Chapter

Corticotrophin-Releasing Factor (CRF) Through CRF1 Receptor Facilitates the Expression of Morphine-Related Positive and Aversive Memory in Mice

Pilar Almela, Juan A. García-Carmona, Elena Martínez-Laorden, María V. Milanés and María L. Laorden

Abstract

Different studies have elucidated the mechanisms underlying the formation and expression of drug-related cue memories; corticotrophin-releasing factor (CRF) plays a critical role in reward- and aversion-driven associative learning. In the present chapter, we have evaluated whether CP-154,526, a selective CRF1 receptor (CRF1R) antagonist, or genetic deletion of CRF1R (KO mice) have comparable effects on conditioned place preference (CPP) and conditioned place aversion (CPA) learning. We also investigated CP-154,526 effects on morphine-induced CPP activation of CRF, CREB phosphorylation, and thioredoxin (Trx1) expression in dentate gyrus (DG), a brain region involved in memory consolidation, and the role of hypothalamic-pituitary-adrenocortical (HPA) axis in CPA expression and extinction. The CRF1R antagonist abolished the acquisition of morphine CPP, Trx-1 and BDNF increased expression, and pCREB/Trx-1 co-localization in the DG. The increase in adrenocorticotropic hormone (ACTH) plasma levels observed after CPA expression was attenuated in CRF1R KO mice, suggesting a role of HPA axis in aversive memories. Altogether, these results suggest a critical role of CRF, through CRF1R, in molecular changes involved in memory formation and consolidation and may facilitate the development of effective treatments for opioid addiction.

Keywords: conditioned place preference, conditioned place aversion, morphine, hippocampus, CRF, HPA axis

1. Introduction

Drug addiction is a chronic brain disease with a high rate of relapse [1–3]. Despite years of abstinence from drugs, relapse can occur when addicts encounter cues, including people or places, associated with their prior drug use [4]. Drug-associated memory can persist throughout the lifetime of a patient; therefore, the elimination of this kind of memory is considered to be crucial for the treatment of drug addiction.

In organism and human models, drug reward can be assessed using a Pavlovian conditioning procedure known as conditioned place preference/conditioned place aversion (CPP/CPA) [5–7]. CPP for the drug-paired environment is predicted by self-reported measures of drug liking in humans [6]. CPA for the drug-paired environment is used to infer the dysphoric properties of drugs, including opioid receptor antagonists [8]. Many neurotransmitters, neurotrophic factors, and protein kinases have been delineated in the regulation of the formation and expression of drug-associated reward memories and withdrawal-associated aversive memories [9–13].

Corticotrophin-releasing factor (CRF) in the brain plays a critical role in reward- and aversion-driven associative learning. However, it is not clear whether it does this by a common mechanism or by separated mechanisms that can be dissociated. The knowledge of these mechanisms could lead to more effective treatments for addictive processes. CRF and its CRF1 receptor (CRF1R) are widely distributed and in a highly conserved way in several brain regions, including the hippocampal formation, involved in reward reinforcement, craving and aversive effects of drug of abuse [14–17]. At the extrahypothalamic level, CRF acts as a neuroregulator of the behavioral and emotional integration of environmental and endogenous stimuli associated with drug dependence [18, 19]. In the hippocampal dentate gyrus (DG), an important brain region involved in saving similar experiences and contexts [20], CRF is released from inhibitory interneurons [21] through CRF1R [14] by environmental signals. CRF1R activation stimulates Gαs protein, promoting the induction of the protein kinase A/cAMP response element binding protein (CREB) pathway [22]. CREB activity in the brain is critical for learning and memory processes [23], and it has been reported to be involved in the expression of opioid dependence. The activation of CREB, as one of the main downstream effectors of extracellular signal-regulated kinase (ERK), accelerates the transcription of CREB-dependent genes such as the brain-derived neurotrophic factor (BDNF). With respect to hypothalamus, CRF release from paraventricular nucleus (PVN) controls the hypothalamic-pituitary-adrenal (HPA) axis responses to stress and drug addiction [24–26]. CRF neurons in the PVN and CRF fibber in DG have direct connexion with dopaminergic neurons located in the ventral tegmental area (VTA) projecting to nucleus accumbens (NAc) [27, 28].

2. Role of CRF in the rewarding effects of morphine

CPP is an animal model widely used to evaluate the correlation between contexts and drugs. Different substances of abuse display differential ability to produce CPP. Opiates induce strong CPP over a wide range of experimental conditions [5]. Previous studies from our laboratory [29–32] and others [33, 34] have demonstrated that morphine administration evokes significant CPP for the drug-associated environment. Different neurobiological substrates have been involved in the rewarding properties of drugs of abuse, although the mesolimbic dopaminergic pathway has been pointed out to be the critical system for drug reward. Recently, it has been suggested that PVN may have a role in the reinforcing effects of opioids [35]. Various studies have elucidated the mechanisms underlying the formation and expression of drug-related cue memories. CRF in the brain plays a critical role in reward-driven associative learning. During the formation or consolidation process (CPP expression), the majority of the CRF-positive neurons in the PVN, central nucleus of amygdale (CeA), and bed nucleus of stria terminalis (BNST) coexpresses pCREB after morphine-induced CPP, suggesting that drug-paired context could trigger neuronal activity in the brain stress system [29]. Morphine-treated mice in their home cage do not show any changes in total CRF/CREB positive neurons, indicating

that the exposure to drug-paired environments is necessary for CRF activation in the brain stress system [29]. Anatomical and functional studies reveal connections between CRF and the mesolimbic dopaminergic system. Thus, VTA and NAc receive CRF-positive projections from the PVN and stress extrahypothalamic areas [36, 37], which have been proposed to regulate dopamine release. The rewarding effect of morphine (CPP expression) is decreased by pretreatment with CP-154,526, a selective CRF1 antagonist, suggesting an important role of CRF/CRF1 receptor in memory formation and consolidation [30].

2.1 Implications of different signaling pathways in the rewarding effects of morphine. Role of CRF1 receptors

Hippocampus is a brain region known to participate in associative processes such as declarative memory, and PVN is an important stress area. Both structures are related with mesolimbic pathways [38]. Our group has studied the implication of different signaling pathways in both areas, because the understanding of how the formation of drug-reward memories alters the neurobiology of the hippocampal DG and PVN, and may shed light on the later and more persistent aspect of addiction.

