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A high-fat diet or galanin in the PVN decreases phosphorylation of CREB in the nucleus accumbens

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Abstract

A high-fat diet (HFD) can increase hypothalamic galanin (GAL). GAL has recently been shown to inhibit opiate reward, which in turn, decreases cAMP response element-binding protein (CREB) in the nucleus accumbens (NAc). We hypothesized that injection of GAL into the PVN, or consumption of a HFD, would be associated with a decrease in NAc CREB. In Exp. 1, GAL in the paraventricular nucleus (PVN) of naïve rats decreased phosphorylated-CREB (pCREB) in the NAc compared to saline injected controls. In Exp. 2, rats fed *ad libitum* HFD for 4 wks had reduced NAc pCREB levels compared to rats with sporadic tastes of the HFD. Body weight, serum triglyceride and leptin levels were also raised in the chronic HFD-fed rats. These data suggest that PVN GAL or chronic intake of a HFD can decrease NAc pCREB. The implications of these findings may help to explain the lack of opiate-like withdrawal that has been reported in response to overeating a high fat diet, thereby providing a potential mechanism underlying behavioral differences seen with addiction-like overconsumption of different types of palatable foods.

Keywords

Addiction; CREB; dependence; high-fat diet; nucleus accumbens; obesity

1.1

cAMP response element-binding protein (CREB) is a transcription factor with an established role in rewarding behaviors. Stimuli that are associated with natural reward, such as tone cues associated with food, lead to increases of phosphorylated CREB (pCREB), the transcriptionally active form of the protein, in the NAc (Shiflett et al., 2009). In addition,

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drugs of abuse, such as cocaine, amphetamine, opiates and Δ9-tetrahydrocannabinol, have been shown to increase CREB or pCREB activity in multiple brain regions, including the NAc and dorsal striatum (Carlezon et al., 2005, Edwards et al., 2007, Briand and Blendy, 2010) as well as the prefrontal cortex and hippocampus (Rubino et al., 2007). It has also been proposed that CREB contributes to the negative emotional state that emerges during withdrawal from some drugs of abuse. For example, withdrawal from chronic nicotine exposure produced an increase in pCREB protein levels in the NAc (Kivinummi et al., 2011).

It may be that CREB, and thus its role in rewarding behaviors, is affected by the neuropeptide galanin (GAL). GAL decreases CREB phosphorylation in striatal slices (Hawes et al., 2006). Further, GAL can have an inhibitory role in opioid-mediated reward. Intraventricular GAL inhibits morphine place preference (Zachariou et al., 1999), and GAL can protect against behavioral signs of opiate withdrawal in transgenic animals (Zachariou et al., 2003, Hawes et al., 2008).

Fat intake is known to increase hypothalamic GAL (Leibowitz, 2005), and injection of GAL into the paraventricular nucleus (PVN) causes an increase in food consumption in rats (Kyrkouli et al., 1986, Kyrkouli et al., 1990), particularly with regards to fat intake (Tempel et al., 1988, Tempel and Leibowitz, 1990), although this latter observation has not been universally supported (Smith et al., 1996). The PVN has also been shown to regulate the NAc, with, for example, GAL administration in the PVN decreasing extracellular levels of acetylcholine and increasing extracellular dopamine in the NAc (Rada et al., 1998).

Collectively, it appears that both reward and withdrawal are associated with an increase in pCREB levels in the NAc. Further, GAL has been shown to decrease CREB in striatal slices and appears to inhibit opiate-mediated reward as well as opiate withdrawal. In light of these findings and research suggesting that the PVN plays a role in regulating the NAc, this study was designed to assess whether microinjections of GAL into the PVN of the hypothalamus or consumption of a HFD, which endogenously elevates GAL levels in the PVN, are associated with changes in NAc CREB. The results of this study may help to elucidate potential brain mechanisms that are associated with the different behavioral profiles that emerge in rats with a history of overeating a diet high in fat versus sugar.

2. Experimental Procedures

2.1 Animals

Male Long Evans rats (250–300 g) were obtained from Taconic Farms (Germantown, NY, USA) and housed individually on a 12-h reversed light/dark cycle, with lights on at 18:00 and off at 6:00. All rats had unrestricted access to water and were fed with standard rodent chow (LabDiet #5001, PMI Nutrition International, Richmond, Indiana, USA; 10% fat, 20% protein, 70% carbohydrate, 3.02 kcal/g), unless otherwise specified. All procedures were approved by the Princeton University Institutional Animal Care and Use Committee.

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2.2. Exp. 1. The effect of microinjections of GAL vs. saline in PVN on pCREB in the NAc

Surgery and Microinjections—Rats (n=9/group) were implanted with cannula aimed at the PVN. For surgery, animals were anesthetized with ketamine (80 mg/kg, i.p.) supplemented by xylazine (10 mg/kg, intraperitoneal). Stainless steel guide shafts (21 gauge) were unilaterally implanted, on the left side in half of the rats and on the right side in the other half, above the PVN using the following coordinates: B −1.8 mm, L 0.3 mm, and V 3.2 mm, with reference to bregma, midsagittal sinus, and the level skull. Stylets (26 gauge, 10 mm long) were inserted into the guide cannulas to keep them patent. Rats were allowed to recover for at least 1 week before microinjections. Microinjectors were made of fused silica tubing (37 μm i.d. × 160 μm o.d.) that extended 5.0 mm beyond the guide shaft to reach a ventral coordinate of 8.2 mm below the skull surface.

