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# Severe stress switches CRF action in the nucleus accumbens from appetitive to aversive

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## Abstract

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]. Here we report that corticotropin releasing factor (CRF), a neuropeptide released in response to acute stressors[5] and other arousing environmental stimuli[6], acts in the nucleus accumbens of naïve mice to increase dopamine release through co-activation of CRF R1 and R2 receptors. Remarkably, severe stress exposure completely abolished this effect without recovery for at least 90 days. This loss of CRF’s capacity to regulate dopamine release in the nucleus accumbens is accompanied by a switch in the reaction to CRF from appetitive to aversive, indicating a diametric change in the emotional response to acute stressors. Thus, the current findings offer a biological substrate for the switch in affect which is central to stress-induced depressive disorders.

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CRF initiates neuroendocrine signaling in the hypothalamic-pituitary-adrenal axis, and also regulates neurotransmission directly via two receptor subtypes, CRF R1 and CRF R2, which

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**Author contributions**

J.C.L. performed immunohistochemistry. J.C.L. and N.G.H. carried out fast-scan cyclic voltammetry experiments. J.C.L., M.J.W. and J.S.S. performed the behavioral experiments. B.A.S.R. and E.J.V. provided transmission electron microscopy data. J.C.L., M.J.W., C.C. and P.E.M.P. developed the conceptual and experimental framework, and J.C.L. and P.E.M.P. wrote the paper.

**Author information**

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are distributed widely throughout the brain[7,8]. In the nucleus accumbens, CRF facilitates cue-elicited motivation[9] and social bonding[10], behaviors thought to be mediated by dopamine transmission[11,12]. Therefore, we sought evidence for CRF-dopamine interactions in the nucleus accumbens, first using fluorescent immunohistochemistry. Dense CRF immunoreactivity was present throughout the rostro-caudal axis of the nucleus accumbens core and lateral shell and in the most rostral portion of the medial shell in sparsely located large cell bodies (cholinergic interneurons, see Supplementary Fig. 1) and fiber terminals that were interdigitated with tyrosine-hydroxylase (TH) immunoreactive fibers that are indicative of dopamine-containing axons (Fig. 1a). Immunoreactivity for the CRF R1 receptor displayed punctate staining with co-localization of TH immunoreactivity on fiber segments in addition to localization on cell bodies within the nucleus accumbens (Fig. 1b and Supplementary Fig. 2). CRF R2 immunoreactivity had a more diffuse, but still, punctate pattern of staining, similar to that in other regions[13], with some co-localization with TH-immunoreactivity (Fig. 1c and Supplementary Fig. 3). Expression of CRF receptors on subcellular profiles in the nucleus accumbens, including TH-positive terminals, was confirmed at higher spatial resolution using transmission electron microscopy (Fig. 1d; quantified in Supplementary Table 1). Together, these data indicate that the localization of CRF and its receptors in the nucleus accumbens is well suited for modulation of dopamine release.

To directly test the functional effects of CRF on dopamine release in the nucleus accumbens, we selectively monitored dopamine release evoked by a single biphasic electrical pulse (2 ms/phase, 100-500 μA delivered once per minute) in acute coronal brain slices using fast-scan cyclic voltammetry at carbon-fiber microelectrodes (Fig. 2a and Supplementary Fig. 4). Vehicle or CRF (10, 100 or 1000 nM) was applied to the slice for 15 minutes following five minutes of stable baseline and the resultant effect was quantified by averaging the evoked dopamine current in the last ten minutes. Following application of vehicle, there was a modest decrease (~7 %) in dopamine release (Fig. 2b), whereas CRF increased dopamine release in a concentration-dependent manner eliciting effects significantly greater than vehicle at 100 and 1000 nM (27.8 ± 6.7 and 30.0 ± 8.4 % respectively, mean ± s.e.m.; $F_{3, 49} = 5.026$, $p < 0.01$, one-way ANOVA with Dunnett's post-hoc t-tests; Fig. 2b and Supplementary Fig. 5). Interestingly, this effect could be blocked by application of either the selective CRF R1 antagonist, antalarmin (1 μM), or the selective CRF R2 antagonist, anti-sauvagine 30 (ASVG 30; 250 nM) to the slice beginning 20 minutes before CRF application ($F_{2, 50} = 5.142$, $p < 0.01$, one-way ANOVA with Dunnett's post-hoc t-tests; Fig. 2c) indicating that co-activation of both receptors is required. Consistently, CRF (10, 100, 1000 nM) failed to increase dopamine release in the nucleus accumbens of mice with deletion of either the CRF R1[14] or R2[15] gene (Fig. 2d). Application of the selective CRF R1 agonist Stressin 1 (100 or 300 nM) or the selective CRF R2 agonist Urocortin 3 (100 or 300 nM) failed to significantly increase dopamine release when applied individually ($p > 0.05$ compared to respective vehicles; Fig. 2e and f), but significantly increased dopamine release when co-applied ($F_{3,36} = 3.528$, $p < 0.05$ vs vehicle, one-way ANOVA with Dunnett's post-hoc t-tests). The effect of the agonists together could be blocked by pre-treatment with Antalarmin and ASVG 30 (unpaired t-test, $p > 0.05$; Fig. 2g). Together these data provide