The transcription factor CREB is critical in the conversion from short-term to long-term memory, and it is involved in the creation of long-term memory. Learning and memory and drug addiction share certain intracellular signaling pathways and depend on activation of CREB [39]. According to previous studies [40, 41], our laboratory has demonstrated that the number of pCREB positive neurons in PVN and DG is significantly increased after morphine-induced CPP expression (**Figure 1**). Since CRF1R is coupled to stimulatory G protein Gαs and can thus activate PKA and, subsequently, CREB [22], our group has investigated if CRF1R signaling is involved in CREB activity after morphine-induced CPP. Administration of the CRF1R antagonist, CP-154,526, completely revoked pCREB positive neuron enhancement induced by morphine in PVN and slightly in DG. CREB involvement in morphine dependence has been previously supported by studies demonstrating that CREB mutant mice do not respond to the reinforcing properties of morphine in a conditioned place preference paradigm [42], suggesting that specific CREB functions are necessary for the rewarding properties of this drug.

Although it is known that CRF signaling is involved in the drug withdrawal-induced anxiogenic-like and negative behavioral response [43], no definitive data are available about the role in the positive reinforcing properties of opiates. CRF-immunoreactive fibers densely innervate many intrahypothalamic and extrahypothalamic brain areas, such as hippocampus. Besides, CRF, through CRF1R, increases neuronal activity propagation from DG, the classical hippocampal input region, to the hypothalamic structure CA1 [44]. CRF is present in GABAergic hippocampal neurons of the pyramidal cells [14]. The supramammillary (SuM) region of the hypothalamus acts a connection nucleus between limbic and hypothalamic structures involved in controlling cognitive aspects [45]. Thus, SuM sends robust and direct inputs to DG. For example, it has been shown that mild stress could activate the SuM cells that project to the hippocampus [46]. Our group has previously shown that most of the CRF positive neurons in PVN coexpresses pCREB during morphine CPP. In addition, we have observed an enhancement in CRF fibers density in DG after morphine administration. Both changes were antagonized by injection of CP-154,526 (**Figure 2**). CRF binding to CRF1R results in activation of heterotrimeric G-proteins. The physiological functions of CRF1R in the central nervous system and in the periphery have been mainly associated to an increase in intracellular cAMP levels. This is consistent with a predominant coupling to Gαs

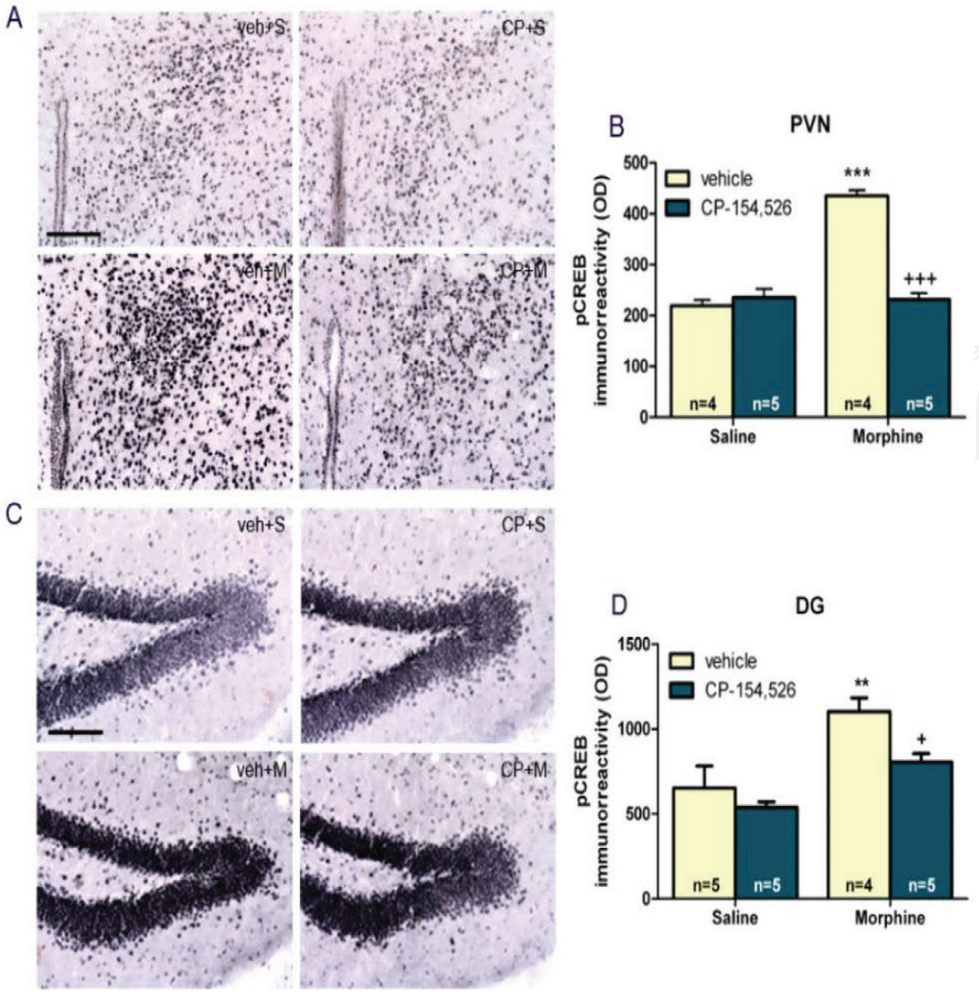

Figure 1.
CREB activation in PVN (A) and DG (C) after morphine-induced CPP. Scale bar 100 μm. Quantitative analysis of pCREB immunodetection in PVN (B) and DG (D). Data are expressed as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$ versus vehicle (veh) + saline (S); $+p < 0.05$, $+++p < 0.001$ versus veh + morphine (M). CP-154,526 (CP). Optical density (OD).

(cAMP/PKA/CREB). However, CRF through CRFR1 is capable of activating other Gα types such as Gαs and activate inositol triphosphate (IP3) cascade. An enhancement in the concentration of secondary messengers (cAMP, IP3, and Ca^{2+}) in cells, induced by CRF1R agonists, promotes the activation of several transcriptional factors such as CREB, AP-1, NF-κB, and the calcium response element (CARE) [47–53]. In this sense, the antagonist of the CRF1R, CP-154,526, by blocking the postsynaptic CRF1R, inhibited CREB phosphorylation in PVN and DG. Moreover, morphine treatment induced an increase in CRF fiber immunodetection in DG, suggesting an elevated CRF release, which was prevented by pretreatment with this antagonist. Since CRF1R activation increases Ca^{2+} levels, it is possible that CP-154,526 inhibits CRF release by blocking presynaptic CRF1R in PVN.