GAL (American Peptide Co., Inc., Sunnyvale, CA, 1.0 nmol, n=9), at a dose previously used in our laboratory to affect alcohol intake behavior (Schneider et al., 2007), dissolved in saline, or saline as a control (n=9), was injected into the PVN by means of a syringe pump set to deliver 0.5 μL in 45 s. The injectors were left in place for an additional 45 s to reduce reflux. Microinjections were conducted 4 to 5 h into the dark cycle, daily, for 7 days.

2 h following the final injection, rats were sacrificed via rapid decapitation. Brains were immediately extracted and chilled, and bilateral tissue punches (1 mm, disposable biopsy punches, Integra Miltex) of the NAc shell were obtained from 2 mm coronal slices based on coordinates obtained from Paxinos and Watson (1998): coordinates +1.2 mm anterior to bregma, 0.8 mm lateral to midsagittal sinus, and 9.0 mm ventral to the surface of the level skull). Tissue was rapidly frozen on dry ice and kept at −80°C until processed using Western blot.

Brain sections containing the PVN were collected for histology (Fig. 1). The injectors were primarily located in the PVN, and there were not enough off-target injections to determine how GAL may affect behavior or CREB levels when injected in other brain regions, which may be considered a limitation of the current study. While we visualized and plotted injector placement, we did not perform a histological verification of the lesion created by repeated injections. However, others have repeatedly injected similar volumes of GAL into the PVN without noting a lesion (Rada et al., 2004, Schneider et al., 2007). Further, the procedures used with the control group were identical to those used with the experimental group, so any tissue lesions would have been similar between the groups. For these reasons, we are confident that lesions are not responsible for the findings in this experiment.

Blood samples were collected and processed for serum levels of triglycerides using enzymatic hydrolysis (Cayman Chemicals, kit #10010303) and leptin using an enzyme immunoassay (Cayman Chemicals, kit #10007609), following the manufacturers' instructions.

2.3. Exp. 2. The effect of chronic vs. acute HFD diet on pCREB in the NAc

Rats (n=12/group) were weight matched and divided into two groups. The Chronic HFD group was maintained on a high-fat diet (5.15 Kcal/g), while the Acute HFD group was maintained on a low-fat diet (5.05 Kcal/g), with weekly access to a 15 Kcal taste of the

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HFD. Diets were provided in glass jars. Previous research has shown that rats maintained for 4 weeks on the same HFD showed more than 75% increases in PVN GAL compared to rats maintained for the same amount of time on the low-fat diet used here (Leibowitz, 1998).

The diets, which are described in prior publications (Leibowitz et al., 2004, Dourmashkin et al., 2006), were nutritionally complete, similar in content, palatability and texture, but different in fat content, thus making them adequate for comparison. The macronutrient composition of the diets was calculated as percentage of total kcal. The HFD contained 50% fat, 25% carbohydrate, and 25% protein, and the low-fat diet contained 10% fat, 65% carbohydrate, and 25% protein. The HFD consisted of (1) fat from 75% lard (Armour, Omaha, NE) and 25% vegetable oil (Wesson vegetable oil, Omaha, NE); (2) carbohydrate from 30% dextrin, 30% cornstarch (ICN Pharmaceuticals, Costa Mesa, CA), and 40% sucrose (Domino®, Yonkers, NY), and (3) protein from casein (Bioserv, Frenchtown, NJ). The low-fat diet consisted of (1) fat from vegetable oil (Wesson vegetable oil, Omaha, NE), (2) carbohydrate from 33% dextrin, 33% cornstarch (ICN Pharmaceuticals, Costa Mesa, CA), and 34% sucrose (Domino®, Yonkers, NY), and (3) protein from casein (Bioserv, Frenchtown, NJ). Both diet mixtures were supplemented with minerals (USP XIV Salt Mixture Briggs; ICN Pharmaceuticals) and vitamins (Vitamin Diet Fortification Mixture; ICN Pharmaceuticals), such that they were nutritionally complete diets. Diets were stored at 4°C until use. The rats were maintained on their respective diets for 4 wks, with standard rodent chow and the mixed diet available on days 1–3 to allow for acclimation. Diets were replaced every other day, and food intake was recorded at those times. At the end of 4 wks, animals were sacrificed as described in Exp. 1 and tissue and blood were collected.

2.4. Western Blot

Tissue samples were homogenized in 5 μM Hepes lysis buffer (1% SDS) containing protease and phosphatase inhibitors. 50 μg samples of total protein lysates were electrophoresed on 4–20% precast polyacrylamide gels (BioRad). Proteins were transferred to nitrocellulose membranes (BioRad) and blocked overnight in Licor Blocking Buffer. Membranes were then incubated in rabbit anti-pCREB (1:1,000, Millipore), rabbit anti-CREB, (1:1,000, Cell Signaling) and mouse anti-β-Tubulin antibodies (1:60,000, Millipore) overnight at 4°C. Membranes were then incubated for 2 h at room temperature with the appropriate IRDye secondary antibodies (Li-Cor, 1:10,000), and bands were visualized using Odyssey Imager (Li-Cor). National Institutes of Health Image J software was used to quantify bands, which were further normalized to β-tubulin, as previously described in the literature (Cao et al., 2010), to control for equal loading.

