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# Lifelong, central corticotropin-releasing factor (CRF) overexpression is associated with individual differences in cocaine-induced conditioned place preference

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ABSTRACT

Stress, through corticotropin-releasing factor (CRF), influences all aspects of cocaine addiction. Earlier studies suggest that individual differences in responsivity to stress affect susceptibility to develop addiction. We have previously found that CRF over-expression alters individual differences in behavioural responses to novelty stress in mice. Therefore, we hypothesised that post-natal, long-term over-expression of brain CRF may alter the rewarding effects of cocaine in a manner that is sensitive to individual differences. In this study we specifically investigated cocaine-induced conditioned place preference (CPP) in transgenic mice over-expressing CRF (CRF-OE) and in wild-type (WT) littermates after determining their individual locomotor and emotional responsivity to inescapable novelty. CRF-OE mice showed decreased overall locomotor activity and increased anxiety-like behaviour in response to novelty compared to WT mice. Low behavioural reactivity to novelty (LR) was associated with heightened anxiety-like behaviour in CRF-OE, but not in WT, mice. WT and CRF-OE mice developed CPP equally to both low (5 mg/kg) and high (20 mg/kg) doses of cocaine. However, LR CRF-OE mice expressed significantly stronger cocaine CPP than transgenic mice with high locomotor response to novelty (HR). In WT mice, on the other hand, stronger CPP induced by 20 mg/kg of cocaine was found in the HR animals. Furthermore, there was a strong negative correlation between locomotor reactivity to novelty and CPP in CRF-OE, but not in WT, mice. Collectively, these results suggest that long-term, post-natal CRF over-expression increases the rewarding effects of cocaine in individuals with high emotional response to stress.



## 1. Introduction

CRF is a 41-amino acid peptide released from neurons in the brain that contribute to the behavioural, hormonal and autonomic response to stress (Heinrichs and Koob, 2004). Acute, central administration of CRF mimics effects of stress and CRF receptor antagonists can prevent stress-induced behavioural responses (Heinrichs and Koob, 2004). Activity of the brain CRF system, as the principal mediator of the biological stress response, has been consistently implicated in different aspects of psychostimulant addiction, including acute behavioural and neuroendocrine effects, reward/reinforcement, withdrawal and relapse (Corominas et al., 2010; Koob, 2010; Sarnyai et al., 2001). For example, blockade of endogenous CRF signalling by an antiserum or a receptor antagonist inhibits cocaine-induced corticosterone response (Sarnyai et al., 1992a), locomotor hyperactivity (Sarnyai et al., 1992b), amphetamine-induced behavioural sensitisation (Cole et al., 1990), cocaine-induced CPP (Lu et al., 2003), cocaine self-administration (Goeders and Guerin, 2000) and behavioural

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effects of cocaine withdrawal (Basso et al., 1999; Sarnyai et al., 1995) as well as stress-induced reinstatement of cocaine seeking (Shalev et al., 2010 for review). Therefore, we hypothesised that chronic over-activity of brain CRF may affect the individual's sensitivity to the rewarding effects of the psychostimulant cocaine.

Although many people experiment with drugs at some point in their lives, far fewer develop substance use disorders. The existence of marked individual differences in behavioural responsiveness to novel, stressful stimuli has been recently recognised (Blanchard et al., 2009). In a series of seminal studies Piazza and co-workers have shown that outbred Sprague-Dawley rats which are characterised as 'high-responders' (HR) on the basis of their locomotor response to a novel environment fundamentally differ from 'low-responders' (LR) in a number of behavioural and physiological indices related to stress pathology, such as stress-induced corticosterone response, propensity to self-administer drugs of abuse, cognitive function in later life and brain neurochemistry (Dellu et al., 1993, 1994; Piazza et al., 1989). The role of individual differences in cocaine-induced CPP is less conclusive, with some studies finding no association (Erb and Parker, 1994; Gong et al., 1996), and others finding a negative association (Brabant et al., 2005; Shimosato and Watanabe, 2003) between locomotor response in a novel environment and the magnitude of CPP. HR and LR rodents differ in other behavioural and molecular indices as well. For example, LR animals are more anxious and express higher levels of CRF mRNA in the central nucleus of amygdala than HR animals (Kabbaj et al., 2000). Therefore, we hypothesised that CRF over-expression may influence individual differences in susceptibility to the rewarding effects of cocaine.

The primary aim of the present study was to investigate the effects of post-natal, life-long brain CRF over-expression on the sensitivity to the rewarding effects of cocaine by using the CPP procedure. We specifically investigated individual differences in locomotor and anxiety-like response to environmental novelty, and their relationship to cocaine-induced CPP. The results will be discussed in relation to the behavioural response to novelty and the rewarding properties of cocaine under the influence of heightened brain CRF expression in mice.