Several evidences suggest that CREB phosphorylation represents a site of convergence for various signaling pathways and alters gene expression [40]. CREB activation can also be regulated by the family of the redox protein Trx-1 [54]. In addition to its antioxidant activity, Trx-1 has been shown to play a crucial role in cellular signaling by controlling several important members of the signal transduction pathway. Thus, NF-κB, p38 mitogen-activated protein kinases, activator protein-1, CREB (as mentioned before), estrogen receptor, glucocorticoid receptor, and p53 are the targets of Trx-1 [55]. Data from our laboratory have shown that morphine-induced CPP increases Trx-1 expression in DG (**Figure 3**). Trx-1 might activate CREB

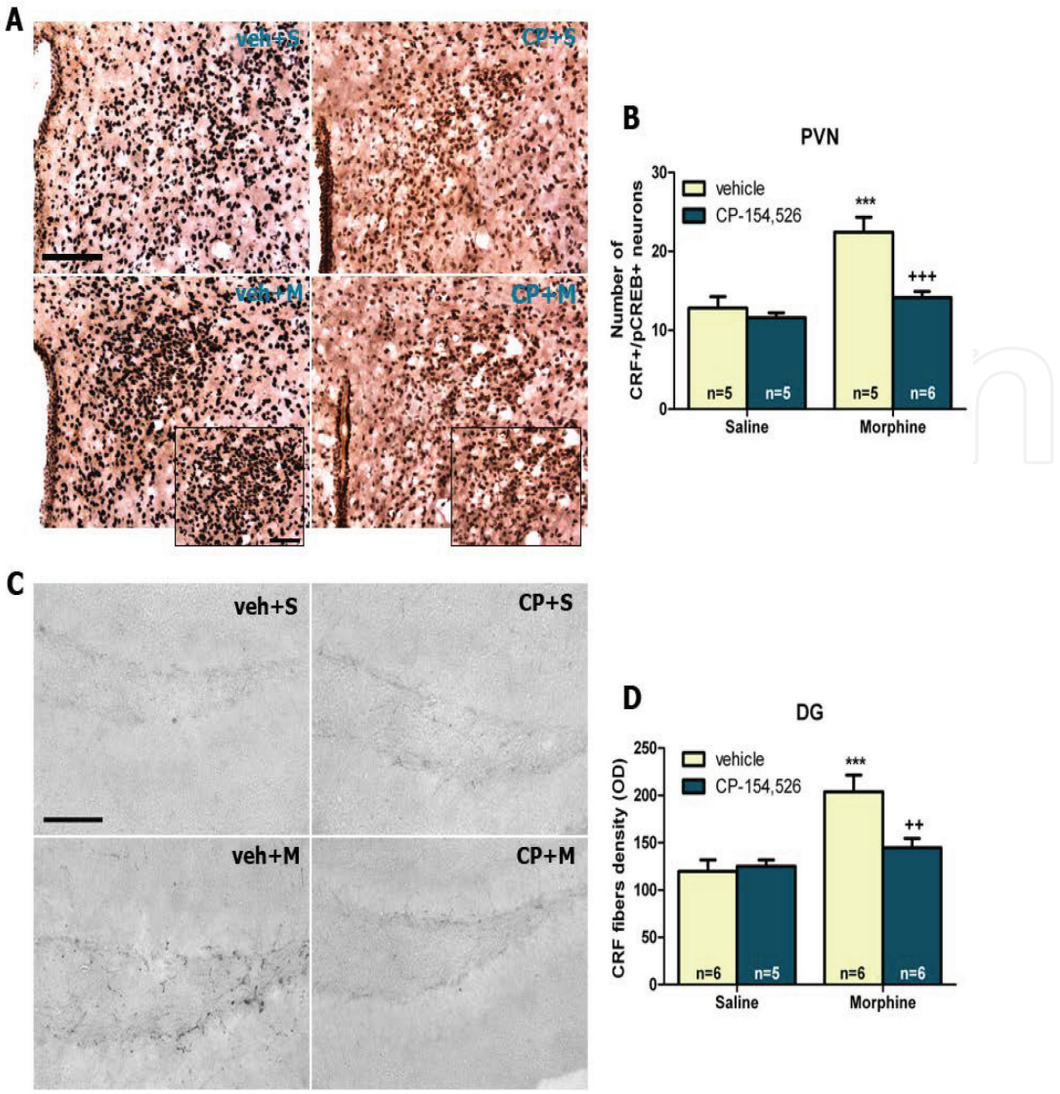

Figure 2.
*CRF/pCREB double-labeling photomicrographs in PVN (A). The upper right side of the figure shows the quantitative analysis of double-labeled neurons (B). CRF fiber photomicrographs in the DG (C). The down right side of the figure shows the CRF fiber density in the DG (D). Scale bar 100 or 50 μm. Data are expressed as mean ± SEM. ***$p < 0.001$ versus vehicle (veh) + saline (S); ++$p < 0.01$, +++$p < 0.001$ versus veh + morphine (M). CP-154,526 (CP). Optical density (OD).*

phosphorylation, thus increasing the rewarding effects of morphine. In agreement with our results, other studies have also observed an increased Trx-1 expression following morphine or methamphetamine administration [56]. Upregulation of CREB activity induced by methamphetamine was suppressed by Trx-1siRNA, which suggests that Trx-1 is necessary for CREB activation [55, 56]. Moreover, morphine-induced Trx-1 expression is blocked by naloxone, indicating that morphine induces Trx-1 expression via activating opioid receptors [57]. Results from our laboratory showing a positive relationship between morphine rewarding effects, and Trx-1 expression are in contrast with another study [58] demonstrating that geranylgeranylacetone induces Trx-1 and, concomitantly, reduces morphine-induced CPP. These variations could be explained by the differential regulating roles of NAc and hippocampus. Besides, CREB expression has been shown to be increased in hippocampus but decreased in NAc after morphine conditioning [40], which suggests that CREB activity is differently regulated depending on the brain area studied. Our investigations have demonstrated a large number of pCREB/Trx-1 double-labeled neurons in DG (**Figure 3**). These neuron colocalizations in DG suggest that CREB might be activated by Trx-1 in this brain nucleus involved in memory consolidation processes.