3. Results

3.1. Exp. 1. GAL in the PVN decreased pCREB in the NAc, with no effect on circulating levels of triglycerides or leptin

After the final injection, there was no difference in bodyweight observed between rats given GAL vs. saline injections ($t(13)=0.45$, p=n.s.; GAL: 321.9 ± 8.0 g, saline: 317.4 ± 6.1 g). Nor were there differences in circulating levels of serum triglycerides ($t(16)=0.53$, p=n.s., GAL: 74.3 ± 8.7 mg/dL, saline: 76.3 ± 7.2 mg/dL) or leptin ($t(16)=0.59$, p=n.s., GAL: 314.8

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± 58.3 pg/mL, saline: 265.4 ± 59.3 pg/mL). There was, however, a difference in normalized pCREB levels (pCREB/tubulin), with less pCREB in the NAc shell of rats that had PVN GAL injections compared to saline ($t(13)=2.82$, $p=0.015$; Fig. 2). There was also a trend towards statistical significance in normalized CREB levels (CREB/tubulin), with less CREB in the NAc shell of rats that had PVN GAL injections compared to saline ($t(13)=2.04$, $p=0.06$; GAL: 1.25 ± 0.06, saline: 1.46 ± 0.07).

3.2. Exp. 2. Chronic HFD reduced pCREB in the NAc, while increasing body weight and circulating levels of triglycerides and leptin

After 4 wks on the diets, rats in the Chronic HFD group weighed significantly more than rats in the Acute HFD group ($t(22)=2.93$, $p=0.008$; chronic HFD: 454.5 ± 8.3 g, acute HFD: 415.4 ± 10.4 g). No interaction was found between the kcal intake of the two groups ($F(3,48)=0.561$, $p=0.644$), with no effect of week ($F(3,38)=1.268$, $p=0.296$), and no effect of diet condition ($F(1,16)=0.942$, =0.346). There was a significant difference between the groups in serum triglyceride ($t(21)=5.85$, $p<0.0001$; chronic HFD: 197.1 ± 13.7 mg/dL, acute HFD: 89.3 ± 11.3 mg/dL) and leptin levels ($t(22)=3.60$, $p=0.002$; chronic HFD: 1117.8 ± 128.1 pg/mL, acute HFD: 553.2 ± 90.3 pg/mL). Further, the rats in the Chronic HFD group had decreased levels of normalized pCREB (pCREB/tubulin) compared to the Acute HFD group ($t(20)=2.78$, $p=0.01$; Fig. 3). There was also a significant difference in normalized CREB levels (CREB/tubulin), with less CREB in the NAc shell of rats that had Chronic HFD compared to Acute HFD ($t(20)=2.70$, $p=0.014$; chronic HFD: 0.59 ± 0.06, acute HFD: 0.85 ± 0.07).

4. Discussion

The current study suggests that either local injections of GAL in the PVN, or chronic access to a HFD, decreases pCREB in the NAc, with similar trends in CREB levels. Given that fat intake is known to endogenously increase GAL levels in the PVN (Leibowitz et al., 2004), this provides a mechanism by which fat consumption may affect CREB levels.

Role of GAL in Appetitive Behaviors

GAL is best known for its role in appetite for food (Morganstern et al., 2011, Parker and Bloom, 2012). However, GAL has been shown to have a role in other appetitive behaviors, including addiction, drug dependence and withdrawal (Picciotto, 2010). Several lines of evidence support the role of GAL in modulating the effects of drugs of abuse such as morphine, cocaine and amphetamine (Jackson et al., 2011). For example, there is evidence for a link between alcohol consumption and GAL levels in the PVN; alcohol consumption, like fat consumption, leads to elevated levels of GAL in the PVN (Leibowitz et al., 2003, Chang et al., 2007). Further, administration of the GAL antagonist, M40, into the PVN, or GAL knockout, reduces alcohol intake in rats (Rada et al., 2004, Karatayev et al., 2010). In support of these data, clinical research has identified variants in the GAL and GALR3 genes that are associated with risk for alcoholism (Belfer et al., 2007).

Increased GAL levels, via transgenic animals that overexpress GAL, reduce signs of opiate withdrawal, and this effect seems to be occurring via the GALR2 receptor (Holmes et al.,

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2012). In addition, mice overexpressing GAL show decreased morphine withdrawal signs, while GAL knockout mice show increased withdrawal signs (Zachariou et al., 2003). Further, galnon, a GAL receptor agonist, attenuates morphine preference and withdrawal in the mouse (Zachariou et al., 1999, Zachariou et al., 2003). Transgenic mice that overexpress GAL in the brain are less sensitive to amphetamine-induced increases in locomotor activity (Kuteeva et al., 2005). Collectively, these data suggest that GAL has a protective role against progression to drug dependence and inhibits the expression of signs of withdrawal in dependent animals.