## 2. Materials and methods

### 2.1. Subjects

CRF-OE mice were generated as previously described (Dirks et al., 2002) to yield founder animals which gave rise to a line (CRF-OE 2122 line) that was further bred at the local breeding facilities (Central Laboratory Animal Institute, Utrecht University, The Netherlands) and used for the present study. Breeding consisted of mating between transgenic male and C57BL/6J female mice. Tail DNA from offsprings, extracted with High Pure PCR Template Preparation Kit (Boehringer, Mannheim, Germany), was screened using PCR with transgene specific primers. The forward primers were specific for rat CRF and the reverse primers originated from the Thy-1 promoter, thus excluding the possibility that the endogenous CRF and Thy-1 genes were amplified. Total of 20 male C57BL/6J WT (18–35 weeks old) and 20 male CRF-OE (16–34 weeks old) mice were used for these experiments. Mice were housed ($n$=2–3 per cage) in plastic cages ($12 \times 22 \times 15$ cm$^3$, Techniplast, Bugugiatte, Italy) enriched with bedding (EnviroDri®, BMI, Helmond, The Netherlands), a piece of PVC tubing (diameter 5 cm) and nesting material at constant room temperature ($21 \pm 2$ °C) and relative humidity (40–50%). Standard rodent food pellets (Special Diet Services Ltd., Essex, U.K.) and tap water was freely available. Animals were maintained on a 12 h light–12 h dark cycle (lights on from 6:00 a.m. until 6:00 p.m.). All experimental procedures were conducted during the light phase of the cycle, between 9:00 a.m. and 4:30 p.m. All studies and procedures were approved by the ethical committee on animal experiments of the Faculties of Pharmacy, Biology, and Chemistry of Utrecht University, The Netherlands, according to the Dutch law for animal experimentation and the Declaration of Helsinki.

### 2.2. Cocaine conditioned place preference

#### 2.2.1. Apparatus

A Plexiglas three-compartment conditioned place preference (CPP) chamber was placed in a sound-attenuated room to allow recording of undisturbed behaviour. The two larger, outer compartments (17.5 cm × 20 cm × 20 cm) were separated by a central compartment (7 cm × 20 cm × 20 cm) and differed in both visual and tactile cues. One compartment had white walls and sand-coloured rough grained floor ('light' compartment) and another had black walls and blue smooth rubber floor ('dark' compartment). The central compartment had grey walls and a Plexiglas floor, and allowed free movement between the two outer compartments, unless barred by removal Plexiglas partitions, which were of the same colour as the walls of the central compartment. These partitions were designed to restrict travel between the two outer compartments during conditioning sessions. The entire experiment was video-recorded.

#### 2.2.2. Preconditioning session: novelty-induced activity

Prior to any drug treatments, mice were habituated to experimenter handling for a minimum of 5 days. WT and CRF-OE mice were randomly assigned to each group with respect to cocaine dose and compartment pairing. In a preconditioning session animals were placed into the centre compartment and then allowed to freely explore and habituate to all the compartments of CPP chamber for 20 min on two consecutive days. The activity was scored by counting the frequency of entries into each compartment, visually on a PC screen connected to the video camera that was used to record the experiment. The time spent in each compartment was also recorded, while watching the video recording of the experiment at a later time. The locomotor activity, as measured by the total number of entries made into all compartments during the first day of preconditioning, was regarded as an index for locomotor response to the novel environment. Upon the completion of the CPP experiment, subjects were retrospectively assigned to low-responder (LR) and high-responder (HR) groups, which showed locomotor activity during the first preconditioning session being below and above, respectively, the median value in each genotype (WT: $121.5 \pm 5.9$; CRF-OE: $79.0 \pm 5.5$). Anxiety-like behaviour expressed upon the first exposure to the CPP apparatus was measured as described by Shimosato and Watanabe (2003). This approach is similar to the traditional 'light–dark box' in that it takes advantage of the contrasting 'dark' vs 'light' compartments of the apparatus and the animals' propensity to enter more often and spend more time in the 'dark' compartment over the 'light' compartment when anxiety-like behaviour increases. Anxiety was quantified by calculating the ratio of 'dark' compartment entry and time over number of entries into and time spent in the other, 'light', compartments.

#### 2.2.3. Conditioning sessions

Conditioning phase started 24 h after the second habituation and took place over a period of 8 days. Each conditioning session lasted for 20 min. During the conditioning phase, the partitions were closed to restrict access of the mice from an outer compartment to the other compartments. For the two cocaine treatment groups, cocaine (cocaine–hydrochloride; 5 mg/kg and 20 mg/kg)