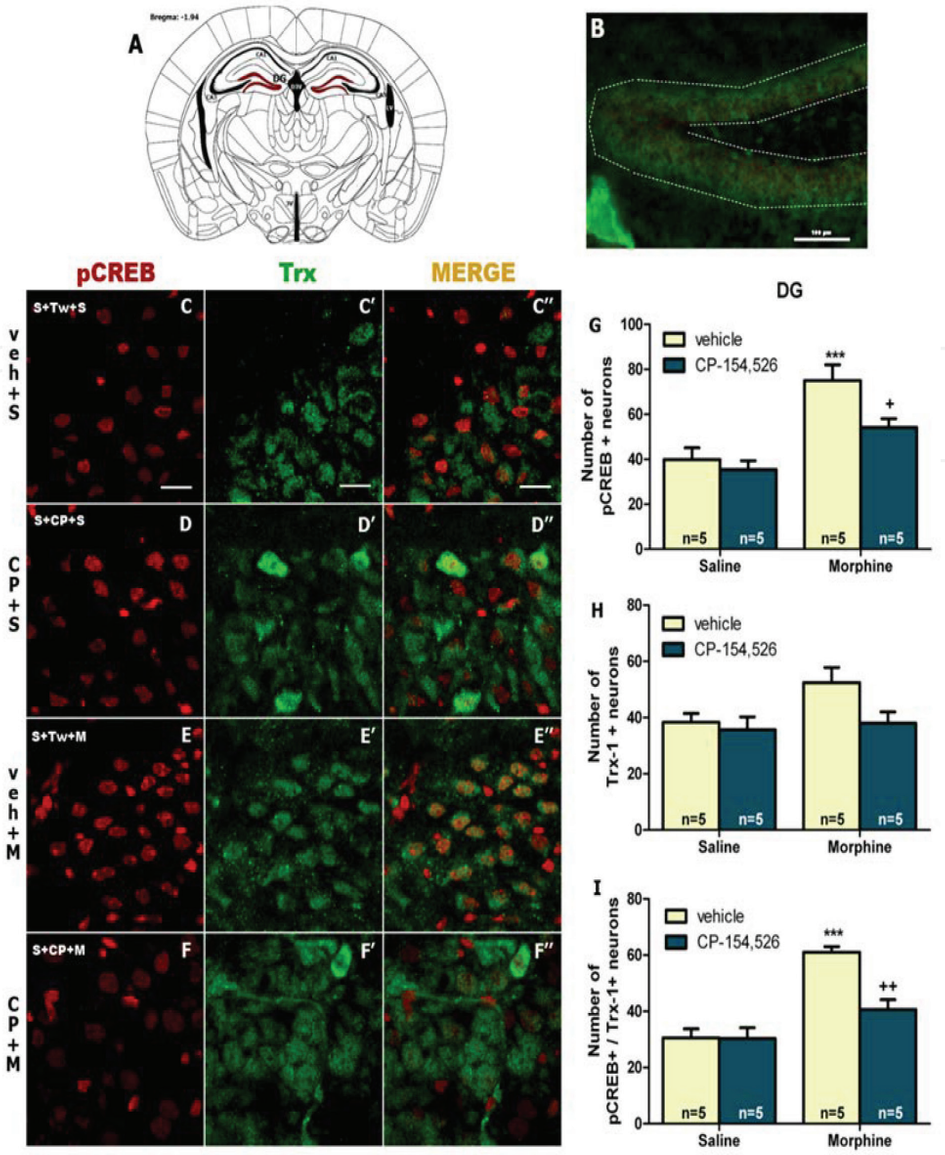

Figure 3.
*Characterization of pCREB and Trx-1 immunostaining in the dentate gyrus (DG) after morphine-induced CPP. (A) Schematic illustration showing the analyzed region of the DG (diagram modified from Franklin & Paxinos) [59]. Coordinate –1.94 mm from Bregma. (B) High-magnification image of a mouse midbrain coronal section immunostained for pCREB and Trx-1. Scale bar 100 μm. Representative confocal images of pCREB (red) (C–F) and Trx-1 (green) (C′–F′). Colocalization (pCREB/Trx-1) is shown in C″–F″ by yellow-orange neurons in the merged images. Scale bar 20 μm. Graphs on the right indicate the mean total number of pCREB (G), Trx-1 (H), and double-labeled (pCREB/Trx-1) neurons (I). Data are expressed as mean ± SEM. ***p < 0.001 versus vehicle (veh) + saline (S); +p < 0.05, ++p < 0.01 versus veh + morphine (M). CP-154,526 (CP).*

Due to the important role of TRX-1 in regulating the cellular redox balance, the induction of TRX-1 expression following morphine CPP could be associated to a mechanism of neural protection against a stressful situation.

Pretreatment with CP-154,526 completely blocks morphine-induced CPP elevation of Trx-1 expression in DG (**Figure 3**).

We have also shown an increase in the number of pCREB neurons coexpressing Trx-1 following morphine-induced CPP, so CRF1R could be involved in CREB phosphorylation, probably through a Trx-1-dependent way. The exact mechanism by which the CRF system participates in Trx-1 signaling regulation in DG is not completely understood. One possible explanation could indicate that pCREB binds to CRE in the 5′-upstream sequence of Trx-1 gene, thus inducing Trx-1 expression to regulate its phosphorylation. In agreement with this hypothesis, other authors have demonstrated that ephedrine promotes Trx-1 expression via the β-adrenergic

receptor/cyclic AMP/PKA/DARPP-32 signaling pathway [60]. Besides, methamphetamine-induced CREB activity in rat pheochromocytoma cells was shown to be regulated by Trx-1 [56].

As shown in **Figure 4**, morphine-induced CPP increases the number of pCREB-positive neurons in PVN, an increase that was blocked by CP-154,526 treatment. However, there are no changes in the number of Trx-1 positive neurons or in the double labeled neurons (pCREB/Trx-1).