Role of NAc CREB in Appetitive/Aversive Behaviors

Elevated expression of CREB in the NAc is associated with increased depressive-like behaviors that are similar to those behaviors and dysphoria observed during drug withdrawal (Pliakas et al., 2001). Further, correlations between the timing of up-regulated activity within cAMP pathways (which activate CREB) and signs of withdrawal raise the possibility that activation of cAMP-regulated molecules (such as CREB) may contribute to the aversive or dysphoric states associated with drug withdrawal (Carlezon et al., 1998). However, others have shown that decreased CRE-dependent transcription using viral gene transfer of a dominant negative mutant of CREB promotes anxiety (Barrot et al., 2005) and the inhibition of CREB activity increases anxiety-like behavior (Barrot et al., 2002). Based on these conflicting findings, the precise role that CREB activity may have in contributing to or protecting against withdrawal remains unclear.

Numerous CREB targeted genes have been identified, including the opioid peptide dynorphin. Viral-mediated elevations of CREB in the NAc shell increase local dynorphin mRNA, while overexpression of dominant-negative CREB in the NAc shell decreases it (Carlezon et al., 1998). These changes in dynorphin are driven by CREB, with the cAMP-CREB cascade triggering the induction of prodynorphin, a precursor to dynorphin (Nestler et al., 2002). Behaviorally, increased dynorphin expression is associated with aversive or depressive-like effects often seen during drug withdrawal. Further, repeated exposure to drugs of abuse increases dynorphin concentrations, which is of interest because the CREB-mediated transcription of dynorphin decreases dopamine function, as it feeds back and suppresses dopaminergic signaling to the NAc (Carlezon et al., 1998, Carlezon et al., 2005). This has been shown to lead to drug tolerance and withdrawal symptoms (Berke and Hyman, 2000).

Role of Diet Components in Appetitive/Aversive Behaviors

Overeating palatable foods can produce behavioral and neurochemical signs that resemble dependence in laboratory animal models and in clinical studies (Avena et al., 2008b, Corwin et al., 2011, Volkow et al., 2011). When fasted or administered an opioid antagonist, signs of withdrawal have been noted in rats with a history of binge eating sugar (Colantuoni et al., 2002, Avena et al., 2008a), however, these signs are not readily seen in rats with a history of binge eating a HFD similar to the one used in the current study (Bocarsly et al., 2011). This suggests that overconsumption of foods consisting primarily of different nutrients may activate select neural mechanisms, manifesting in different behavioral phenotypes. Further research would be needed to assess whether the effects on NAc pCREB reported here are

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observed in rats fed a high sugar diet. If not, these findings may provide a mechanism by which withdrawal is inhibited in fat-consuming animals.

Recently it has been shown that mice maintained on a HFD for 6 weeks do show signs of anxiety during spontaneous withdrawal from the diet but no significant change in pCREB in the NAc (Sharma et al., 2012). While these findings are inconsistent with our data, they are not necessarily in conflict with the hypothesis that pCREB may mediate withdrawal, as anxiety may not have been observed if pCREB levels were reduced. It is relevant to note that increased anxiety-like behavior has also been found in dietary-induced obese rats fed a HFD for 12 weeks without removal of the diet, raising the possibility that anxiety may not be a sufficient measure of withdrawal within this context (Sharma and Fulton, 2013). The lack of a significant change in NAc pCREB levels in the HFD group in the former study is in contrast to the findings presented here. It is possible that there were significant differences in the amount of diet consumed by the rats and mice beyond what might be expected for these two species.

Conclusion

In summary, the findings from the present study suggest that PVN GAL or chronic intake of a HFD can decrease NAc pCREB. Future research investigating the effect of a high sugar diet on NAc pCREB may aid in determining whether the decreased pCREB levels in the NAc reported here suggest the mechanism by which opiate-like withdrawal is inhibited in rats with a history of overeating fat. Further examination into the neurobiological mechanisms associated with overconsumption of different palatable macronutrients may help to enhance the current understanding of both normal and pathological feeding behaviors.

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References

Avena NM, Bocarsly ME, Rada P, Kim A, Hoebel BG. After daily bingeing on a sucrose solution, food deprivation induces anxiety and accumbens dopamine/acetylcholine imbalance. Physiol Behav. 2008a; 94:309–315. [PubMed: 18325546]

Avena NM, Rada P, Hoebel BG. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. Neuroscience and biobehavioral reviews. 2008b; 32:20–39. [PubMed: 17617461]

Barrot M, Olivier JD, Perrotti LI, DiLeone RJ, Berton O, Eisch AJ, Impey S, Storm DR, Neve RL, Yin JC, Zachariou V, Nestler EJ. CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:11435–11440. [PubMed: 12165570]

Barrot M, Wallace DL, Bolanos CA, Graham DL, Perrotti LI, Neve RL, Chambliss H, Yin JC, Nestler EJ. Regulation of anxiety and initiation of sexual behavior by CREB in the nucleus accumbens. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:8357–8362. [PubMed: 15923261]

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Belfer I, Hipp H, Bollettino A, McKnight C, Evans C, Virkkunen M, Albaugh B, Max MB, Goldman D, Enoch MA. Alcoholism is associated with GALR3 but not two other galanin receptor genes. Genes, brain, and behavior. 2007; 6:473–481.

