Neuropharmacology 105 (2016) 500–507

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

# The $CRF_1$ and the $CRF_2$ receptor mediate recognition memory deficits and vulnerability induced by opiate withdrawal

Nadège Morisot [a, b, 1], Angelo Contarino [a, b, *]

[a] Univ. Bordeaux, INCIA, UMR 5287, 33000 Bordeaux, France
[b] CNRS, INCIA, UMR 5287, 33000 Bordeaux, France

ARTICLE INFO

*Article history:*
Received 25 November 2015
Received in revised form
12 February 2016
Accepted 15 February 2016
Available online 18 February 2016

*Keywords:*
Corticotropin-releasing factor (CRF) system
$CRF_1$ receptor-deficiency
$CRF_2$ receptor-deficiency
Opiate withdrawal
Recognition memory
Stress vulnerability

ABSTRACT

Opiate use disorders are associated with impaired cognitive function and altered stress-responsive systems. The corticotropin-releasing factor (CRF) system mediates stress responses *via* $CRF_1$ and $CRF_2$ receptors and may be implicated in substance use disorders. However, the specific role for each of the two known CRF receptor subtypes in cognitive impairment induced by opiate administration and withdrawal remains to be elucidated. In the present study, $CRF_1$−/−, $CRF_2$−/− and their respective wild-type mice are injected with escalating doses of morphine and cognitive function assessed by the novel object recognition (NOR) memory task throughout relatively long periods of opiate withdrawal. Early (2 days) phases of opiate withdrawal impair NOR memory in wild-type, $CRF_1$−/− and $CRF_2$−/− mice. However, the duration of opiate withdrawal-induced NOR memory deficits is prolonged in $CRF_1$−/− *but* shortened in $CRF_2$−/− mice, as compared to their respective wild-type mice, indicating opposite roles for the two CRF receptor subtypes. Nevertheless, following apparent recovery, exposure to an environmental stressor induces the reemergence of NOR memory deficits in long-term opiate-withdrawn wild-type *but not* $CRF_1$−/− or $CRF_2$−/− mice, indicating an essential role for both CRF receptor subtypes in stress vulnerability. These findings bring initial evidence of a complex physiopathological role for the CRF system in cognitive deficits and the long-lasting vulnerability induced by opiate drugs.



## 1. Introduction

Opiate use disorders patients often display impaired cognitive function, especially during drug abstinence periods (APA, 2013). For instance, opiate-abstinent patients perform more poorly than healthy controls on tests measuring episodic memory and executive function, as measured 5 days–3 weeks after the last drug intake (Fishbein et al., 2007; Rapeli et al., 2006). Notably, after the cessation of opiate intake cognitive deficits may persist for relatively longtime periods (APA, 2013), underlie poor treatment compliance and outcome and trigger relapse to opiate drug abuse (Verdejo-Garcia et al., 2004). Accordingly, cognitive dysfunction is found in rodents tested in the eight-arm radial maze and the novel object recognition (NOR) paradigms, both during early (4–14 h) and long-term (6–9 months) opiate withdrawal phases (Rabbani et al., 2009; Sala et al., 1994). Thus, pharmacological remediation of opiate-induced cognitive dysfunction appears as a new strategy for treating opiate use disorders.

Opiate withdrawal promotes the activation of the corticotropin-releasing factor (CRF) system, a major coordinator of neuroendocrine and behavioral responses to stressors. For instance, early (8–48 h) morphine withdrawal is associated with increased CRF mRNA expression in the central nucleus of the amygdala (CeA) and the paraventricular nucleus of the hypothalamus (PVN), brain regions implicated in the effects of substances of abuse (Ingallinesi et al., 2012; Maj et al., 2003; Papaleo et al., 2007). The biological actions of CRF-like peptides are modulated by a CRF-binding protein (CRF-BP), which is highly conserved in mammalian species (Seasholtz et al., 2002). Studies show a role for the CRF-BP in the effects of substances of abuse. Indeed, administration into the ventral tegmental area (VTA) of $CRF_{6\text{-}33}$, a CRF fragment that competes for the CRF binding site on the CRF-BP, reduces the reinstatement of cocaine-seeking behavior elicited by CRF or UCN I in rats (Wang et al., 2007). Moreover, intra-VTA administration of