On the other hand, BDNF, an important neurotrophin for synaptic plasticity, is one of the molecular candidates underlying the development of persistent

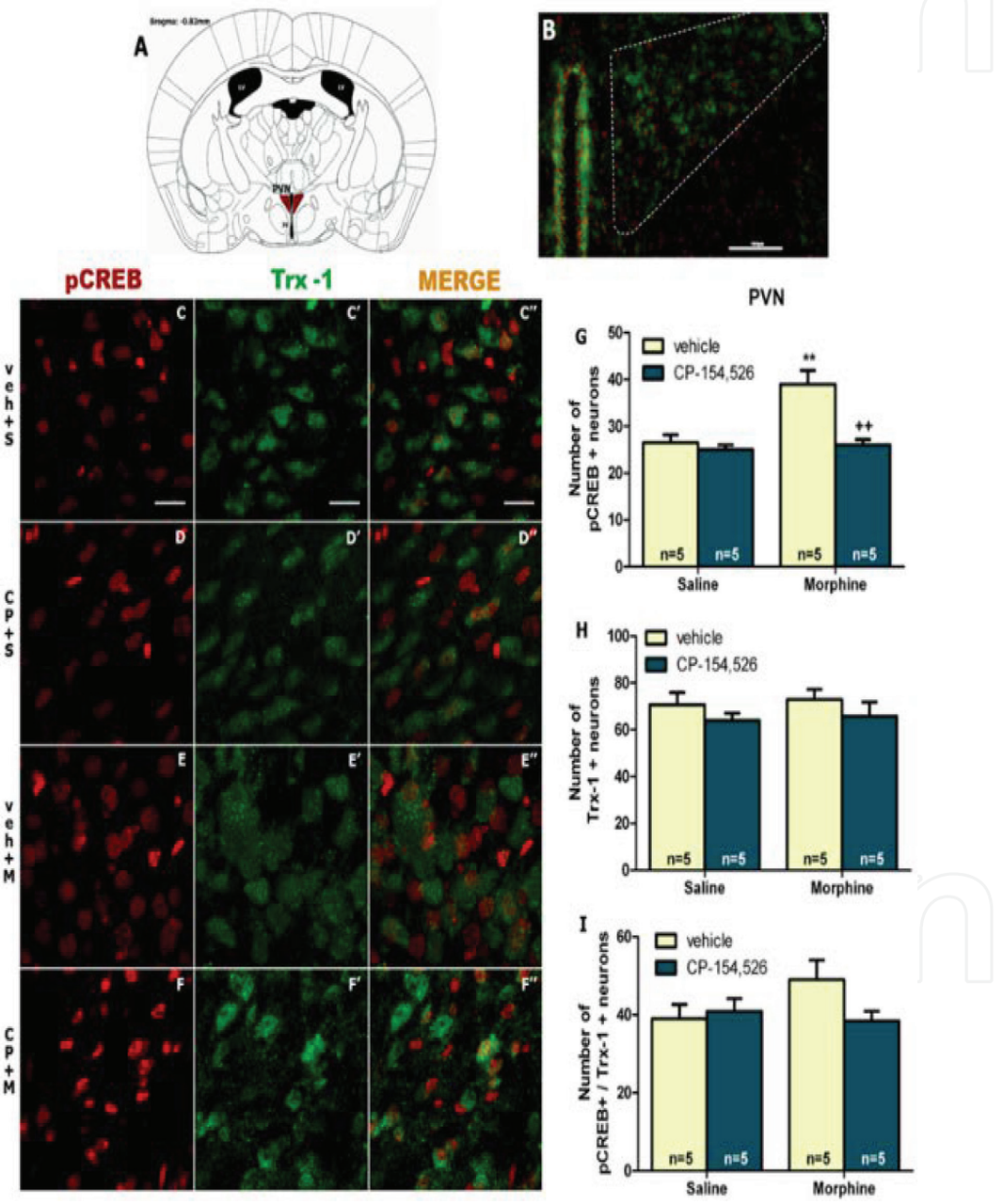

Figure 4.
*Characterization of pCREB and Trx-1 immunostaining in the paraventricular nucleus (PVN) after morphine-induced CPP. (A) Schematic illustration showing the analyzed region of the PVN (diagram modified from Franklin & Paxinos) [59]. Coordinate −0.82 mm from Bregma. (B) High-magnification image of a mouse midbrain coronal section immunostained for pCREB and Trx-1. Scale bar 100 μm. Representative confocal images of pCREB (red) (C–F) and Trx-1 (green) (C'–F'). Colocalization (pCREB/Trx-1) is shown in C"–F" by yellow-orange neurons in the merged images. Scale bar 20 μm. Graphs on the right indicate the mean total number of pCREB (G), Trx-1 (H), and double-labeled (pCREB/Trx-1) neurons (I). Data are expressed as mean ± SEM. **p < 0.01, versus vehicle (veh) + saline (S); ++p < 0.01, versus veh + morphine (M). CP-154,526 (CP).*

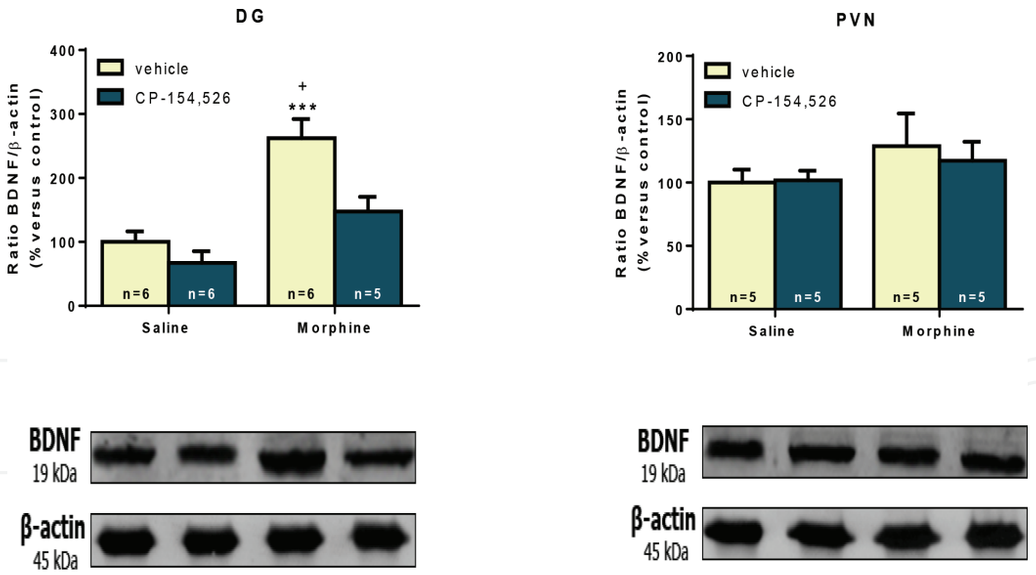

Figure 5.
*Western-blotting analysis of BDNF in the dentate gyrus (DG) and paraventricular nucleus (PVN) from animals pretreated with vehicle (veh) or CP-154,526 (CP) before saline or morphine. The immunoreactivity corresponding to BDNF is expressed as a percentage of that in the control group defined as 100% value. ***p < 0.001 versus morphine + CP; +p < 0.05 versus saline + veh.*