Berke JD, Hyman SE. Addiction, dopamine, and the molecular mechanisms of memory. Neuron. 2000; 25:515–532. [PubMed: 10774721]

Bocarsly ME, Berner LA, Hoebel BG, Avena NM. Rats that binge eat fat-rich food do not show somatic signs or anxiety associated with opiate-like withdrawal: implications for nutrient-specific food addiction behaviors. Physiol Behav. 2011; 104:865–872. [PubMed: 21635910]

Briand LA, Blendy JA. Molecular and genetic substrates linking stress and addiction. Brain Res. 2010; 1314:219–234. [PubMed: 19900417]

Cao JL, Vialou VF, Lobo MK, Robison AJ, Neve RL, Cooper DC, Nestler EJ, Han MH. Essential role of the cAMP-cAMP response-element binding protein pathway in opiate-induced homeostatic adaptations of locus coeruleus neurons. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:17011–17016. [PubMed: 20837544]

Carlezon WA Jr, Duman RS, Nestler EJ. The many faces of CREB. Trends Neurosci. 2005; 28:436–445. [PubMed: 15982754]

Carlezon WA Jr, Thome J, Olson VG, Lane-Ladd SB, Brodkin ES, Hiroi N, Duman RS, Neve RL, Nestler EJ. Regulation of cocaine reward by CREB. Science. 1998; 282:2272–2275. [PubMed: 9856954]

Chang GQ, Karatayev O, Ahsan R, Avena NM, Lee C, Lewis MJ, Hoebel BG, Leibowitz SF. Effect of ethanol on hypothalamic opioid peptides, enkephalin, and dynorphin: relationship with circulating triglycerides. Alcohol Clin Exp Res. 2007; 31:249–259. [PubMed: 17250616]

Colantuoni C, Rada P, McCarthy J, Patten C, Avena NM, Chadeayne A, Hoebel BG. Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. Obesity research. 2002; 10:478–488. [PubMed: 12055324]

Corwin RL, Avena NM, Boggiano MM. Feeding and reward: perspectives from three rat models of binge eating. Physiol Behav. 2011; 104:87–97. [PubMed: 21549136]

Dourmashkin JT, Chang GQ, Hill JO, Gayles EC, Fried SK, Leibowitz SF. Model for predicting and phenotyping at normal weight the long-term propensity for obesity in Sprague-Dawley rats. Physiol Behav. 2006; 87:666–678. [PubMed: 16513148]

Edwards S, Graham DL, Bachtell RK, Self DW. Region-specific tolerance to cocaine-regulated cAMP-dependent protein phosphorylation following chronic self-administration. Eur J Neurosci. 2007; 25:2201–2213. [PubMed: 17439498]

Hawes JJ, Brunzell DH, Narasimhaiah R, Langel U, Wynick D, Picciotto MR. Galanin protects against behavioral and neurochemical correlates of opiate reward. Neuropsychopharmacology. 2008; 33:1864–1873. [PubMed: 17957220]

Hawes JJ, Narasimhaiah R, Picciotto MR. Galanin attenuates cyclic AMP regulatory element-binding protein (CREB) phosphorylation induced by chronic morphine and naloxone challenge in Cath.a cells and primary striatal cultures. J Neurochem. 2006; 96:1160–1168. [PubMed: 16417577]

Holmes FE, Armenaki A, Iismaa TP, Einstein EB, Shine J, Picciotto MR, Wynick D, Zachariou V. Galanin negatively modulates opiate withdrawal via galanin receptor 1. Psychopharmacology. 2012; 220:619–625. [PubMed: 21969124]

Jackson KJ, Chen X, Miles MF, Harenza J, Damaj MI. The neuropeptide galanin and variants in the GalR1 gene are associated with nicotine dependence. Neuropsychopharmacology. 2011; 36:2339–2348. [PubMed: 21796100]

Karatayev O, Baylan J, Weed V, Chang S, Wynick D, Leibowitz SF. Galanin knockout mice show disturbances in ethanol consumption and expression of hypothalamic peptides that stimulate ethanol intake. Alcohol Clin Exp Res. 2010; 34:72–80. [PubMed: 19860804]

Kivinummi T, Kaste K, Rantamaki T, Castren E, Ahtee L. Alterations in BDNF and phospho-CREB levels following chronic oral nicotine treatment and its withdrawal in dopaminergic brain areas of mice. Neurosci Lett. 2011; 491:108–112. [PubMed: 21232579]

Kuteeva E, Hokfelt T, Ogren SO. Behavioural characterisation of transgenic mice overexpressing galanin under the PDGF-B promoter. Neuropeptides. 2005; 39:299–304. [PubMed: 15944026]

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Kyrkouli SE, Stanley BG, Leibowitz SF. Galanin: stimulation of feeding induced by medial hypothalamic injection of this novel peptide. European journal of pharmacology. 1986; 122:159–160. [PubMed: 2420618]

Kyrkouli SE, Stanley BG, Seirafi RD, Leibowitz SF. Stimulation of feeding by galanin: anatomical localization and behavioral specificity of this peptide's effects in the brain. Peptides. 1990; 11:995–1001. [PubMed: 1704616]

