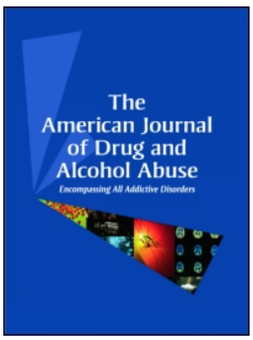

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# Augmentation of morphine-conditioned place preference by food restriction is associated with alterations in the oxytocin/oxytocin receptor in rat models

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**ABSTRACT**

*Background*: Studies indicate that food restriction (FR) reinforces the effects of morphine. The exact mechanisms by which FR influences the reward circuitry of morphine have not yet been determined.

*Objectives*: We hypothesized that the effects of FR on the oxytocin (OXT) system and HPA axis can be associated with substance abuse disorders. In this study, the serum levels of OXT and corticosterone, and the expression of *OXT*/*OXT* receptor (*OXTR*), glucocorticoid receptor (*GR*), and brain-derived neurotrophic factor (*BDNF)* in the hippocampus, prefrontal cortex, and nucleus accumbens were investigated in an FR model.

*Methods*: First, the male rats (n = 8 per group) were subjected to FR for 3 weeks. Then, morphine-induced conditioned place preference (CPP) was observed using two doses of morphine (3 and 5 mg/kg). The serum concentrations of corticosterone and OXT were determined by ELISA and the expression of genes was examined by qPCR.

*Results*: FR induced an enhanced preference in the animals for the 5 mg/kg dose of morphine compared to the controls. Serum corticosterone levels increased after FR but OXT levels decreased. Meanwhile, FR actuated downregulation of *GR, BDNF*, and *OXT* genes, while inducing the overexpression of *OXTR*.

*Conclusion*: We propose the inclusion of OXT and OXTR alterations in the enhancement of morphine-induced CPP and addiction vulnerability following FR. Moreover, we conclude that altered BDNF levels and HPA axis activity may be the mechanisms involved in the effects of FR on morphine-induced behavior.

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Food restriction; morphine-conditioned place preference; HPA; glucocorticoids; oxytocin; oxytocin receptor; BDNF

## Introduction

Opioid abuse is a global epidemic, that causes numerous physical and mental health problems, and imposes a considerable economic burden on society (1); it is a recurring chronic brain disorder, the emergence of which is triggered by socioeconomic and familial factors (2).

It is well-established that most psychosocial risk factors are patterned by socioeconomic conditions (2). Exposure to stressful conditions in lower socioeconomic strata is related to many harmful health effects. Socioeconomic inequalities can seriously jeopardize physical and mental health (3). Degradation of the quality of life is an important topic in psychiatric studies, and has a variety of detrimental effects on health (4). A stressor involves physiological and emotional reactions, followed by a variety of neural and hormonal mechanisms attempting to restore homeostasis.

Reinforcers like food can mediate the rewarding effects of abused drugs by activating the brain's reward system. The reward system is based on the functions of the prefrontal cortex (PFC), cingulate gyrus, amygdala, hippocampus, and nucleus accumbens (NAc) (5), with the NAc mediating the reinforcing effect of food. The striatum receives inputs from limbic cortical structures, such as the hippocampus and PFC (6). Food restriction (FR) augments self-administration and sensitivity to the locomotor-stimulating effects of many psychostimulants (7).

Changes in the effects of psychostimulant drugs induced by food deprivation are partly mediated by the dopamine reward system (8,9).

Evidence shows that the hypothalamic-pituitary-adrenal (HPA) axis and corticosteroids, which are involved in mediating addiction to a variety of narcotics, play a central role in stress-induced behavior (10). The inactivation of the glucocorticoid receptor gene in dopaminoceptive cells markedly decreases cocaine self-

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administration in mice (11). Additionally, plasma concentrations of corticosteroids increase during drug withdrawal. Vulnerability to drug relapse arises with changes in HPA activity under stressful conditions (12).

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is widely expressed and distributed in the central nervous system. It has an important role in the development, survival and plasticity of neurons (13). Stress exposure results in decreased BDNF mRNA levels in several brain areas in rodents (14–16).

Corticosterone levels increase during food deprivation (17,18), which suggests a relationship between increased levels of corticosterone and increased effects of psychostimulants caused by stress (19).

Augmentation of the rewarding effects of psychostimulants by FR is the result of stress that is induced by persistent underfeeding (20). A better understanding of the circuits and cellular mechanisms involved in drug abuse will lead to more effective therapeutic strategies for the prevention and treatment of substance abuse disorders (21). The augmentation of drug reward by FR may have clinical significance; a history of severe dieting is associated with the development of binge eating (22). In fact, the association between eating disorders and substance abuse is well-established (23). Individuals with eating disorders have a 5- to 6-fold higher risk of developing a substance abuse disorder than the general population and the highest prevalence of substance use exists in individuals with anorexia/bulimia (24).

Although FR reportedly changes neurotransmitter systems (25,26), further studies are needed to find the exact mechanism through which FR affects the brain's reward pathway for addictive drugs.