* Corresponding author. Univ. Bordeaux, INCIA, UMR 5287, 146 rue Leo Saignat, F-33000 Bordeaux, France.
*E-mail address:* angelo.contarino@u-bordeaux.fr (A. Contarino).
[1] Present address: University of California San Francisco, Department of Neurology, San Francisco CA 94143, USA.

http://dx.doi.org/10.1016/j.neuropharm.2016.02.021
0028-3908/

$CRF_{6-33}$ reduces ethanol binge drinking in mice (Albrechet-Souza et al., 2015). In mammals, CRF signaling is mediated through two distinct receptors named $CRF_1$ and $CRF_2$ (Hauger et al., 2003). Initial studies using pharmacological agents show that CRF receptor antagonism attenuates either the somatic signs or the negative affective-like states of naloxone-precipitated opiate withdrawal (Heinrichs et al., 1995; Iredale et al., 2000; Lu et al., 2000; Stinus et al., 2005). However, more recent studies using genetic mouse models bearing a targeted inactivation of only one CRF receptor subtype show opposite roles for the $CRF_1$ and the $CRF_2$ receptor in the somatic expression of opiate withdrawal. Indeed, $CRF_1$ receptor-deficiency exacerbates whereas $CRF_2$ receptor-deficiency reduces the somatic signs of opiate withdrawal, as compared to wild-type mice (Papaleo et al., 2008, 2007). Moreover, despite either $CRF_1$ or $CRF_2$ receptor-deficiency abolishes the negative affective-like states of opiate withdrawal, $CRF_1-/-$ mice show impaired whereas $CRF_2-/-$ mice show unaltered ability to cope with the stressful condition of opiate withdrawal (Contarino and Papaleo, 2005; Ingallinesi et al., 2012; Papaleo et al., 2007). Thus, the latter studies indicate that the two known CRF receptor subtypes may have distinct or opposite roles in the behavioral effects of opiate administration and withdrawal. However, the relative contribution of the $CRF_1$ or the $CRF_2$ receptor subtype to cognitive dysfunction and the long-lasting vulnerability to stressful events following opiate withdrawal remains poorly understood.

Thus, in the present study $CRF_1$ and $CRF_2$ receptor-deficient mice are used to assess the specific role for each of the two known CRF receptor subtypes in opiate withdrawal-induced cognitive dysfunction. For this purpose, the NOR task, a paradigm commonly employed to examine recognition memory in rodents, is used (Bevins and Besheer, 2006). The NOR task is based on the innate tendency of rodents to explore more a novel than a familiar object, which *per se* are devoid of reinforcing properties, making it suitable to *specifically* monitor cognitive function in substance-treated and/or -withdrawn animals showing altered motivational processes (Barr and Phillips, 1999; Rouibi and Contarino, 2012). Thus, the effect of either $CRF_1$ or $CRF_2$ receptor-deficiency upon NOR memory is first examined in drug-naive mice. Then, studies investigate whether the genetic inactivation of only one CRF receptor subtype affects NOR memory deficits induced by withdrawal from intermittent injections of escalating morphine doses. Moreover, following apparent recovery from the opiate withdrawal-induced NOR memory dysfunction, the implication of the $CRF_1$ and the $CRF_2$ receptor in the stress-induced reemergence of recognition memory deficits is assessed relatively longtime after the cessation of morphine administration.

## 2. Methods

### 2.1. Subjects

Littermate wild-type ($CRF_1+/+$, n = 19; $CRF_2+/+$, n = 23), $CRF_1$ or $CRF_2$ receptor null mutant ($CRF_1-/-$, n = 20; $CRF_2-/-$, n = 24) female mice with a mixed C57BL/6Jx129 background are used throughout (Bale et al., 2000; Smith et al., 1998). The mice derive from mating $CRF_1+/-$ or $CRF_2+/-$ mice, their genotype is determined by PCR analysis of tail DNA and are 5–6 months old at the beginning of the experiment. The mice are group-housed (2–4/cage) in a colony room (22 ± 2 °C, relative humidity: 50–60%) on a 12-h light/dark cycle (lights on at 08:00) with standard laboratory food (3.3 kcal/g; SAFE, Augy, France) and water available *ad libitum*. All studies are conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and are approved by the local Animal Care and Use Committee. All efforts are made to minimize animal suffering and to reduce the number of animals used.