Leibowitz SF. Regulation and effects of hypothalamic galanin: relation to dietary fat, alcohol ingestion, circulating lipids and energy homeostasis. Neuropeptides. 2005; 39:327–332. [PubMed: 15944030]

Leibowitz SF, Avena NM, Chang GQ, Karatayev O, Chau DT, Hoebel BG. Ethanol intake increases galanin mRNA in the hypothalamus and withdrawal decreases it. Physiol Behav. 2003; 79:103–111. [PubMed: 12818715]

Leibowitz SF, Dourmashkin JT, Chang GQ, Hill JO, Gayles EC, Fried SK, Wang J. Acute high-fat diet paradigms link galanin to triglycerides and their transport and metabolism in muscle. Brain Res. 2004; 1008:168–178. [PubMed: 15145753]

Morganstern I, Barson JR, Leibowitz SF. Regulation of drug and palatable food overconsumption by similar Peptide systems. Current drug abuse reviews. 2011; 4:163–173. [PubMed: 21999690]

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron. 2002; 34:13–25. [PubMed: 11931738]

Parker JA, Bloom SR. Hypothalamic neuropeptides and the regulation of appetite. Neuropharmacology. 2012; 63:18–30. [PubMed: 22369786]

Picciotto MR. Galanin and addiction. Exs. 2010; 102:195–208. [PubMed: 21299070]

Pliakas AM, Carlson RR, Neve RL, Konradi C, Nestler EJ, Carlezon WA Jr. Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. J Neurosci. 2001; 21:7397–7403. [PubMed: 11549750]

Rada P, Avena NM, Leibowitz SF, Hoebel BG. Ethanol intake is increased by injection of galanin in the paraventricular nucleus and reduced by a galanin antagonist. Alcohol. 2004; 33:91–97. [PubMed: 15528006]

Rada P, Mark GP, Hoebel BG. Galanin in the hypothalamus raises dopamine and lowers acetylcholine release in the nucleus accumbens: a possible mechanism for hypothalamic initiation of feeding behavior. Brain Res. 1998; 798:1–6. [PubMed: 9666056]

Rubino T, Sala M, Vigano D, Braida D, Castiglioni C, Limonta V, Guidali C, Realini N, Parolaro D. Cellular mechanisms underlying the anxiolytic effect of low doses of peripheral Delta9-tetrahydrocannabinol in rats. Neuropsychopharmacology. 2007; 32:2036–2045. [PubMed: 17287821]

Schneider ER, Rada P, Darby RD, Leibowitz SF, Hoebel BG. Orexigenic peptides and alcohol intake: differential effects of orexin, galanin, and ghrelin. Alcohol Clin Exp Res. 2007; 31:1858–1865. [PubMed: 17850217]

Sharma S, Fernandes MF, Fulton S. Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. Int J Obes (Lond). 2012

Sharma S, Fulton S. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. Int J Obes (Lond). 2013; 37:382–389. [PubMed: 22508336]

Shiflett MW, Mauna JC, Chipman AM, Peet E, Thiels E. Appetitive Pavlovian conditioned stimuli increase CREB phosphorylation in the nucleus accumbens. Neurobiol Learn Mem. 2009; 92:451–454. [PubMed: 19248836]

Smith BK, York DA, Bray GA. Effects of dietary preference and galanin administration in the paraventricular or amygdaloid nucleus on diet self-selection. Brain research bulletin. 1996; 39:149–154. [PubMed: 8866690]

Tempel DL, Leibowitz KJ, Leibowitz SF. Effects of PVN galanin on macronutrient selection. Peptides. 1988; 9:309–314. [PubMed: 2453854]

Tempel DL, Leibowitz SF. Diurnal variations in the feeding responses to norepinephrine, neuropeptide Y and galanin in the PVN. Brain research bulletin. 1990; 25:821–825. [PubMed: 1705161]

Volkow ND, Wang GJ, Fowler JS, Tomasi D, Baler R. Food and Drug Reward: Overlapping Circuits in Human Obesity and Addiction. Current topics in behavioral neurosciences. 2011

Zachariou V, Brunzell DH, Hawes J, Stedman DR, Bartfai T, Steiner RA, Wynick D, Langel U, Picciotto MR. The neuropeptide galanin modulates behavioral and neurochemical signs of opiate withdrawal. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:9028–9033. [PubMed: 12853567]

Zachariou V, Parikh K, Picciotto MR. Centrally administered galanin blocks morphine place preference in the mouse. Brain Res. 1999; 831:33–42. [PubMed: 10411981]

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Highlights

- Galanin in the PVN led to decreased pCREB in the NAc
- Galanin in the PVN had no effect on body weight, serum leptin and triglyceride levels
- High-fat diet, which is known to increase galanin, led to decreases in NAc pCREB
- High-fat diet led to increased body weight, and serum leptin and triglyceride levels