convergent evidence that CRF increases dopamine release in the nucleus accumbens through co-activation of CRF R1 and R2.

If this ability for CRF to positively regulate dopamine in the nucleus accumbens has specific motivational relevance to the behaving animal, we would predict that it would cause conditioned place preference when restricted to the nucleus accumbens, even though centrally administered CRF elicits robust conditioned place aversion[16]. Therefore, we used a balanced place-conditioning apparatus consisting of two visually distinct test chambers separated by a smaller neutral compartment. On day 1, mice were allowed to freely roam the apparatus, and the time they spent in each chamber was recorded. On days 2 and 3, mice received CRF bilaterally into the nucleus accumbens (500 ng per side in 200 nl artificial cerebrospinal fluid; cannulae placements shown in Supplementary Fig. 6) or vehicle infusions and then were isolated in one of the test chambers for 30 minutes. Four hours later they received the alternative infusion and were isolated in the other test chamber for 30 minutes. On day 4, mice were again allowed free access to the apparatus. Mice exhibited a significant preference for the CRF-paired context, demonstrating that intra-accumbens CRF (500 ng) was an appetitive stimulus to these animals (conditioning by drug, $F_{1,12} = 6.435$, $p < 0.001$ two-way repeated-measures ANOVA, Fig. 3a). Similarly, unilateral infusions of CRF (500 ng/200 nl) also produced conditioned place preference (conditioning by drug, $F_{1,12} = 11.77$, $p < 0.001$ two-way repeated-measures ANOVA; Fig. 3b and Supplementary Fig. 7a). This dose of CRF is within the range that produces selective effects *in vivo*[11], but it is difficult to ascertain the steady-state concentration at receptors as CRF undergoes both radial diffusion and active clearance[17]. Nonetheless, even at a lower dose of CRF (5 ng/ 200 nl), conditioned place preference was observed (conditioning by drug, $F_{1,14} = 5.415$, $p < 0.05$, two-way repeated measures ANOVA; Fig. 3b and Supplementary Fig. 7b). Taken together, these data indicate that CRF acts in the nucleus accumbens to produce a positive affective state.

To test whether this positive affective state is dependent upon CRF's ability to increase dopamine release, we used the catecholaminergic-neuron-selective neurotoxin, 6-OHDA. We compared unilateral CRF place conditioning in animals that had received ipsilateral infusions of 6-OHDA (2 μg in 500 nl) or vehicle (0.09% NaCl,0.1% ascorbate) into the nucleus accumbens seven days earlier. CRF (500 ng in 200 nl) produced place preference in sham animals (conditioning by drug, $F_{1,18} = 6.95$, $p < 0.05$ two-way repeated-measures ANOVA; Supplementary Fig. 8a), of similar magnitude to controls (treatment by drug, $F_{1,30} = 0.35$, $p > 0.05$, two-way ANOVA). However, place preference to intra-accumbens CRF was absent in animals that received 6-OHDA (conditioning by drug, $F_{1,18} = 0.00$, $p > 0.05$, two-way repeated-measures ANOVA; Supplementary Fig. 8b) demonstrating a significant change in the subjective effects of CRF ($p < 0.05$, unpaired t-test, Fig. 3b). This treatment produced a significant dopamine depletion on the side of the injection ($p < 0.001$; Supplementary Fig. 8c), but did not alter locomotor activity ($p > 0.05$, unpaired t-test, Supplementary Fig. 8d), demonstrating that the unilateral lesions did not produce a general deficit in motor function. These data demonstrate that the positive affective state produced by CRF in the nucleus accumbens is dependent upon its ability to increase dopamine release.