### 2.2. NOR and elevated platform apparatuses

The NOR apparatus is a grey polypropylene box (40 × 30 × 23 cm; length, width, height). A glass-made rectangle and a ceramic bowl are used as objects; they are about 5 cm high, too heavy to be displaced by the mouse and located 5 cm away from a corner of the apparatus. The mice do not show any preference for one or the other of the two objects (Morisot & Contarino, unpublished observations). The elevated platform is a grey polypropylene square (10 × 10 cm) elevated 40 cm above the floor.

### 2.3. Experimental procedure

Prior to the beginning of the experiment, during three consecutive days each animal is handled for 1 min per day. Notably, the mice are repeatedly tested in the NOR task (Fig. 1). All testing is conducted during the light phase of the 12 h light/dark cycle in a quiet room dimly illuminated (30 lux). During the two days preceding the first NOR test and on the day preceding the subsequent NOR tests, each mouse is allowed to freely explore the empty (without objects) apparatus for 10 min (habituation trial). The NOR test consists of 2 trials (T1 and T2) separated by an inter-trial time interval (ITI). On T1 (acquisition trial), the mice are placed in the apparatus containing two identical objects (F1 and F2) for 10 min. The mice are then returned to their home cage and, following a variable ITI (15 min - 2 h), they are placed back in the NOR apparatus containing a familiar (F) and a novel object (N) for 5 min (T2, restitution trial). The role (familiar or novel) and the position (left or right) of the two objects is counterbalanced and randomized within each experimental group. Between each trial, the NOR apparatus is cleaned with water and the objects with 50% ethanol. Exploration is defined as the animal directing the nose within 0.5 cm of the object while looking at, sniffing or touching it, excluding accidental contact with it (backing into, standing on the object, etc.). The experiments are recorded on a video system and

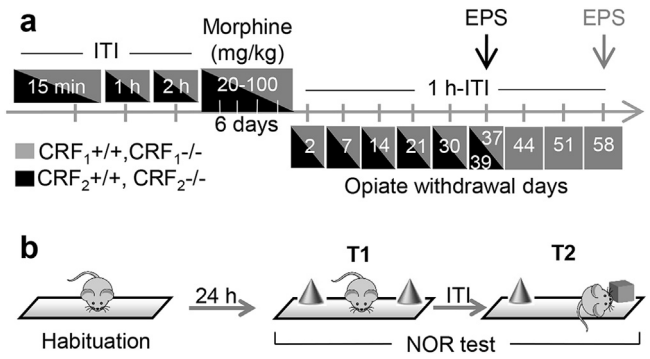


**Fig. 1.** Experimental procedure. (A) $CRF_1+/+$, $CRF_1-/-$, $CRF_2+/+$ and $CRF_2-/-$ mice are tested (ticks) once a week in the novel object recognition (NOR) memory task using a 15 min, a 1 h and a 2 h inter-trial interval (ITI) between the acquisition (T1) and the restitution (T2) trial. Then, over a 6-day period morphine is injected i.p. twice a day (08:00–20:00) at the dose range indicated, except for the last day when only one injection is given in the morning. Following cessation of morphine administration, NOR memory is assessed using a 1 h ITI throughout a relatively longtime period, up to the apparent recovery of opiate withdrawal-induced memory deficits in all of the experimental groups. Then, to assess the role for the $CRF_1$ and the $CRF_2$ receptor in stress vulnerability, on opiate withdrawal day 39 ($CRF_2-/-$) or 58 ($CRF_1-/-$), the null mutant and their respective wild-type mice are exposed to an elevated platform stressor (EPS) 1 h prior to the acquisition trial (T1) of the NOR test. (B) Drawing illustrating the habituation to the NOR apparatus (without the objects), the T1 and the T2 trial of the NOR test. Habituation trials are carried out during the two days preceding the first NOR test and on the day preceding each subsequent NOR test.