of exposure and returned to house chow (n = 9 control, n = 8 ΔFosB). Twenty-four hours following withdrawal, mice were exposed to the open-field test in accordance with our previously published dietary withdrawal paradigm (10). Briefly, the mouse was placed in the center of the open-field apparatus and monitored for five minutes. Total line crosses, fecal boli, time in the center, and crosses into the center were measured.

Statistics

All data except Western blots were analyzed using a two-way ANOVA followed by Fisher's PLSD test with doxycycline treatment (ΔFosB expression) and diet condition as the independent variables. For RT-PCR analyses, a reduced P value was utilized to correct for multiple comparisons within groups of related genes (see supplemental materials). Western blots were analyzed using a student's t-test with doxycycline treatment as the independent variable, comparing optical densities within the same blot. All data are presented as mean ± SEM.

Results

Basal biochemical differences

To elucidate the molecular pathways that underlie the enhanced reward sensitivity in Δ FosB-overexpressing mice, levels of several key signaling molecules were examined in the NAc. There was a trend for increased levels of Cdk5 in the NAc of Δ FosB mice compared to littermate control animals maintained on doxycycline ($F = 5.1$, $P = 0.08$; Fig. 1A). Δ FosB mice expressed significantly reduced levels of pCREB ($F = 7.4$, $P < 0.05$; Fig. 1B) as well as total levels of CREB ($F = 5.4$, $P = 0.05$; Fig. 1C). A significant reduction in BDNF was also observed in the NAc of Δ FosB mice ($F = 10.6$, $P < 0.05$; Fig. 1D).

Food intake and body weight on high fat diet

We next examined the effects of a naturally rewarding HF diet on alterations in signaling molecules in the Δ FosB-overexpressing mice. There were no differences between Δ FosB mice and controls in food intake on either house or HF. However, there was an overall decrease in caloric intake normalized to body weight when exposed to HF that was specific to the Δ FosB mice ($F = 11.2$, $P < 0.01$; Fig. 2A). At the end of six weeks of diet exposure, mice receiving HF weighed significantly more than those on chow diet ($F = 17.2$, $P < 0.001$), and Δ FosB mice weighed less overall than controls ($F = 5.6$, $P < 0.05$; Fig. 2B). This effect was specific to differences between groups on the chow diet ($P < 0.05$).

Biochemical differences on high fat diet

To determine how basal differences in NAc signaling might be altered by HF diet, the same signaling proteins studied at baseline were examined in animals that had received six weeks of HF. There were no significant differences in Cdk5 levels (Fig. 3A). Levels of pCREB and total CREB were no longer different after six weeks of HF (Fig. 3B,C). Levels of BDNF were significantly elevated in Δ FosB mice following six weeks of HF exposure ($F = 6.5$, $P = 0.05$; Fig. 3D).

Dopamine receptor autoradiography

We used receptor autoradiography to assess whether the Δ FosB-induced alterations in dopamine signaling in the NAc are related to changes in dopamine receptor expression (Fig. 4A). High fat diet appeared to increase slightly the density of D1 dopamine receptor binding ($P = 0.14$), and this difference was greater in Δ FosB mice (Fig. 4B). There was also a trend toward an increase in D1 binding area following HF ($P = 0.06$), and post hoc testing showed this to be significant in the Δ FosB mice ($P < 0.05$; Fig. 4C). In contrast to D1 receptors, no changes in D2 receptor binding density (control chow = 97.6 ± 6.9 , control HF = 101.1 ± 8.2 , Δ FosB chow = 91.6 ± 1.0 , Δ FosB HF = 94.8 ± 9.5) or binding area (control chow = 47.3 ± 3.4 , control HF = 53.8 ± 6.0 , Δ FosB chow = 51.9 ± 3.7 , Δ FosB HF = 49.0 ± 3.3) in the NAc were observed.