To ascertain the role of *endogenously released* CRF in the nucleus accumbens in mediating appetitive behaviors, we tested the effect of CRF antagonism on the response to an arousing stimulus by assaying novel object exploration, a behavior that requires intact dopamine transmission[18]. We bilaterally infused the CRF antagonist, α-helical CRF (500 ng in 200 nl per side) or vehicle (lactated ringer's with 1% acetic acid), into the nucleus accumbens, placed animals into an arena and then 15 minutes later, introduced a novel object into the center. While α-helical CRF had no effect on baseline exploration of the center of the arena compared to vehicle, it significantly attenuated the appetitive effects (i.e., eliciting of approach and exploration) of the novel object (treatment by stimulus, $F_{1,18} = 4.62$, $p < 0.05$, two-way repeated-measures ANOVA; Fig. 3c). These data demonstrate that endogenous CRF in the nucleus accumbens is utilized under physiological conditions to mediate appetitive responses to arousing environmental stimuli.

Exposure to severe or chronic stress can produce profound alterations in normal stress signaling that can be detrimental to physical and mental health, predisposing individuals to depression[19]. To model this phenomenon, we employed a modified Porsolt paradigm in which mice are exposed to two days of repeated swim stress. Animals were placed in a vessel of water (29.0 - 31.0 °C) for 15 minutes followed by four additional 6-minute swim sessions (separated by 6-minute recovery periods) 24 hours later. This protocol has been shown to produce escalating immobility across sessions indicating a depression-like phenotype[20]. We prepared coronal slices of the nucleus accumbens from these animals thirty minutes after the final stress exposure and found that the ability for CRF to potentiate dopamine release was completely abolished (stress exposure by drug, $F_{4,116} = 12.61$, $p < 0.001$ two-way ANOVA, Fig. 4a). Notably, we established that this change in the ability of CRF to regulate dopamine release was not a generalized change in stress-related peptide signaling as the effect of a kappa-opioid agonist to reduce dopamine release was unaffected by the two-day stress-exposure paradigm (Supplementary Fig. 9). Therefore, these data demonstrate that severe stress selectively abolishes CRF's ability to modulate dopamine release in the nucleus accumbens. Surprisingly, there was no recovery of the action of CRF on dopamine release in the nucleus accumbens 7, 30 or even 90 days after stress exposure (stress exposure by drug, $F_{4,116} = 4.852$, $p < 0.01$, two-way ANOVA; Fig. 4a). This time period is consistent with the protracted course of stress-induced depressive disorders[21], and indeed, a depression-like phenotype was maintained across this 90-day post-stress period as assessed by swim immobility (Supplementary Fig. 10). Importantly, the loss of the CRF response was neither due to a baseline change in evoked dopamine release (Supplementary Fig. 11) nor simply an age-related phenomenon (Supplementary Fig. 12). Therefore, we have demonstrated that severe stress produces a persistent dysregulation of CRF-dopamine interactions that normally produce a positive affective state.

Stress-induced depressive disorders are associated with altered levels of several neurochemicals that interact with the CRF system, including serotonin[22], dynorphin[23] and glucocorticoids[4,24]. Therefore, we targeted these systems to gain mechanistic insight into the stress-induced loss of CRF's regulation of dopamine release. We pretreated animals (10 ml/kg intraperitoneal) with vehicle, fluoxetine (selective serotonin-reuptake inhibitor; 10 mg/kg), norBNI (kappa-opioid-receptor antagonist; 10 mg/kg) or RU486 (glucocorticoid-

receptor antagonist; 30 mg/kg) prior to stress exposure on each of the swim-stress days. The animals were allowed to recover for seven days, then slices were prepared and the CRF response was tested. While acute regimens of fluoxetine do not alleviate pre-existing depression-related symptoms in patients or animal models, they have been shown to prevent the induction of some depression-like responses to stress[25]. Nonetheless, this treatment did not affect the abolition of CRF modulation of dopamine release by stress ($p > 0.05$; Supplementary Fig. 13). Likewise, this stress-induced perturbation was not significantly affected by norBNI ($p > 0.05$; Supplementary Fig. 13); however, it was prevented by RU486 (30 mg/kg; $p < 0.001$; Fig. 4a and Supplementary Fig. 13), even at the lower dose (10 mg/kg; $p < 0.01$; Supplementary Fig. 13). These data demonstrate that glucocorticoid signaling is a critical component of the profound stress-induced dysregulation of CRF-dopamine interactions in the nucleus accumbens.