neuroplastic adaptation that regulates drug addiction [61]. Several lines of evidence indicate that chronic morphine treatment triggers ERK activation in different brain regions [62]. ERK phosphorylates CREB and active (phosphorylated) CREB stimulates the expression of target genes, including BDNF [63–65]. Chronic morphine use has been shown to increase the expression of BDNF in the NAc and hippocampus [61, 66, 67]. According to these data, our findings demonstrated that morphine-induced CPP activates BDNF signaling in the DG without any changes in the saline group (**Figure 5**), demonstrating that repeated morphine with context exposure, but not merely the context, increases BDNF expression in DG, suggesting that BDNF is implicated in drug-induced contextual memory formation. Therefore, BDNF is a crucial signal molecule involved in morphine dependence. However, whether this molecule is regulated in a CRF1R-dependent manner remains largely unknown: CP-154,526 attenuated CREB-BDNF expression (**Figures 4** and **5**) and prevented morphine-induced CPP [29]. Taken together, CRF1R-mediated CREB-BDNF signaling changes may regulate morphine reward through modulating contextual memory in the hippocampus.

3. Role of CRF1 receptor in the aversive effects induced by naloxone-precipitated withdrawal

The physical component of morphine withdrawal syndrome can be assessed by scoring some somatic withdrawal signs after morphine exposure [68]. Recent results from our group have demonstrated significant alterations in some morphine withdrawal signs such as body weight loss, rearing, rubbing, grooming, diarrhea, freezing, and time to first immobility in wild type morphine-withdrawn animals compared with controls treated with saline (**Figure 6**). Besides, and in agreement with previous studies [69–71], our laboratory has shown that body weight loss (**Figure 6H**), freezing (**Figure 6F**), and diarrhea (**Figure 6E**) are significantly attenuated in CRF1R KO mice although an increase in jumping in CRF1R KO mice was observed (**Figure 6A**), as it has been described previously by other authors [72]. Jumping is a sensitive and commonly used index of naloxone-induced withdrawal [73–76]. However,

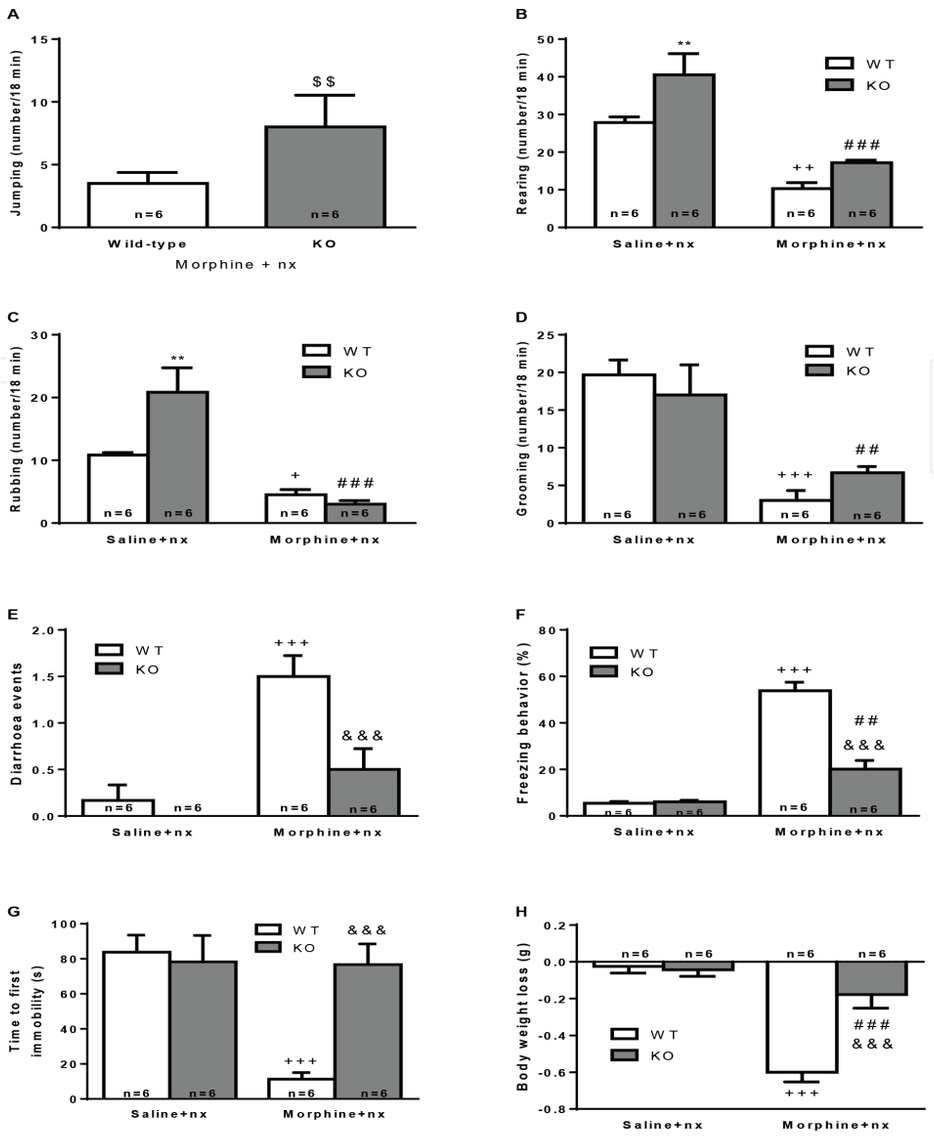

Figure 6.
*Behavior effects by naloxone (nx)-precipitated morphine withdrawal in wild type (WT) or knockout (CRF1R KO) mice. The following somatic signs, (A) jumping, (B) rearing, (C) rubbing, (D) grooming, (E) diarrhea, (F) freezing behavior, and (H) body weight loss, induced after nx (1 mg/kg, s.c.)-injection to morphine or saline-treated mice during 18 min, were evaluated. The time to first immobilization (G) was also evaluated. Data are expressed as the mean ± SEM. $$p < 0.01 versus WT mice treated with morphine + nx; **p < 0.01 versus WT mice treated with saline + nx; +p < 0.05, ++p < 0.01, +++p < 0.001 versus WT mice treated with saline+nx; ##p < 0.01, ###p < 0.001 versus KO mice treated with saline + nx; &&&p < 0.001 versus WT mice treated with morphine + nx.*