Oxytocin (OXT), a neuropeptide produced by the hypothalamus, is best known for stimulating the contraction of uterine smooth muscles during childbirth and the contraction of the muscles around milk-glands during breastfeeding. OXT is also involved in the regulation of social behavior including pair-bonding and social cognition (27). Importantly, centrally administered OXT affects feeding behavior and promotes the cessation of food intake (28). Interestingly, the rewarding effects of some psychostimulants are directly affected by OXT (29).

Oxytocin receptor (OXTR) expression has been identified in different parts of the central nervous system, such as in areas involved in reward processing (30). OXTRs in the NAc are implicated in pair-bond formation. Infusion of OXTR antagonist into the NAc blocks this phenomenon (31). Hippocampal projections to the PFC, hypothalamus, or amygdala promote OXTR-dependent influences on social behavior (32). Chronic stress induces an increase in *OXTR* mRNA expression in the hypothalamus (33). Additionally, acute stress induces higher localization of OXTR in the heart (34).

Recent studies have shown that the use of oxytocin receptor ligand reduces morphine- (35) and methamphetamine- (36) seeking behavior. Injection of OXT into the NAc decreases ethanol CPP (37). Neurochemical studies emphasize the relationship between OXT and dopamine in modulating the reward pathways. Central administration of OXT reduces the release of methamphetamine-stimulated dopamine in rats (38).

Although OXT is known to regulate food intake homeostasis, recent studies have revealed its effect on food reward and its key role in the reduction of food intake for pleasure and food preference (39). Behavioral and neuroendocrine processes related to food intake and addiction can overlap. For example, FR can increase self-injection of addictive drugs, while anorectic drugs have an opposite effect (39).

Considering the issues itemized above, this study examined the effects of FR on morphine-induced CPP, systemic OXT levels, and the mRNA expression of *OXT*/*OXTR* in three important areas of the mesocorticolimbic reward pathway including NAc, PFC, and hippocampus by qPCR. Moreover, we investigated the role of BDNF and HPA pathways as potential mechanisms involved in the effects of FR on morphine-induced behavior.

Two animal models have been widely used to examine the features of addiction; these are: drug self-administration and the conditioned place preference (CPP) paradigms. Unlike the self-administration paradigm, CPP paradigm does not require surgical implantation of a catheter for intravenous drug administration, nor does it require an extensive operant conditioning procedure; it is thus commonly used as a reliable model for testing the rewarding or aversive influences of drugs or experiences (40,41). The CPP paradigm consists of two distinct conditioning chambers that are repeatedly paired with drug and saline administrations; the times spent in each of the two sides are taken as a measure of conditioned incentive effects. An increase in preference for the drug-paired side reveals the motivational properties of the objects or treatments.

## Methods

### Animals

Adult male Wistar rats weighing between 200 and 240 g (2 months old) were obtained from Kerman Neuroscience Research Center's animal house and were housed in standard cages in groups of 4–5 rats. They were maintained under controlled temperature (22 ± 2°C) with a 12 h light/dark cycle (light on 08:00–20:00). Rats were randomly assigned to 6 groups: saline control group,

saline FR group, two morphine control groups, and two morphine FR groups. Animals were acclimated for at least one week before the experiment started. All animal experiments followed the NIH guidelines for Care and Use of the Laboratory animals and were approved by a local ethical committee.

### Drugs

Morphine sulfate was obtained from Temad (Iran) and was dissolved in 0.9% sterile saline immediately prior to use. Morphine and saline were injected intraperitoneally (i.p.) in a volume of 1 ml/kg.

### Food regime

Control groups received food *ad libitum*. The FR groups were given 10 gm meal every day at 08:00 h AM, which shows 40–50% of ad-lib food intake. After 2 weeks of this regimen, the initial body weight of rats decreased by 20%. Food intake was then titrated for 1 week to keep them at their reduced body weight (42). Animals remained in this restricted feeding till the end of the experiment. The FR groups were kept in a separated room, unable to see the feeding of the rats with full access to food.

### CPP apparatus

A three-compartment CPP apparatus was used. The CPP apparatus was made of wood (88 × 36 × 34 cm) and consisted of two equal-sized chambers (45 × 45 × 30 cm) separated by a small gray central (neutral) chamber (25 × 10 × 30 cm). Lateral compartments were connected through guillotine doors (10 × 10 cm) to the central chamber and had different colored walls and floor textures. One patterned with vertical black-and-white stripes with a smooth floor and the other designed with horizontal stripes with a rough floor.

### CPP paradigm

A CPP procedure was performed, which was counterbalanced, lasting ten days, comprised of three phases: preconditioning, conditioning, post-conditioning (43,44). CPP was carried out during the light period (10 a.m.–2 p.m.). In the pre-conditioning phase (lasting one day), we placed animals into the central chamber and granted them full access to the CPP apparatus for a quarter of an hour. The time spent in each chamber was recorded and the initial place preferences were determined. The rats that had displayed greater than 65% preference to one chamber were omitted from the study. During the conditioning phase (8 days), the morphine control groups and morphine FR groups were conditioned with morphine (3, 5 mg/kg, i.p.) four times on days 1, 3, 5, and 7 and four times with saline on days 2, 4, 6 and 8. Immediately after the injection, the animals were placed in the assigned conditioning chambers with the connection doors closed and were allowed 45 minutes to explore the chamber. On the day after the last conditioning session, or the post-conditioning phase (1 day), without any injection, guillotine doors were left open and the animals had full access to all chambers for 15 minutes. The duration spent by each rat in the compartments was recorded by a video camera and analyzed using the Ethovision software. Conditioning scores were assessed as the time spent in the morphine-paired compartment minus the time spent in the saline-paired compartment.