DARPP-32 expression in the NAc

In situ hybridization was used to determine expression levels of DARPP-32 in the NAc (Fig. 4D). High fat diet significantly increased DARPP-32 expression in this brain region ($F = 5.1$, $P < 0.05$), and there was a significant interaction between diet and Δ FosB expression ($F = 8.9$, $P < 0.05$), with Δ FosB mice showing a greater diet-induced change (Fig. 4E). A basal difference in DARPP-32 expression between control and Δ FosB mice was revealed by post hoc testing ($P < 0.01$), as well as a significant increase in DARPP-32 expression in the Δ FosB mice on HF ($P < 0.01$).

Gene expression in the VTA

QRT-PCR was utilized to assess changes in gene expression in the VTA, targeting several key genes previously implicated in the regulation of reward. All samples were normalized to β -actin. To ensure that β -actin expression was not altered by treatment, a separate assay was run to compare β -actin to a second internal control, GAPDH. There were no significant differences in β -actin expression (ΔC_T values, β -actin - GAPDH: control chow = 2.29 ± 0.21 , control HF = 2.01 ± 0.04 , Δ FosB chow = 2.32 ± 0.49 , Δ FosB HF = 2.37 ± 0.10).

A trend for an interaction between Δ FosB expression and diet treatment was observed for expression of tyrosine hydroxylase ($F = 3.6$, $P < 0.06$; Fig. 5A). Six weeks of exposure to HF appeared to decrease tyrosine hydroxylase expression in control mice and increase expression in Δ FosB mice. A significant interaction between Δ FosB expression and diet exposure was observed for expression of the dopamine transporter ($F = 6.7$, $P < 0.03$; Fig. 5B). Similar to tyrosine hydroxylase, exposure to HF reduced dopamine transporter expression in control mice and significantly increased expression in Δ FosB mice ($P < 0.05$). The basal difference in dopamine transporter expression between control and Δ FosB mice did not reach significance ($P = 0.16$), but after 6 wks of HF, Δ FosB mice expressed significantly elevated levels of dopamine transporter compared to controls ($P < 0.05$).

There was a trend indicating an effect of increased Δ FosB expression to reduce TrkB levels in the VTA ($F = 5.7$, $P < 0.04$; Fig. 5C). Although there were no main effects on κ -opioid receptor expression, there was a trend toward reduced expression in Δ FosB mice ($P = 0.08$; Fig. 5D). Expression of the leptin receptor was also determined in the VTA. A significant effect of diet exposure was found ($F = 6.1$, $P < 0.03$), with HF significantly reducing levels of the leptin receptor in the VTA in both Δ FosB and control mice (Fig. 5E). Expression of orexin receptor 1 in the VTA was also examined. There was a significant effect of diet on expression of the orexin receptor ($F = 9.0$, $P < 0.02$), with mice exposed to HF expressing higher levels in the VTA (Fig. 5F). There was also a trend for Δ FosB mice to express overall higher levels of orexin receptor 1 in this brain region ($P < 0.05$).

Orexin expression in the lateral hypothalamus

We measured levels of orexin in the lateral hypothalamus, the origin of orexinergic innervation of the VTA, by in situ hybridization (Fig. 6A). There was a significant interaction between Δ FosB expression and diet exposure on orexin expression ($F = 9.1$, $P < 0.01$), with HF significantly increasing orexin levels in control mice ($P < 0.05$) and decreasing expression in Δ FosB mice (Fig. 6B). Although there were no significant differences in orexin expression in the basal state, following 6 wks of HF, Δ FosB mice expressed significantly reduced levels of orexin compared to controls ($P < 0.05$).

Behavioral Analyses

To evaluate alterations in arousal and emotionality due to dietary change, mice were exposed to the open-field test 24 hrs after withdrawal of the HF diet (10). Total line crosses, which were scored as a measure of arousal, were significantly affected by Δ FosB expression ($F = 6.6$, $P < 0.05$) and diet ($F = 4.6$, $P < 0.05$; Fig. 7A). Δ FosB mice were more active in the novel environment than controls, and post hoc testing showed that mice withdrawn from HF were significantly more active than those exposed to chow ($P < 0.05$). Fecal boli were counted as a measure of anxiety-like behavior (10). There was a main effect of Δ FosB expression ($F = 10.2$, $P < 0.01$), with Δ FosB-overexpressing mice producing more fecal boli in the novel environment, particularly in the house chow and HF withdrawal groups (Fig. 7B). Δ FosB mice maintained on HF diet produced fewer fecal boli than those maintained on chow and those withdrawn 24 hrs prior to the test. Control mice did not appear to be affected by diet. There were no significant effects of either Δ FosB expression or diet on time spent in the center of

the open field (control chow = 14.5 ± 3.1 sec, control HF = 18.0 ± 3.2 sec, control W/D = 15.4 ± 1.9 sec, Δ FosB chow = 16.9 ± 2.4 sec, Δ FosB HF = 13.1 ± 3.9 sec, Δ FosB W/D = 19.8 ± 2.6 sec).