it is important to clarify that different neural elements mediate several withdrawal behaviors [77, 78]. Thus, it is not easy to extrapolate naloxone-precipitated jumping in CRF1R KO mice to other physical symptoms like body weight loss.

4. Role of CRF1 receptor in CPA expression and extinction

It is commonly accepted that affective drug withdrawal symptoms are of major motivational significance in contributing to relapse and continued drug use; thus, it is important to understand the mechanisms that mediate affective behaviors during morphine withdrawal. CPA paradigm is a highly sensitive animal model for the measurement of the negative affective component of drug withdrawal as well as to investigate the neural substrates underlying the aversive memory associated with drug withdrawal [79, 80]. In this model, a morphine-dependent animal undergoing

withdrawal is exposed to a particular environment for a period of time. When later is given the opportunity to freely explore the apparatus, animals trained in this way tend to avoid the previously paired context due to the association between the context and aversive memories of drug withdrawal [79].

The extinction of this aversion occurs if the association is weakened by repeated exposure to the withdrawal-associated context in the absence of the conditioned stimulus, and the initial response (CPA) can be reinstated by a drug priming injection, stress or by conditioned cues. Extinction is complete when animals no longer avoid the previously cue-paired compartment. Typically, while memory reconsolidation requires single context reexposure, extinction requires multiple cue reexposures [81]. For example, fear conditioning studies suggest that the extinction process does not eliminate the initial context, but the organism learns that this cue does not cause the previous stimulus [82]. Thus, extinction requires associative learning, consolidation, and the formation of a new memory [83].

Recently, our group has investigated the mechanism underlying CPA expression and extinction. These experiments showed that morphine administration induced a significant place aversion for the naloxone-paired compartment, compared to the saline group. However, CRF1R KO mice presented less aversion than wild type mice (**Figure 7A**).

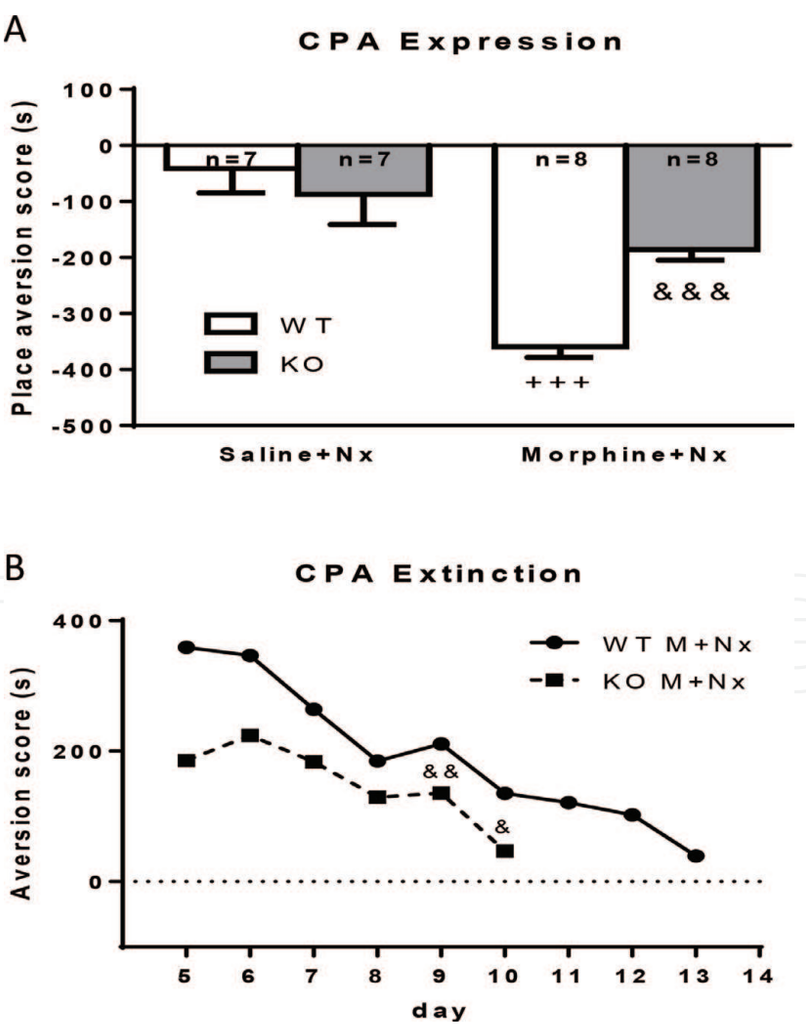

Figure 7.
(A) CPA expression induced by naloxone (nx, 1 mg/kg, s.c.) in wild type (WT) or knockout (CRF1R KO) mice treated with morphine or saline. The score was calculated for each mouse as the difference between the postconditioning and the preconditioning time spent in the naloxone-paired compartment. (B) Extinction of CPA training. Aversion scores from day 5 to 13 for WT and CRF1R KO mice are shown. Data are expressed as the mean ± SEM. +++p < 0.001 versus WT mice treated with saline + nx, &p < 0.05, &&p < 0.01, &&&p < 0.001 versus WT mice treated with morphine + nx.

There is much information about the neurobiological mechanisms involving extinction of reward memory of drug taking [84–86]. However, little information is known about extinction of aversive memory of drug withdrawal [87]. Previous studies have demonstrated that the aversive effects of opiates might be related to basal genotype differences in the brain systems [88]. Accordingly, we have clearly demonstrated that the genetic disruption of the CRF/CRF1R pathway decreases the period of CPA extinction (**Figure 7B**).