### Experimental design

In the current study, we used rats in two different conditions. As shown in Figure 1, for CPP assay, after 3 weeks of food schedule, rats (n = 48) were subjected to CPP test. For hormone assays and gene expression experiments, separate groups of rats (n = 16) were used, and rats were sacrificed the day after the end of 3 weeks of food schedule.

### Tissue isolation and RNA extraction

Rats were decapitated the day after the end of food schedule. The hippocampus, NAc, and PFC were quickly removed, frozen on dry ice and stored at −80°C.

Total RNA was extracted from each tissue sample by the Trizol reagent (Sigma, Taufkirchen, Germany). Total RNA treated with DNase (RNase-free DNase set from Roche, Manheim, Germany). The final RNA pellets were resuspended in 30 µl diethyl-pyrocarbonate-treated water (DEPC-treated water). The amount of the purified RNA (A260⁄A280 ratio was ≥1.9) was determined by Nanodrop and the integrity of RNA samples was analyzed on a 1.5% agarose gel (Sigma). RNA was stored at −80°C until further analysis.

### Reverse transcription

Briefly, the reaction was performed using Oligo-dT primer and M-MuLV reverse transcriptase (Thermo Fisher Scientific, Germany) based on the manufacturer's protocol. The reproducibility of single results was determined with two strategies: two time measurement of cDNA aliquots; analysis of two different cDNA prepared from the same RNA extract.

### Quantitative polymerase chain reaction (qPCR)

Quantification of relative RNA expression followed established method using qPCR with the SYBR green reporter dye and protocol. The 2Xuniversal master mix (Takara, Japan) was used in the PCR reactions. Thermal cycling utilized a Bio-Rad iQ5 detection System (Bio-Rad, Richmond, CA, USA). A final melting curve of fluorescence versus temperature was generated to screen for primer dimers and to document single product formation. Primer sequences, RT-PCR fragment lengths and NCBI accession numbers are reported in Table 1.

All primer pairs produced a single band on agarose gel electrophoresis corresponding to the predicted size. The amount of PCR products were normalized with housekeeping (*GAPDH*) primers in separate reactions. All samples were assayed in triplicate. Linearity and efficiency of PCR amplification were assessed using standard curves generated by decreasing amount of cDNA, using five points, diluted over a twofold range. In qPCR, the relative mRNA levels were calculated by the expression $2^{-\Delta\Delta CT}$ (45).

### Hormone assay

At the end of the experiment, trunk blood was collected. Sera were aliquoted and kept at −80°C until assays. Serum levels of corticosterone and OXT were quantified by rat-specific ELISA Kits (ZellBio GmbH, Ulm, Germany) according to the manufacturer's instructions. The assay sensitivity of corticosterone and OXT were 1.63 ng/L and 1 ng/L, respectively. Intra-assay and inter-assay coefficients of variability for corticosterone were 4.7% and 6.3% and for OXT they were lower than 10% and 12% respectively.

### Data analysis

In the CPP study, for determining the effect of FR on CPP scores, a two-way (morphine dose × feeding condition) analysis of variance (ANOVA) was used. A two-way repeated measures ANOVA [between-subjects factors: feeding condition, morphine dose; within-subjects factors: conditioning day (pre-conditioning, post-conditioning)] was used to determine if morphine has a significant effect