Discussion

In obesity treatment, there is a critical need for identification of factors that influence susceptibility to overeating and weight gain. Brain reward pathways play an important role in the motivation for and response to palatable foods and dietary changes (6,10,15,16). As orexigenic and anorexigenic signals can directly influence reward signaling via a hypothalamus-VTA-NAc circuit, elucidation of genes responsive to energy-rich palatable diets within reward centers may provide novel therapeutic targets in obesity treatment (17,18). Therefore, we examined biochemical and molecular markers of reward and energy balance signaling along the hypothalamus-VTA-NAc circuit in response to a HF diet in Δ FosB-overexpressing mice as a model of enhanced sensitivity to changes in reward (13,19,20), and the behavioral sensitivity following diet withdrawal. We hypothesized that basal dysregulation of dopamine signaling in Δ FosB mice would be normalized by the rewarding effects of a HF diet, encompassing the intersection of energy balance signals and the dopamine system.

To examine markers indicative of a dysregulation in dopamine signaling in the NAc, we examined D1 receptor levels and downstream effectors. Although there were no significant differences in D1 receptor binding, there was a trend for HF exposure to increase binding area in the Δ FosB mice. This is interesting as induction of Δ FosB by drug and natural rewards appears to predominate in the dynorphin-positive subtype of medium spiny neurons that primarily express D1 receptors (9,21). Levels of the downstream dopamine signaling target pCREB were significantly reduced in Δ FosB mice, supportive of reduced D1 receptor activation in this brain region (22,23). Interestingly, we also detected a significant decrease in total CREB levels in Δ FosB mice, suggesting a further reduced capacity for dopamine signal transduction that may be secondary to feedback resulting from a prolonged decrease in pCREB (24). BDNF expression is regulated by pCREB, is elevated with D1 activation, and is an important mediator of reward-related neuroplasticity in the NAc (25,26). Accordingly, we detected a significant decrease in BDNF protein in the NAc of Δ FosB mice.

All medium spiny neurons in the NAc express DARPP-32 (27). Its numerous downstream effectors make it a vital player in reward pathways (28), and it has been implicated in drug addiction and in other disorders involving the dopamine system including affective disorders and schizophrenia (27,29). We detected profound basal reductions in DARPP-32 expression in the NAc of Δ FosB mice. DARPP-32 expression is regulated by BDNF, and therefore the reduced expression may be directly related to the reductions in BDNF levels detected in Δ FosB mice (27,29,30). Even moderate changes in the phosphorylation state of DARPP-32 can lead to substantial alterations in intracellular signaling within the NAc (27). Previous studies have reported no change in DARPP-32 protein in Δ FosB mice following a 12-wk removal from doxycycline when a more broad striatal assessment was conducted (31), suggesting that effects of Δ FosB on DARPP-32 may be time and region specific.

We hypothesized that the dramatic reductions in indices of dopamine signaling in the NAc of Δ FosB mice likely involved changes in the VTA dopamine projection neurons, even though Δ FosB is not overexpressed within these neurons. Therefore, we examined expression of dopamine-related genes in the VTA, including tyrosine hydroxylase and the dopamine transporter. Levels of tyrosine hydroxylase and dopamine transporter are positively correlated with dopamine output. There was a trend for Δ FosB mice to exhibit reduced tyrosine hydroxylase and a significant reduction in dopamine transporter, in keeping with the dysregulation of dopamine signaling in the NAc. As these basal reductions in dopamine-related