This robust loss of the neurochemical response to CRF in the nucleus accumbens following severe stress suggests a long-lasting alteration in its subjective qualities. To test this notion, we utilized the place conditioning paradigm in animals that had been exposed to the two-day swim-stress regimen. Mice that underwent repeated swim stress seven days prior to conditioning spent significantly less time in the CRF-paired chamber compared to the vehicle-paired chamber following conditioning, establishing that CRF in the nucleus accumbens is now aversive to these animals (conditioning by drug, $F_{1, 10} = 5.824$, $p < 0.01$, two-way ANOVA, Supplementary Fig. 14a). Therefore, severe stress produces a diametric shift in the subjective qualities of CRF in the nucleus accumbens from positive to negative (Fig. 4b). Consistent with the enduring loss of CRF regulation of dopamine observed *in vitro*, the absence of CRF conditioned place preference persisted for at least 90 days following repeated stress exposure ($F_{2,20} = 6.870$, $p < 0.05$, one way ANOVA with Dunnett's post-hoc; Fig. 4b and Supplementary Fig. 14b). Likewise, endogenously released CRF no longer stimulated exploration of a novel object when tested seven days after stress exposure (stimulus by drug, $F_{1,16} = 0.004$, $p > 0.05$, two-way repeated measures ANOVA; Supplementary Fig. 15) demonstrating that severe stress abolished the function of CRF in the nucleus accumbens to stimulate appetitive responses to arousing stimuli (unpaired t-test, $p < 0.05$, Fig. 4c). Therefore, these findings demonstrate the long-term loss of a regulatory mechanism of motivated behavior following severe stress.

Major Depressive Disorder has a lifetime prevalence of 17 %, making it one the World's largest public health concerns[26]; yet, its molecular foundation has been elusive. Patients suffering from this disorder present with constellations of symptoms including loss of affect, cognitive impairment and homeostatic imbalance[27], symptoms that are presumably precipitated by dysregulation of multiple brain regions[4]. It is established that glucocorticoid-dependent hippocampal atrophy is a critical mediator of cognitive impairment in depression such as memory loss[4]. More recently, disruption of nucleus accumbens function has been implicated in the affective symptoms of depression[4]. In the current work, we studied the actions of CRF on neurotransmission within this brain region in an attempt to connect pathological-stress-related neuroadaptation with the shift in affect observed in depressed patients.

CRF receptors are distributed widely throughout the brain[8] and mediate disparate effects (see Supplementary Discussion). Our data highlight the specificity of the local action of both exogenously applied and endogenously released CRF in the nucleus accumbens in producing a positive, rather than negative, subjective state by increasing dopamine release. Importantly, we demonstrate that severe stress disables this capacity of CRF to positively regulate dopamine, removing CRF's appetitive qualities, leaving a negative perceptual bias. This dysregulation is mediated by glucocorticoid, but not kappa, receptors and is not ameliorated by acute prophylactic administration of a selective serotonin-reuptake inhibitor. Glucocorticoid signaling has been shown to have genomic repressive effects of the CRF system, in particular the downregulation of CRF R1[24]. Indeed, genetic deletion of the CRF R1 gene selectively from dopamine neurons increases anxiety-like behavior[28], further demonstrating that disruption of CRF-dopamine interactions alone is sufficient to produce a negative affective state similar to that following severe stress[29].

Collectively, we demonstrate a specific defect in the regulation of dopamine transmission in the nucleus accumbens as a consequence of exposure to stress that induces depression-like behavior. Indeed, depressive disorders produce a profound change in the perception and behavioral response to acute stressors and other arousing environmental stimuli that elicit CRF signaling. Taken together, our findings provide a neurobiological mechanism for the affective shift from engagement of the environment to withdrawal following severe stress, central to the manifestation of Major Depressive Disorder.