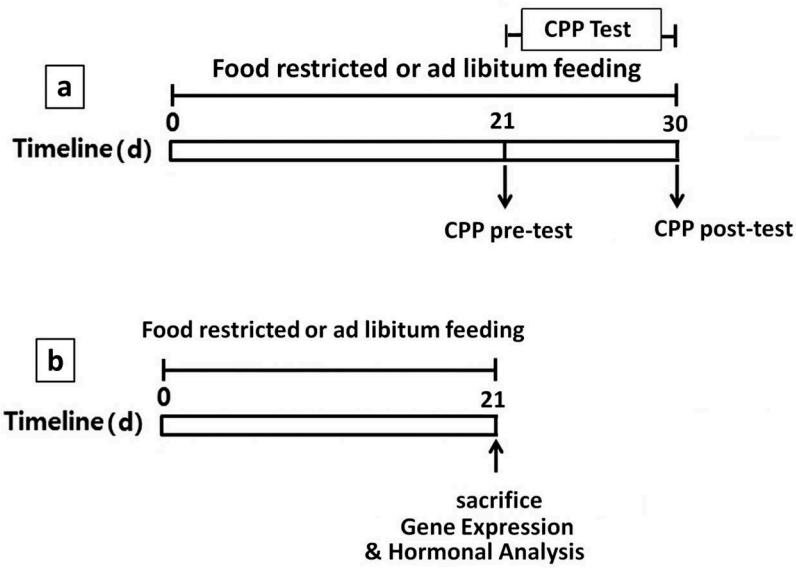


**Figure 1.** Timeline of the experiments. (a) CPP test. (b) Hormone assays and gene expression experiments.

**Table 1.** Primer sequences.

| Primer name | Primer sequence | Size of PCR product | NCBI accession number |
|---|---|---|---|
| GAPDH | F: GTCTTCACCACCACGGAGAAGGC<br>R: ATGCCAGTGAGCTTCCCGTTCAGC | 392 | NM_017008.4 |
| BDNF | F: CGTGATCGAGGAGCTGTTGG<br>R: CTGCTTCAGTTGGCCTTTCG | 343 | NM_001270630.1 |
| OXT | F: CCTGGATATGCGCAAGTGTCTTC<br>R: TCGGAGAAGGCAGACTCAGG | 267 | NM_012996.3 |
| OXTR | F: GGCTGCCGAGGGGAATGAC<br>R: ATGGCAATGATGAAGGCAGAAGC | 222 | NM_012871.3 |
| GR | F: ACCCTGCATGTATGACCAATGT<br>R: TTAGGAACTGAGGAGAGAAGCAGTA | 123 | NM_012576.2 |

on the CPP scores compared to pre-conditioning day. Tukey's post hoc test is used when applicable. Independent samples T-test was performed for analysis of the gene expression and hormone assay results. $P < .05$ was regarded statistically significant.

## Results

### Effect of food restriction on morphine-induced CPP

Two-way repeated measures ANOVA revealed a significant effect of conditioning day [$F(1,42) = 490.2$, $P < .001$], and conditioning day × morphine dose interaction [$F(2,42) = 154.2$, $P < .001$]. Tukey's test showed that control and food-restricted rats conditioned with 3 mg/kg, and 5 mg/kg morphine spent significantly more time ($P < .001$) in the drug-paired side in post-conditioning, based on CPP scores, compared to pre-conditioning (Figure 2).

Two-way ANOVA revealed a significant effect of feeding condition [$F(1,42) = 5.44$, $P < .05$], and morphine dose [$F(2,42) = 211.36$, $P < .001$]. Tukey's test showed that food-restricted rats experienced greater place preference, based on CPP scores, than the control rats at 5 mg/kg morphine ($P < .01$), but there was not a significant difference for dose of 3 mg/kg morphine (Figure 2).

### Hormone levels

Independent Samples T-test was used to compare hormone concentrations in food-restricted rats with controls. The statistical analysis of data revealed that corticosterone levels were higher in animals exposed to FR ($t = -5.35$, $P < .001$) than the control group (See Figure 3).

Figure 4 depicts serum concentration levels of OXT in the groups. The statistical analysis of data by independent Samples T-test showed that the serum OXT levels were lower in FR ($t = 6.38$, $P < .001$) compared to control group.

### qPCR results

All primer pairs produced a single band on agarose gel electrophoresis corresponding to the predicted size as depicted in Figure 5.

The gene expression results were analyzed by independent samples T-test. The effect of FR on *GR* mRNA expression levels is shown in Figure 6. The statistical analysis of data by independent Samples T-test showed that FR decreased the expression of *GR* mRNA in the hippocampus ($t = -7.03$, $P < .001$), PFC ($t = -7.62$, $P < .001$), and NAc ($t = -5.99$, $P < .001$) compared to control group.

The effect of the FR on *BDNF* mRNA expression levels is shown in Figure 7. The statistical analysis of data by independent Samples T-test showed that FR decreased the expression of *BDNF* mRNA in the hippocampus ($t = -4.05$, $P < .01$), and NAc ($t = -2.84$, $P < .05$) compared to control group. Decrease of *BDNF* mRNA expression in the PFC wasn't statistically significant.

The effect of the FR on *OXT* mRNA expression levels in the hippocampus, PFC, and NAc is shown in

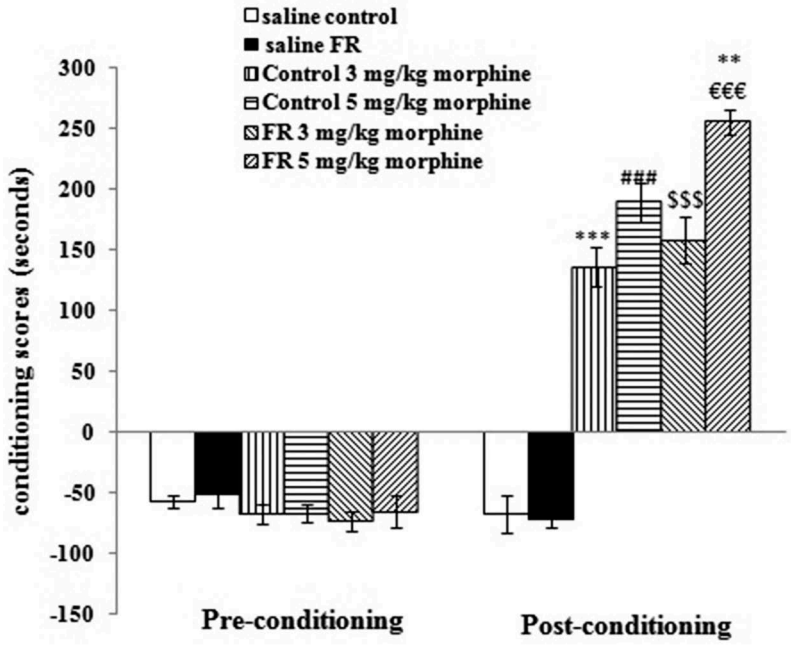


**Figure 2.** Effect of FR on morphine-induced CPP. Data are presented as Mean ± SEM. *** P< 0.001, ### P< 0.001, $$$ P< 0.001, €€€ P< 0.001: significant differences in CPP scores in post-conditioning compared to pre-conditioning. ** P<0.01, significant differences in CPP scores compared to control 5 mg/kg morphine post-conditioning. FR: food restriction.