genes in the VTA of Δ FosB mice presumably reflect altered feedback from the NAc during long-term Δ FosB overexpression, we examined expression of the BDNF receptor, TrkB, as a possible mechanism of NAc feedback to the VTA (32). Similar to tyrosine hydroxylase and dopamine transporter, TrkB expression also showed a trend to be basally reduced in Δ FosB mice that did not reach significance when corrected for multiple comparisons. The BDNF-TrkB complex can be retrogradely transported and act within the VTA to affect local gene expression and promote cell growth and maintenance (33). Further, BDNF activation of presynaptic TrkB within the NAc can directly stimulate dopamine neurotransmission (32), supporting an underlying diminution of dopamine signaling in these mice.

Dynorphin activation of κ -opioid receptors regulates dopamine signaling and is another mechanism whereby the NAc provides feedback to the VTA (34). We found that κ -opioid receptor expression in the VTA showed a trend to be reduced in Δ FosB mice. As Δ FosB overexpression has been shown to decrease dynorphin expression in the NAc (20), Δ FosB mice likely have profound reductions in net VTA κ -opioid activation. Although dynorphin signaling normally exerts an inhibitory effect on dopamine neurons (35), rats that show enhanced self-administration of drugs of abuse exhibit reduced levels of dynorphin in the NAc, pointing to a role for basally reduced dynorphin signaling in enhancing reward sensitivity (36,37). Dysregulation of the dynorphin - κ -opioid system has been linked to the acquisition and persistence of drug abuse, supporting a critical balance of opioid signaling in normalization of dopamine pathways (38).

Based on the rewarding capacity of an energy-dense HF diet, we hypothesized that a dysregulation in dopamine and opioid reward signaling in Δ FosB mice would predispose these mice to enhanced reward responses to such a diet, thus normalizing the reward system via activation of the hypothalamus-VTA-NAc circuit. During the six week diet exposure, no differences in food intake between Δ FosB and control mice were observed, suggesting that the changes found in biochemical and molecular markers of reward signaling in Δ FosB mice were not due to differences in the calories consumed. As anticipated, basal differences detected in pCREB, total CREB, BDNF, DARPP-32, and κ -opioid receptor levels between Δ FosB and control mice were attenuated, likely due to increased dopamine output in Δ FosB mice on HF (29,39-41).

Examination of both tyrosine hydroxylase and the dopamine transporter in the VTA revealed surprising opposing responses of Δ FosB and control mice following HF. Control mice showed a decrease in tyrosine hydroxylase and dopamine transporter expression, while Δ FosB mice displayed increased expression of both of these dopamine-related genes. Interestingly, tyrosine hydroxylase expression is altered in the VTA by chronic cocaine or methamphetamine administration (42-44), suggesting that Δ FosB mice may find the natural reward of HF more salient than control mice.

In order to examine how potential hypothalamic input to the VTA may be relaying signals that reflect energy balance, expression of the leptin receptor and orexin receptor-1 were also examined. Circulating leptin levels are increased by HF, and leptin can in turn act at the VTA to alter dopamine signaling (18,45). VTA leptin receptor expression was similarly decreased by HF in both Δ FosB and control mice, in keeping with similar weight gain and diet intake while on HF. High fat also increased expression of the orexin receptor-1 in the VTA of both Δ FosB and control mice. Orexin activates dopamine neurons in the VTA, promotes VTA plasticity, and increases dopamine levels in the NAc (46-48). High fat diet has been shown to increase orexin expression in mice, in accordance with our observations (49,50). Thus, increased expression of the orexin receptor as well as changes in leptin signaling in the VTA could promote diet reward in both Δ FosB and control mice, supporting a dissociation between pathways relaying energy balance signals and those tied directly to reward.

To examine the stress-provoking effects of reward withdrawal, mice were examined in an open-field test 24 hrs following the removal of HF. Δ FosB mice were more sensitive to the acute effects of preferred diet withdrawal, showing heightened arousal activity and fecal boli production in the novel open arena compared to all other control and diet groups. Δ FosB mice also showed an interesting behavioral pattern in this test suggestive of reward and stress sensitivity, with the HF diet initially reducing fecal boli production relative to chow, and the withdrawal again increasing this anxiety-related response. This observed increase in open-field activity did not correlate with changes in orexin expression, suggesting a relationship to stress-induced arousal that is not merely an effect of changes in orexin-mediated signaling. Overall, these data support our hypothesis that Δ FosB mice would be more sensitive to the acute effects of preferred diet withdrawal due to their heightened reward sensitivity.