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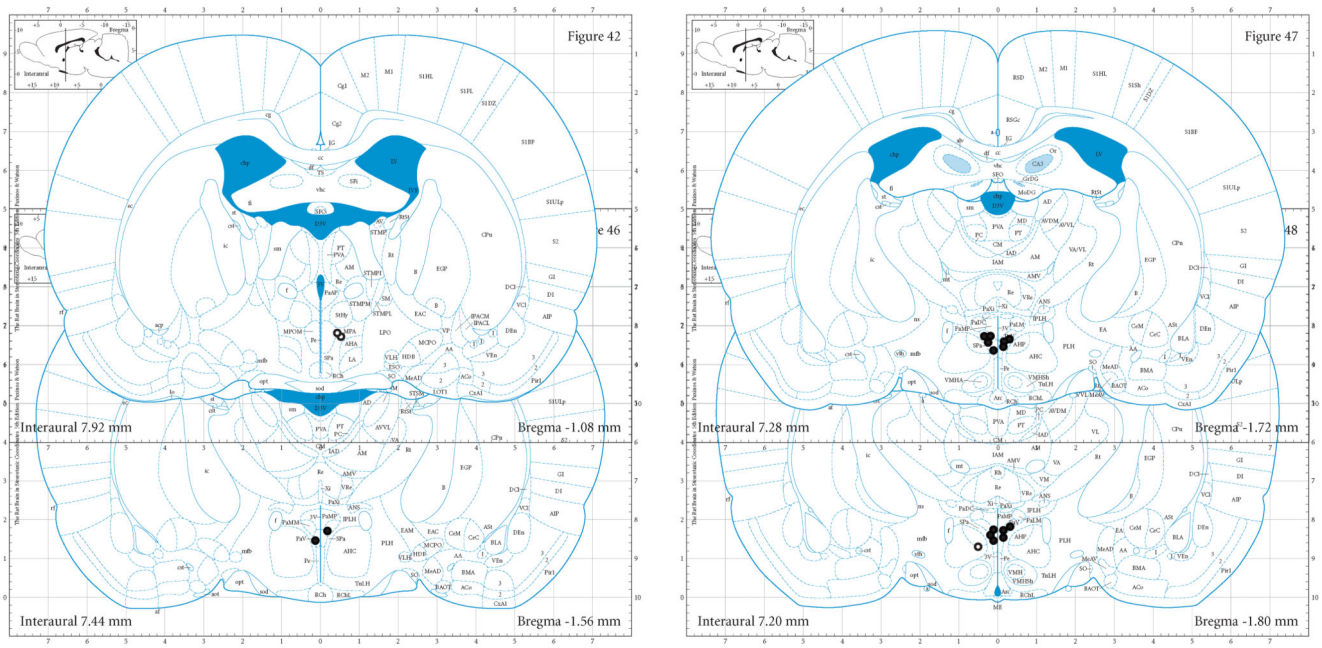

Fig. 1.

Locations of microinjection sites in the PVN. Solid circles indicate correct placement. Open circles indicate placement +0.5 mm from the target site, and thus were not included in the data analysis.

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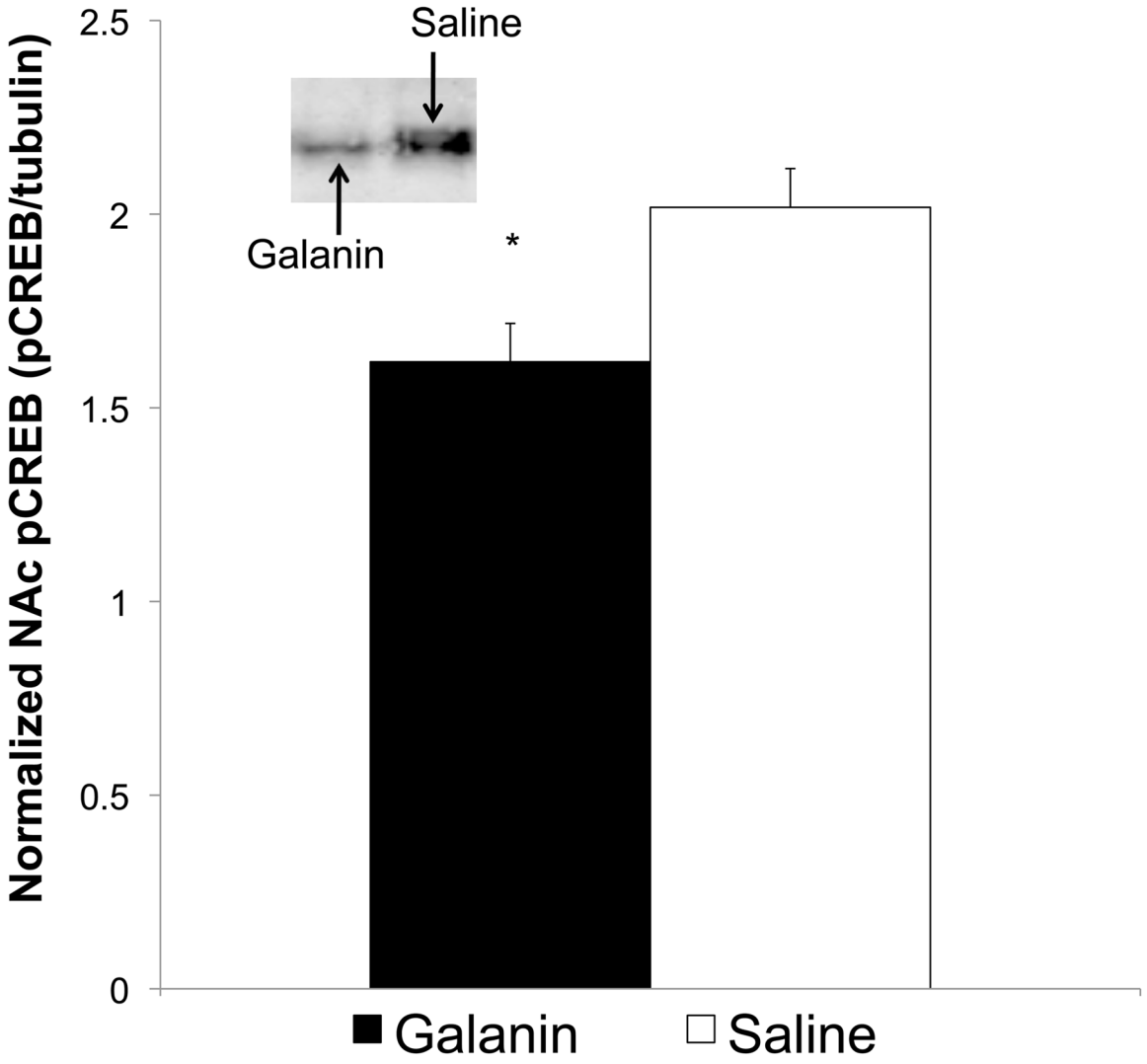