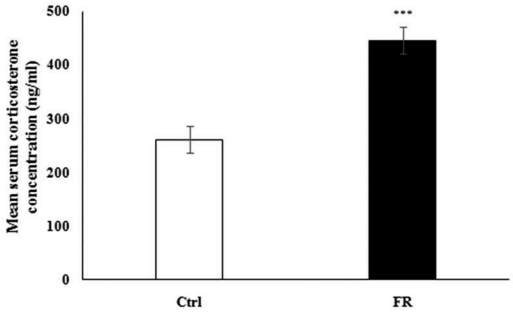


**Figure 3.** The effect of FR on serum corticosterone concentration. Values are means ± SEM. *** $P < .001$ compared to control group. FR: food restriction; Ctrl: control.

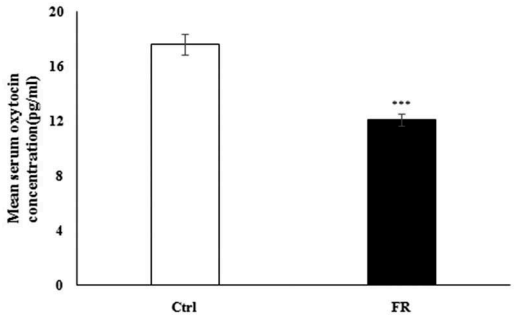


**Figure 4.** The effect of FR on serum OXT concentration. Values are means ± SEM. *** $P < .001$ compared to control group. FR: food restriction; Ctrl: control.

Figure 8. The statistical analysis of data by independent Samples T-test showed that FR decreased the expression of *OXT* mRNA in the hippocampus (t = −8.09, $P < .001$), PFC (t = −3.02, $P < .05$), and NAc (t = −7.47, $P < .001$) compared to control group.

The effect of the FR on *OXTR* mRNA expression levels in the hippocampus, PFC, and NAc is shown in Figure 9. The statistical analysis of data by independent Samples T-test showed that FR increased the expression of *OXTR* mRNA in the hippocampus (t = 3.85, $P < .01$), PFC (t = 3.98, $P < .01$), and NAc (t = 2.97, $P < .05$) compared to control group.

## Discussion

Previous studies have shown that reinforcers like food can augment self-administration and sensitivity to the locomotor-stimulating effects of many psychostimulants (7).

Using a CPP paradigm, the present study investigated the effects of FR on morphine-rewarding effects. We used two doses of morphine to determine that animals exposed to FR were more sensitive to morphine than control animals. Our results showed that a dose of 5 mg/kg of morphine caused a distinction between food-restricted and *ad libitum*-fed rats in morphine CPP, however a statistically significant difference was not found between the two groups for a dose of 3 mg/kg of morphine. The findings obtained from our experiments indicate that FR increases morphine CPP, a result which is in accordance with previous research. Previous studies have indicated that food-deprived rats are more susceptible to the reinstatement of morphine CPP. Further, food deprivation induces heroin-seeking behavior compared to *ad libitum*-fed control animals (46,47). Another study on

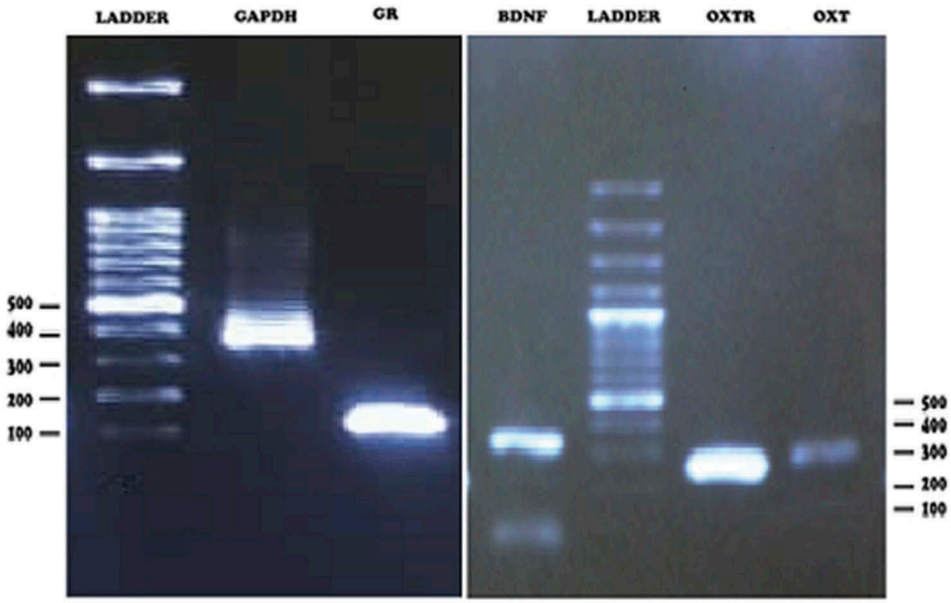


**Figure 5.** Agarose gel electrophoresis of *GAPDH, GR, BDNF, OXTR*, and *OXT* cDNA amplification products under UV light. From left: *GAPDH* (392 bp); *GR* (123 bp); *BDNF* (343 bp); Marker 100 bp–3 kb DNA Ladder (Smobio, Taiwan); *OXTR* (222 bp); *OXT* (267 bp).