How does long-term overexpression of Δ FosB in the NAc lead to such changes in behavior and reward signaling? We have proposed a model of VTA coincident detection wherein altered feedback from the NAc and hypothalamus relays signals regarding reward state to determine the regulation of the dopamine system that may support a link between reward pathway dysregulation and a predisposition to obesity (Fig. 8). During HF exposure, multiple inputs reflecting both energy balance and reward state converge on the VTA. Increases in leptin and orexin signaling as well as altered feedback from the NAc to the lateral hypothalamus may affect how these orexigenic signals respond to HF in the Δ FosB mice (17,18,45,47,51-53). High fat diet-induced elevations in BDNF may provide reward feedback to the VTA, further promoting changes in dopamine-related gene expression.

These results delineate molecular markers of reward sensitivity and indicate that long-term dysregulation of the dopamine system may predispose an individual to addiction and obesity. Further, these data provide an important step toward pinpointing potential new therapeutic targets in the treatment and prevention of obesity and other disorders that may center on the reward system. In the future, it will be important to investigate how this system responds to removal of the HF diet, as well as to investigate any sex differences in sensitivity to reward and high fat diet exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Mice overexpressing Δ FosB exhibited biochemical markers of reduced dopamine signaling in the NAc

(A) Δ FosB mice displayed an indication of higher levels of Cdk5 than controls, similar to previous reports (Ctrl; $P < 0.09$). (B) pCREB levels were significantly reduced in Δ FosB mice compared to controls ($P < 0.05$). (C) Total CREB levels were also significantly reduced in Δ FosB mice ($P = 0.05$). (D) Δ FosB mice showed a significant reduction in BDNF levels in the NAc ($P < 0.05$). For all figures, representative bands are shown for control (C) and Δ FosB overexpressing (Δ F) mice. *Significantly different from control.

Figure 2. Δ FosB overexpressing mice showed no differences in food intake on either chow or high fat (HF) diet

(A) Δ FosB expression did not significantly affect daily food intake normalized to body weight on either chow or HF diet, although daily caloric intake was reduced in the HF groups in the third week of diet exposure ($P < 0.01$), and post hoc testing showed this effect to be specific to the Δ FosB mice ($P < 0.01$). (B) Final body weights were significantly increased in mice that received six weeks of HF diet compared to chow ($P < 0.001$), and were reduced in mice overexpressing Δ FosB ($P < 0.05$), which post hoc testing showed to be significant to the chow group ($P < 0.05$). *Significantly different from chow within expression group, +significantly different from control chow.

Figure 3. High fat (HF) diet ameliorated signaling differences observed in the NAc of Δ FosB overexpressing mice

Following 6 wks of HF diet, levels of (A) Cdk5, (B) pCREB, and (C) CREB were not different between control (Ctrl) and Δ FosB mice. (D) Δ FosB mice displayed elevated BDNF levels relative to controls at the end of 6 wks of HF ($P = 0.05$). Representative bands from control (C) and Δ FosB (Δ F) mice are shown for all figures. *Significantly different from control.

Figure 4. High fat diet (HF) led to changes in D1 dopamine receptor binding and DARPP-32 expression in the nucleus accumbens (NAc) of Δ FosB overexpressing mice
 (A) Representative images of D1 autoradiography. Dotted lines indicate the region of the NAc analyzed. (B) There were no main effects on D1 binding density. (C) A trend toward increased binding area of the D1 receptor was observed following 6 wks of HF diet ($P < 0.06$). Post hoc testing showed this to be significant in the Δ FosB mice ($P < 0.05$). (D) Representative images showing in situ hybridization for DARPP-32 expression in the NAc. Dotted lines indicate the region of the NAc analyzed. (E) There was a main effect of diet ($P < 0.05$), as well as an interaction between diet and Δ FosB expression ($P < 0.05$) on DARPP-32 expression. Post hoc testing revealed that Δ FosB mice expressed reduced levels of DARPP-32 basally ($P < 0.01$),

and showed a significant increase in expression following HF diet ($P < 0.01$). *Significantly different from Δ FosB chow, ⁺significantly different from control chow.