Thus, results obtained by our laboratory regarding CPA expression and extinction suggest an important role for CRF1R in aversive memory.

5. Role of HHA axis in the CPA induced by morphine withdrawal

It is well established that acute withdrawal of all major drugs of abuse dysregulates the HPA axis and alters CRF activity in the PVN of the hypothalamus, with a common response of increased adrenocorticotropic hormone (ACTH) and corticosterone [89], which mediate somatic and aversive components of withdrawal [72, 90–92]. To evaluate whether a causal link exists between CRF1R activation and HPA axis, our group has measured plasma ACTH and corticosterone levels in wild type and CRF1R KO mice after naloxone-induced CPA expression and CPA extinction (**Figure 8**). Our investigations have shown that plasma ACTH levels are increased in wild type mice although plasma corticosterone levels are not changed following CPA expression. These results indicate that ACTH-independent mechanisms could have an important role in the regulation of the adrenal stress system to appropriately adapt its response to physiological necessities, and even the presence of pituitary ACTH is basic for adrenocortical function. Numerous lines of evidence indicate that a large number of neuropeptides, neurotransmitters, growth

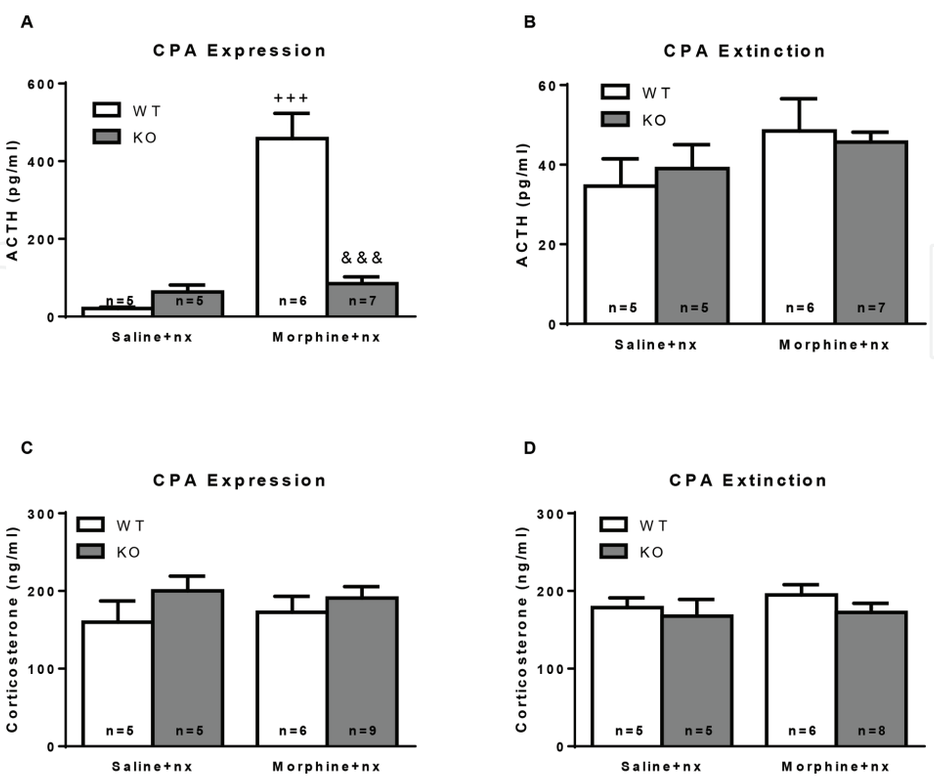

Figure 8.
Effect of CPA expression and CPA extinction training on ACTH (A and B) and corticosterone (C and D) plasma levels in wild type (WT) and knockout (CRF1R KO) mice. Data are expressed as the mean ± SEM. +++p < 0.001 versus WT mice treated with saline + nx, &&&p < 0.001 versus WT mice treated with morphine + nx.

factors, and bacterial ligands can influence the release of adrenal glucocorticoids independently of pituitary ACTH [93]. Adrenocortical cells express a large diversity of receptors for these factors, thus triggering potential direct actions on glucocorticoids release. Damage in the upstream stress regulating pathways in the brain leads to a rupture between ACTH and corticosterone, which suggests that central nervous system neurocircuits can regulate HPA axis response at both pituitary and adrenal sites [94]. Our results also indicate that CPA expression-induced ACTH release is attenuated in CRF1R KO mice. In agreement with these observations, it has been reported fewer ACTH levels in morphine withdrawn animals treated with CRF1R antagonists [70]. Besides, a role for the HPA axis and extra-hypothalamic brain circuitry in somatic, molecular, and endocrine changes induced during opioid withdrawal has been described [72]. ACTH plasma levels returned to basal in wild type and CRF1R KO mice after CPA extinction. These results suggest that CPA expression is, at least, partially due to an increase in plasma ACTH levels which can be decreased after naloxone CPA extinction.

6. Conclusion

CP-154,526 administration or genetic deletion of CRF1R impairs CPP and CPA learning, suggesting that the expression of reward and aversive learning and memory shares some common neural circuits related with CRF/CRF1R signaling. During the formation or consolidation process (CPP expression), the majority of phospho-CREB positive neurons in DG coexpresses Trx-1, in parallel with an increased expression of BDNF, suggesting that Trx-1 could activate CREB and this in turn accelerates the transcription of CREB-dependent genes such as BDNF. However, CP-154,526 diminishes CPP expression, in parallel with a block of phospho-CREB/Trx-1 colocalization and BDNF expression, suggesting that Trx-1-CREB-BDNF signaling could be essential for memory formation or consolidation. In addition, CPA expression training increases plasma ACTH levels, which is critical for the maintenance of aversive memories associated with drug withdrawal. Genetic deletion of CRF1R (KO mice) induces a reduction in CPA expression accompanied with a higher decrease in ACTH plasma levels. CPA extinction period is reduced in KO mice, indicating a role for CRF1R in the aversive memory retrieval. Altogether, these results indicate a critical role for CRF, through CRF1R, in molecular changes involved in reward memory-associated behaviors and in aversive memory expression and extinction. The disruption of these processes by CRF1 antagonists might lead to effective treatments in drug addiction.

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