Stress exposure produces a switch from appetitive to aversive signaling by corticotropin-releasing factor in the nucleus accumbens

Lemos J.C.\textsuperscript{1,2,3}, Wanat M.J.\textsuperscript{1,2}, Smith J.S.\textsuperscript{2}, Reyes B.A.S.\textsuperscript{4}, Hollon N.G.\textsuperscript{1,2,3}, Van Bockstaele E.J.\textsuperscript{4}, Chavkin C.\textsuperscript{2,3}, Phillips P.E.M.\textsuperscript{1,2,3,}\textsuperscript{*}

\textsuperscript{1}Department of Psychiatry and Behavioral Sciences, \textsuperscript{2}Department of Pharmacology, \textsuperscript{3}Graduate Program in Neurobiology and Behavior, University of Washington, Seattle, WA, USA; \textsuperscript{4}Department of Neuroscience, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, PA, USA.

\textsuperscript{*}pemp@uw.edu

Introduction

Stressors motivate an array of adaptive responses ranging from “fight or flight” to an internal urgency signal facilitating long-term goals [1]. However, traumatic or chronic uncontrollable stress promotes the onset of Major Depressive Disorder where acute stressors lose their motivational properties and are perceived as insurmountable impediments [2]. Consequently, stress-induced depression is a debilitating human condition characterized by an affective shift from engagement of the environment to withdrawal [3]. An emerging neurobiological substrate of depression and associated pathology is the nucleus accumbens, a region with the capacity to mediate a diverse range of stress responses by interfacing limbic, cognitive and motor circuitry [4]. Corticotropin releasing factor (CRF) is a neuropeptide released in response to acute stressors and other arousing environmental stimuli [5]. It acts in the nucleus accumbens to facilitate motivation for cued rewards [6] and social bonding behavior [7]. Since these processes are, at least in part, mediated by dopamine [8,9], we hypothesized that CRF acts within the nucleus accumbens to enhance dopamine transmission.

Methods

Immunohistochemistry and transmission electron microscopy were used for anatomical characterization of CRF and its receptors. Fast-scan cyclic voltammetry in brain slices was combined with pharmacology in wildtype and knockout mice for functional characterization of the regulation of dopamine release by CRF. Conditioned-place-preference and novel-object-exploration assays following intracerebral microinjections of CRF agonists and antagonists were used to test the behavioral consequences of CRF-dopamine interactions. A stress-induced depressive-like state was induced by two-day forced swim stress.

Results and Discussion

CRF was present in the nucleus accumbens in cholinergic interneurons and axonal fibers that were interdigitated with dopamine-containing axons. CRF R1 and R2 receptors were present on soma and fibers, including dopamine terminals.
Figure 1. CRF acts in the nucleus accumbens to increase dopamine release and produce appetitive effects that are lost following severe stress exposure. **a.** CRF (100 nM) increased dopamine release in the nucleus accumbens core of stress-naïve mice (blue bars, n = 13-18). However, this effect was incapacitated after severe stress without recovery for 90 days (n = 8-16). This effect of stress was partially averted by treatment with a glucocorticoid-receptor antagonist (RU 486, 30 mg/kg i.p.) prior to stress exposure (n = 6-10). **b.** Microinjected of CRF directly into the nucleus accumbens of stress-naïve mice produced conditioned place preference which was absent following stress and, in fact, produced conditioned place aversion seven days after stress exposure (n = 6-8). The activity plots show the times spent in the vehicle- and CRF-paired chambers representative in representative animals during the post-conditioning test, and the bar graph shows the difference in time spend in these chambers (CRF-vehicle). **c.** Endogenous CRF in the nucleus accumbens stimulated exploration of novel objects in naïve mice, as assessed by the loss of function following microinjection of a CRF-receptor antagonist (α-helical CRF, ) into the nucleus accumbens, an effect that was abolished by severe stress exposure (n = 9-10). The bar graph shows the additional time spent exploring the novel object following vehicle administration versus with CRF receptor antagonism. Data on bar graphs are mean ± s.e.m.; ns p > 0.05, * p < 0.05, ** p < 0.01; § p < 0.05, §§ p < 0.01 for interaction.
CRF produced a concentration-dependent increase in evoked dopamine release in slices of the nucleus accumbens core (Fig. 1a) through co-activation of CRF R1 and R2. The potentiation of dopamine release by CRF was completely ablated by stress exposure without recovery for 90 days. This effect of stress was partially precluded by prophylactic administration of the glucocorticoid-receptor antagonist RU 486 (10 or 30 mg/kg, i.p.), but not the κ-opioid-receptor antagonist, norBNI (10 mg/kg, i.p.), or the selective-serotonin-reuptake inhibitor, fluoxetine (10 mg/kg, i.p.).

Consistent with the ability to increase dopamine release in stress-naïve animals, microinjection of CRF (5 or 500 ng bilateral or 500 ng unilateral in 200 nl) into the nucleus accumbens produced conditioned place preference (Fig. 1b) that was abolished by local dopamine depletion using the neurotoxin 6-OHDA (2 µg unilateral in 500 nl). However, intra-accumbens CRF did not elicit conditioned place preference following stress, but produced robust conditioned place aversion seven days post-stress.

To test the relevance of our finding to endogenous CRF function, we investigated the effects of intra-accumbens microinjection of a CRF antagonist (helical CRF, 500 ng in 200 nl) on novel object exploration. The introduction of a novel object into the center of an open field, dramatically increased the time mice spent in the center, but this effect was greatly reduced by CRF antagonism in the nucleus accumbens. In mice that underwent stress exposure seven days earlier, novel object presentation produced a more modest increase in center time, and CRF antagonism in the nucleus accumbens had no effect on this exploration.

These data describe a neuropeptidergic regulatory system of mesolimbic dopamine and identify a specific and enduring stress-induced defect in this regulation that produces an affective shift in responses to environmental stimuli, characteristic of the cardinal symptom of stress-induced depressive disorders.

**References**