Fig. 2.

Normalized pCREB levels (pCREB/tubulin) in rats with daily injections of galanin were decreased compared to rats with weekly injections of saline. *=p<0.05

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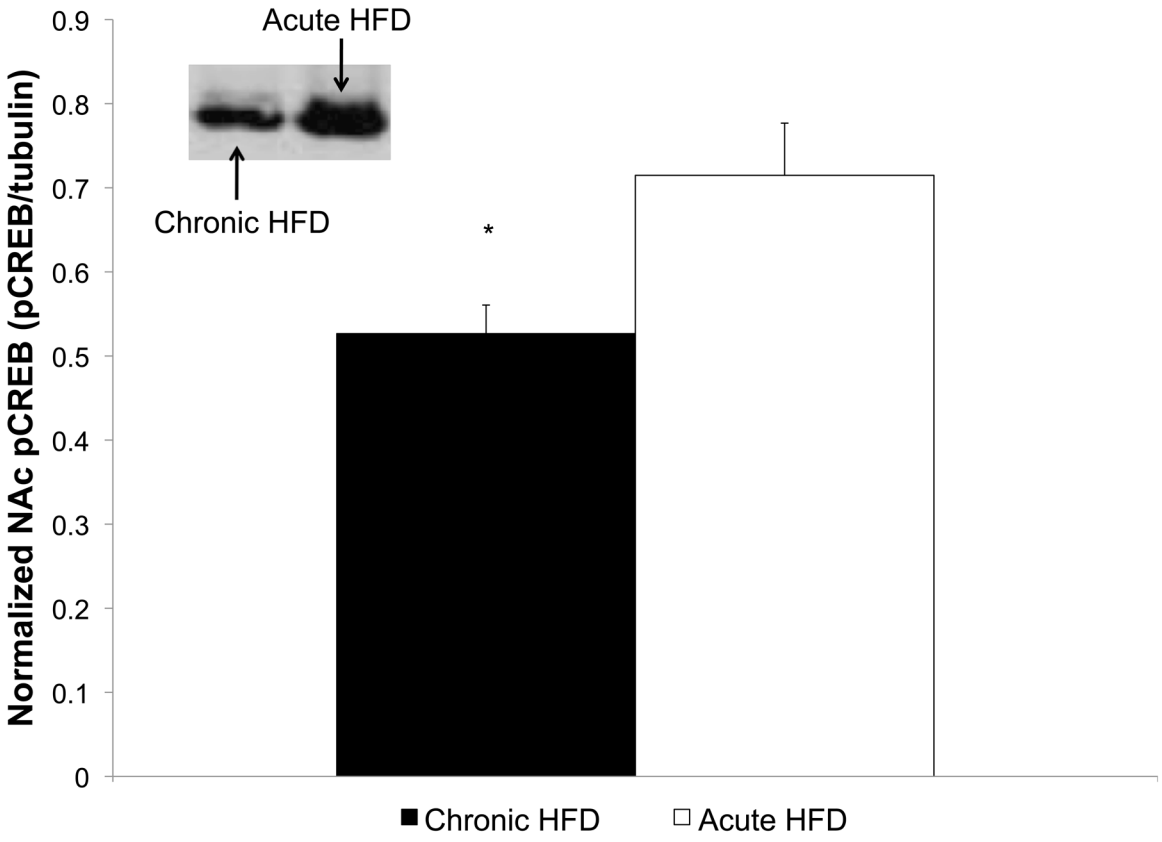

Fig. 3.

Normalized pCREB levels (pCREB/tubulin) in rats in the Chronic HFD group (maintained for 4 wks on a HFD) were decreased compared to rats in the Acute HFD group (maintained on a low-fat diet with weekly exposure to 15 Kcal HFD). *=$p<0.05$