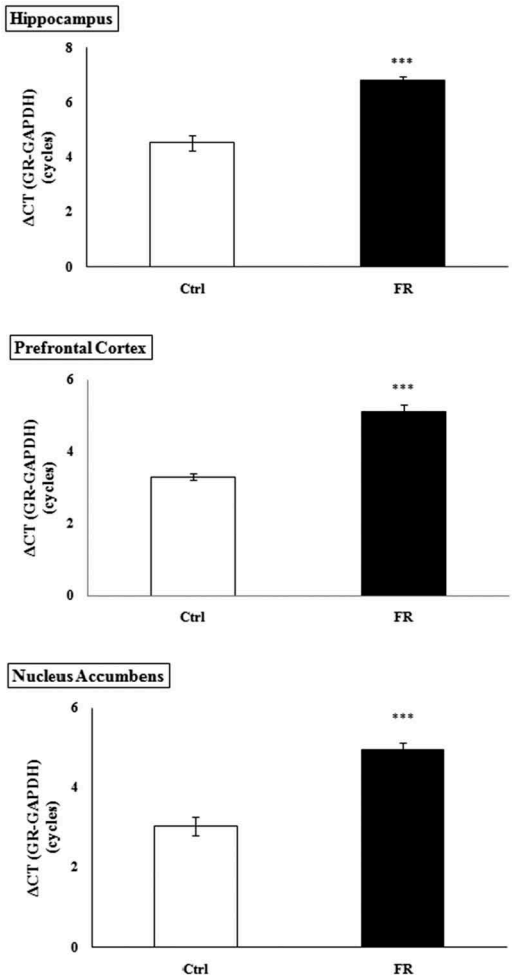


**Figure 6.** Effects of FR on *GR* mRNA levels in the hippocampus, prefrontal cortex, and nucleus accumbens. Each point represents mean ± S.E.M and *** $P < .001$ vs. control. Ctrl: control; FR: food restriction.

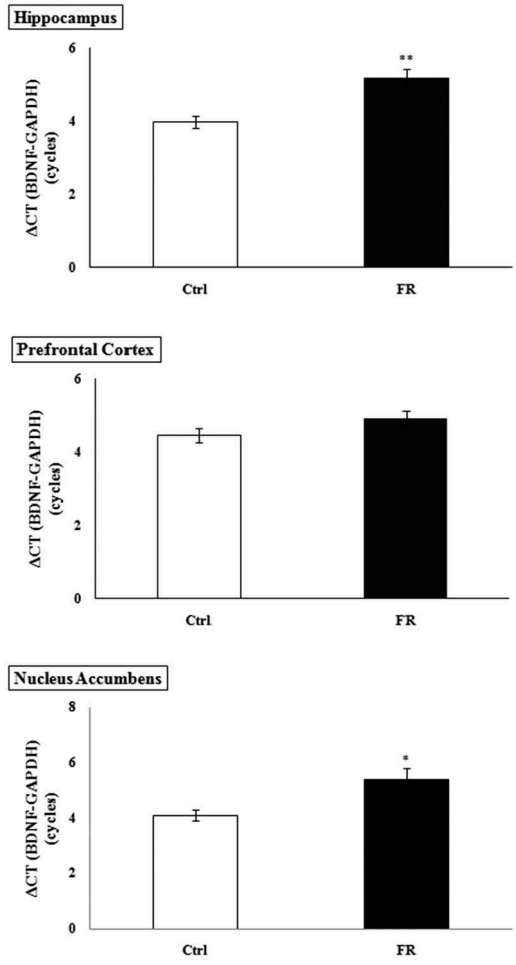


**Figure 7.** Effects of FR on *BDNF* mRNA levels in the hippocampus, prefrontal cortex, and nucleus accumbens. Each point represents mean ± S.E.M and ** $P < .01$ and * $P < .05$ vs. control. Ctrl: control; FR: food restriction.

psychostimulants showed that FR increased the sensitivity of CPP to low doses of amphetamine (48). Additionally, it has been established that food deprivation considerably augments the rewarding effect, and leads to more frequent instances of self-administration of cocaine, thereby promoting the risk of relapse. Food-deprived rats are more susceptible to cocaine in a CPP paradigm than the *ad libitum*-fed ones (49,50).