## Methods Summary

### Subjects

Male C57bl/6 mice aged >50 days had *ad libitum* access to food and water. Mice housed together (2-4 per cage) were subjected to the same behavioral treatments. All animal procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

### Neuroanatomy

Immunohistochemistry was performed as previously described[20]. Sections were incubated for 24 h with a mixture of mouse anti-tyrosine hydroxylase 1:1000 and rabbit anti-CRF (peptide) 1:150 and chicken anti-ChAT antibody 1:150 or rabbit anti-CRF R1 or CRF R2 (1:100 to 1:500), then incubated in the appropriate fluorescently tagged secondary antibodies (1:500), and were imaged using epifluorescent and confocal microscopes. Transmission electron microscopy was carried out as previously described[30].

### Fast scan cyclic voltammetry

250-μm coronal slices containing the nucleus accumbens were continuously perfused (1.5-2.0 ml/min) with oxygenated aCSF maintained at 31-33 °C. The potential at a carbon-fiber electrode was held at −0.4 V versus Ag/AgCl, ramped to +1.3 V and back to −0.4 V (400V/s) every 100 ms. A single bi-phasic electrical pulse (2 ms/phase, 100-500 μA) was applied to the slice to evoke dopamine release.

### Conditioned place preference

A three-compartment place-conditioning apparatus was used to measure preference as previously described[20]. On days 2 and 3, mice received two intra-accumbens microinjections per day: one injection of aCSF and one injection of CRF (500ng/200nl) paired with different chambers. On day 4, mice were allowed free access to the apparatus for 30 minutes. Following the conclusion of behavioral testing, cannulae placements were assessed.

### Novel object exploration

The novel object exploration assay similar to previously described[28]. Animals received bilateral intra-accumbens microinfusions of vehicle or α-helical CRF (500 ng/200 nl) counterbalanced across two testing days. On each testing day, the animal was exposed to a new novel object.

## Methods

### Subjects

Male C57BL/6 mice age > 50 days were maintained under a 12-h light-dark cycle (7a.m. to 7p.m. light) with access to standard food and water *ad libitum.* All procedures on animal subjects were approved by the University of Washington or Thomas Jefferson University IACUC committee. Mice housed together (2 - 4 per cage) were subjected to the same behavioral treatments.

### Immunohistochemistry

We used perfusion, cryosectioning and immunohistochemistry procedures as previously described[20]. Sections (30 μm) were then incubated with a mixture of mouse anti-tyrosine hydroxylase 1:1000 (Sigma, St. Louis, MO) and either rabbit anti-CRF (peptide) 1:150 (Sigma, St. Louis, MO) and chicken anti-ChAT antibody 1:150 (Invitrogen, Carlsbad, CA) or rabbit anti-CRF R1 or CRF R2 (Novus Biologicals, Littleton, CO) in blocking buffer for 24-36 hours at room temperature. Sections were then washed with PBS, and detection was performed using the fluorescent secondary antibody Alexa Fluor 488 goat anti-mouse IgG 1:500, Alexa Fluor 555 goat anti-rabbit IgG and Alexa Fluor 633 goat anti-chicken IgG (Invitrogen, Carlsbad, CA) in blocking buffer for 2 hours at room temperature. Sections were washed in PBS 3 × 10 minutes and PB 2 × 10 minutes and mounted on Superfrost plus slides. Sections were imaged with epifluorescence (Nikon) and confocal microscopes (Leica).

### Transmission electron microscopy

Mice were perfused and brains were sectioned as previously described. 100-nm sections were processed using standard transmission electron microscopy procedures[31]. Sections were incubated in mouse anti-TH (1:1,000; Immunostar Inc., Hudson, WI, USA) and rabbit anti-corticotropin-releasing factor receptor (1:1,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) overnight at RT. Immunoperoxidase detection of TH and silver-intensified immunogold localization of CRFr followed standard procedures[30]. Digital images were

captured using the AMT advantage HR/HR-B CCD camera system (Advance Microscopy Techniques Corp., Danvers, MA). Only tissue sections with good preservation of ultrastructural morphology and with both TH and CRFr immunoreactivity clearly apparent in the tissue were used for the analysis. For immunogold labeling, profiles with at least two immunogold-silver particles within a cellular compartment in a single thin section were considered immunolabeled[30,32]. The cellular elements were classified according to the description by Peters and colleagues[33,34].