Figure 5. High fat diet (HF) exposure and Δ FosB expression led to changes in expression of a number of key molecules in the VTA

Expression levels were normalized to control (Ctrl) chow mice. (A) There was trend toward an interaction between diet exposure and Δ FosB expression on levels of tyrosine hydroxylase (TH; $P < 0.06$). HF diet decreased TH in control mice but increased expression in Δ FosB mice. (B) Expression of the dopamine transporter (DAT) was also differentially affected by both diet exposure and Δ FosB expression ($P < 0.03$). Similar to TH, DAT levels were decreased in control mice following HF diet and increased in Δ FosB mice. (C) Levels of the neurotrophin receptor TrkB showed a trend when corrected for multiple comparisons to be reduced in Δ FosB mice ($P < 0.04$), which appeared to be primarily due to differences in the basal state. (D) Δ FosB mice showed a trend for reduced expression of the κ -opioid receptor (κ -OR; $P < 0.09$). (E) Expression of the leptin receptor (ObRb) was significantly reduced following six weeks of HF diet ($P < 0.03$). (F) Expression of orexin receptor 1 (Ox1) was significantly increased overall following six weeks of HF diet ($P < 0.02$). *Significantly different from Δ FosB chow, +significantly different from control HF.

Figure 6. High fat (HF) diet had differential effects on orexin expression in control (Ctrl) and Δ FosB overexpressing mice
 (A) Representative images showing orexin expression in the lateral hypothalamus by in situ hybridization. (B) There was a significant interaction between diet exposure and Δ FosB expression on orexin expression ($P < 0.01$). Post hoc testing revealed that control mice showed a significant increase in orexin expression following HF diet ($P < 0.05$) and, coupled with a non-significant decrease in expression in Δ FosB mice, this led to a significant difference in orexin expression between the control and Δ FosB mice on HF diet ($P < 0.05$). *Significantly different from control chow, +significantly different from control HF.

Figure 7. Mice over-expressing Δ FosB were more sensitive to the effects of high fat diet (HF) withdrawal

(A) Twenty-four hours following HF withdrawal, there were main effects of both diet status ($P < 0.05$) and Δ FosB expression ($P < 0.05$) on arousal in a novel environment, as measured by total line crosses in an open field arena. Mice withdrawn from HF (W/D) were significantly more active than mice maintained on chow ($P < 0.05$) and tended to be more active than mice maintained on HF ($P < 0.15$). This difference was driven primarily by differences within the Δ FosB mice, which were more active overall than control mice (Ctrl). (B) Δ FosB mice produced more fecal boli in the novel environment than controls ($P < 0.01$), a difference driven primarily by the chow and withdrawal groups, as a measure of anxiety-like behavior.

*Significantly different from other Δ FosB diet groups, ⁺significantly different from control W/D.

Figure 8. High fat (HF) diet normalizes dysregulated reward signaling in Δ FosB mice

Under basal conditions (left panel), long-term overexpression of Δ FosB leads to dysregulation of dopamine (DA) signal transduction and gene expression in the nucleus accumbens (NAc), altering ventral tegmental area (VTA) feedback and changes in DA-related gene expression in the VTA. During HF diet exposure (right panel), signals of reward and energy status from the NAc, hypothalamus, and periphery converge on the VTA as part of a complex network regulating reward status for palatable food. In the dysregulated Δ FosB mice, the HF diet results in normalization of DA signaling in the NAc and VTA. In this model, the VTA acts as a 'coincident detector' for information related to reward and energy balance. Abbreviations not defined in main text: PFC, prefrontal cortex; Hpc, hippocampus; Dyn, dynorphin; GABA, γ -aminobutyric acid; MCH, melanin-concentrating hormone; Arc, arcuate nucleus of the hypothalamus; NPY, neuropeptide Y; α MSH, α -melanocyte-stimulating hormone.