The exact mechanism by which food-restricted rats show higher morphine preference compared to control animals in the CPP paradigm is unknown. Our experiments examined three possible mechanisms. The first mechanism may be through the HPA axis. Oversecretion of glucocorticoid levels is essential for an effective stress response, and prolonged exposure to elevated glucocorticoids endangers the sensitive areas of the brain (51). The PFC region of the brain that is most sensitive to the harmful effects of stress. It is able to regulate emotional responses with extensive connections to subcortical structures (such as the NAc and the hypothalamus) (52). According to our results, corticosterone concentrations were higher in FR groups compared to control subjects. In a previous study, the period for development of withdrawal symptoms was found to decrease with cortisone or adrenocorticotropic hormone treatment and chronic treatment with corticosteroids resulted in delayed withdrawal symptoms (53). Adrenal-mediated neuroadaptation in corticotropin-releasing factor responsiveness is involved in stress-induced reinstatement of cocaine self-administration (54). Cocaine priming-induced reinstatement of cocaine-seeking behavior in squirrel monkeys was not modulated by corticotropin-releasing hormone receptor 1 antagonist, and HPA axis activation was not necessary for cocaine-induced reinstatement in this non-human primate model (55).

In the present study, FR led to elevated serum corticosterone concentrations. Moreover, our results have shown that FR reduces the expression of glucocorticoid receptor (*GR*) mRNA in the PFC, hippocampus and NAc of the

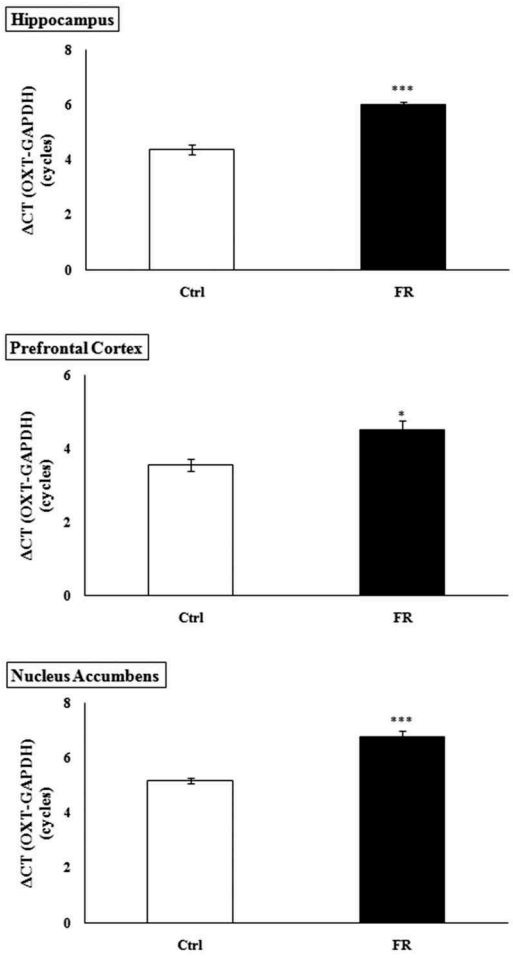


**Figure 8.** Effects of FR on *OXT* mRNA levels in the hippocampus, prefrontal cortex, and nucleus accumbens. Each point represents mean ± S.E.M and *** $P < .001$ and * $P < .05$ vs. control group, Ctrl: control; FR: food restriction.

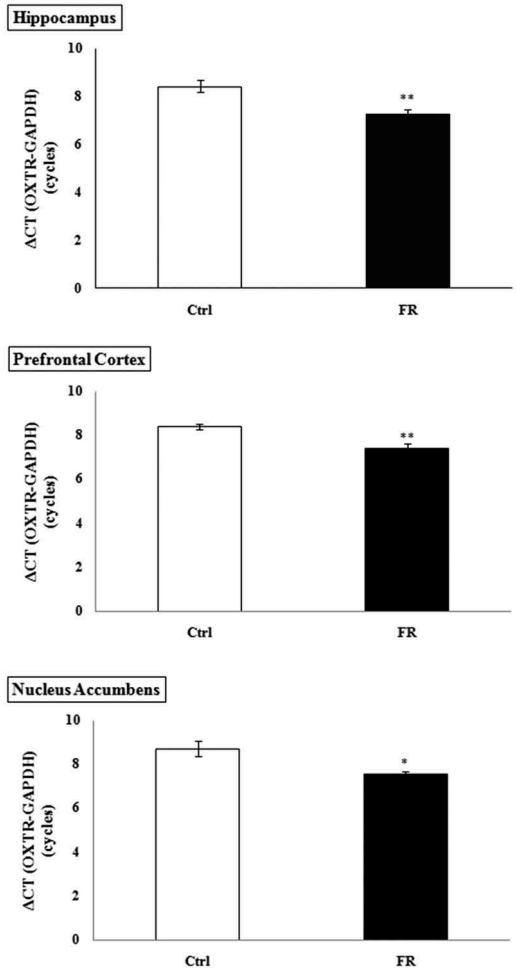


**Figure 9.** Effects of FR on *OXTR* mRNA levels in the hippocampus, prefrontal cortex, and nucleus accumbens. Each point represents mean ± S.E.M and ** $P < .01$ and * $P < .05$ vs. control, Ctrl: control; FR: food restriction.

animal brain. To the best of our knowledge, the effect of FR on the expression of this receptor has not been investigated, but the reductive effect of stress on its expression has been reported. In chronic stress, there is excess secretion of glucocorticoids that reduces GR levels in certain regions of the brain by a negative feedback mechanism (56).

Increased sensitization to the conditioned rewarding effects of morphine in FR may be partly due to greater reward system responsivity. Comparison of the acquisition of cocaine self-administration between controls and rats treated daily with corticosterone showed that cocaine self-administration is facilitated by prior exposure to corticosterone (57). Corticosterone circadian secretions change the reactivity of mesolimbic dopamine (DA) neurons and influence the impact of cocaine and morphine (58). The dopamine-mediated behavioral effects of morphine are facilitated by the action of glucocorticoid hormones on GRs (59). Administration of a GR antagonist to the hippocampus and NAc dose-dependently decreased the cocaine self-administration and morphine-CPP; therefore, GR could provide a potential target to reduce cocaine use disorder (60). A recent study showed that cocaine users had lower GR expression compared to control individuals (61). Inactivation of the *GR* gene within dopaminoceptive neurons diminishes behavioral responses to amphetamine and cocaine (62).

The results of our qPCR study revealed a corresponding difference in the expression of BDNF as an anxiety- and stress-related gene in the hippocampus and NAc of FR exposed rats. BDNF is a member of the neurotrophin family that widely expressed in the central nervous system, and is involved in the development, survival, maintenance and plasticity of neurons. Regulation of BDNF expression by glucocorticoids may play a significant role in stress-induced behavioral changes (63). Previous studies have shown that expression of BDNF decreases under different types of stresses and decreased BDNF may contribute to depression and other mental health diseases (64,65). Studies that have examined the effects of FR

on changes in the BDNF expression levels in the brain have reported both reduction and increase in the levels of *BDNF* mRNA (66–68). A possible explanation for this inconsistency may be due to the type of FR regimen used, which can have different physiological and behavioral impacts in rats. Another possible explanation is that some of the studies investigated *BDNF* mRNA expression in regions of the brain that were different from those examined in our study.

The OXT system is the other possible mechanism that may explain changes in morphine-induced CPP in food-restricted rats. Our results revealed that compared to control animals, plasma OXT concentrations were lower in animals exposed to FR. Many studies have investigated the role of OXT in various drug-related behaviors. Intraperitoneal administration of OXT dose-dependently reduced methamphetamine self-administration and methamphetamine-induced behaviors in rats (69). Other studies reported that exogenous administration of OXT inhibits acute cocaine-induced locomotor hyperactivity, seeking behavior, and sensitization to cocaine in rodents (70,71). A number of reports cite the role of OXT in social behavior and stress regulation. Moreover, *OXT*-knockout mice were more aggressive and showed more social dominance behaviors than the wildtype mice (72). Contrary to these findings, some studies have reported an increase in OXT levels in rodents exposed to a variety of stressful stimuli like fear conditioning and restraint stress (73). Furthermore, increased OXT levels have been reported in humans exposed to uncontrollable noise and different types of psychosocial stresses. Likewise, it is hypothesized that OXT exerts a dampening effect on response to stressful events (74). Experimental enhancement of OXT diminishes response to stress. Plasma OXT levels can be used as diagnostic evidence of personal distress (75).

In this study, we evaluated the expression of OXT and its receptor in the brain. Remarkably, we found significant alterations in *OXT* mRNA levels. Compared to control animals, animals subjected to FR exhibited decreased *OXT* mRNA expression in three important areas of the mesocorticolimbic reward pathway, including the NAc, PFC, and hippocampus.

The medial prefrontal cortex (mPFC) is a region where OXT administration may attenuate anxiety-related behavior. Furthermore, OXT can suppress the anxiety-like behavior of rats through the following: attenuation of glutamatergic transmission in the mPFC; an increase in GABA release; and decreased excitation of glutamatergic projections from the mPFC to the central nucleus of the amygdala (76). Evidence also indicates that stress-induced decrease in OXT levels in the lateral septum and amygdala reinstates the morphine CPP (77).

In our study, in rats exposed to FR, OXT levels decreased in the serum, and there was an increase in the *OXTR* mRNA levels in the NAc, PFC, and hippocampus. The biological effects of OXT are exerted by its binding to its receptor (OXTR). It has been established that the PFC is involved in the response to stress and expresses OXTRs (78). The upregulation of OXTRs in the brain may be a feedback response to the decreased levels of OXT. To the best of our knowledge, few studies have examined the effects of FR on changes in the OXT system in the brain; the expression of OXTR in the PFC and hippocampus of food-restricted rats has not yet been studied. A previous report revealed that *OXTR* mRNA expression in the NAc core was elevated in rats exposed to food deprivation (79).

## Conclusion

In conclusion, these findings demonstrate that FR resulted in the augmentation of morphine-induced CPP. Further, FR increased serum corticosterone levels, but decreased the OXT levels. Our data indicate that disruption of the HPA axis response and alterations in the OXT/OXTR system may contribute to an increase in morphine-induced CPP and vulnerability to addiction following FR. Perhaps these central changes could explain the higher sensitivity to morphine in animals exposed to FR. Further studies are required to identify other mechanisms underlying behavioral and physiological responses to FR.

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## Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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