### Fast scan cyclic voltammetry

Mice were rapidly decapitated and the head placed in preoxygenated ice-cold artificial cerebrospinal fluid (aCSF) in which sucrose (248 mM) was substituted for NaCl. The brain was rapidly removed and blocked to isolate the anterior forebrain. 250-μm coronal slices containing the nucleus accumbens were prepared using methods previously described[35], placed in a recording chamber, and continuously perfused (1.5-2.0 ml/min) with oxygenated aCSF (in mM), NaCl 124, KCl 2.5, $NaH_2PO_4$ 1.25, $MgSO_4$ 2.0, $CaCl_2$ 2.0, Dextrose 10 and $NaHCO_3$ 26) maintained at 31-33 °C. Carbon fiber electrodes were fabricated using a Sutter P-97 puller. Carbon fiber electrodes (working electrodes) were hand cut to approximately 100-150 μm past the capillary tip. The potential at a carbon-fiber electrode was held at −0.4 V versus Ag/AgCl, ramped to +1.3 V and back to −0.4 V (400V/s) every 100 ms. A single biphasic electrical pulse (2 ms/phase, 100-500 μA) was applied to the slice to evoke dopamine release.

### Swim stress

Mice were subjected to either a single 15 minute swim with a 24 hour recovery period, or a two-day swim stress in which they were exposed to a 15-minute swim session on day one, then 24 hours later on day two, were exposed to four 6-minute swim sessions separated by 6 minutes conducted under bright light (690-700 lux) conditions. Water temperature was maintained at 29.0 – 31.0 °C. Animals were removed from the water if they became completely submerged for >1 sec at any time during the paradigm. Some animals were sacrificed at 30 minutes, 7, 30 or 90 days following the final swim session of the two-day protocol and nucleus accumbens slices were prepared.

### Cannulations

Animals were anesthetized with isoflurane and cannulation surgeries were performed using a stereotaxic alignment system similarly to methods previously described[20]. Double guide cannulas (26 gauge, 3.5 mm from pedestal, 2 mm separation; Plastics One, Roanoke, VA) were placed in the nucleus accumbens core at 1.0 mm/-1.0 mm lateral, 1.0 mm posterior from bregma, and 3.5 mm below the skull. Guide cannulas were anchored using dental cement, and dummy internal cannulas were placed inside until injection. Mice were injected intracerebroventricularly by placing a 33 gauge internal cannula (Plastics One, Roanoke, VA) into the guide cannula.

### Conditioned place preference

Animals were allowed to recover from surgery for at least seven days. All animals were handled for four days prior to the pre-test day. Animals assigned to the stress-exposed group were subjected to the two-day swim stress paradigm following recovery; animals were not included if they did not show normal swimming responses. Stress-exposed animals began CRF conditioning 7 or 90 days following the final swim session. A three-compartment place-conditioning apparatus was used to measure preference as previously described[20]. On days 2 and 3, mice received two injections per day: one injection of aCSF and one injection of CRF (500ng/200nl) paired with different chambers at 125 nl/min. On day 4, mice were once again allowed free access to the entire apparatus for 30 minutes. Following the conclusion of behavioral testing, cannulae placements were assessed. Mice with cannula placements outside the accumbens were excluded from the study.

### 6-OHDA lesion and HPLC

Mice were injected with either 6-OHDA (2 μg/500 nl; Sigma) or vehicle (0.9-% NaCl, 0.1-% ascorbate). Following the conclusion of behavioral testing, a tissue core (approximately 2 × 2 × 1 mm) of the ipsilateral and contralateral accumbens of each animal was microdissected, rapidly frozen in liquid nitrogen and stored in microcentrifuge tube at −80 °C until processed for tissue dopamine content. High-performance liquid chromatography (HPLC) was used to measure monoamine content by the Neurochemistry Core Lab at Vanderbilt University's Center for Molecular Neuroscience Research.

### Novel object exploration

Mice were cannulated, allowed to recover from surgery and handled for four days prior to being subjected to a novel object exploration assay similar to previously described[28]. Briefly, on test day 1, mice were given bilateral intra-accumbens microinfusions of either vehicle (lactated ringer's with 1-% acetic acid) or α-helical CRF (2 μg) and were allowed to habituate in an open field for 15 minutes.

Subsequently, a novel object was introduced and exploratory behavior of the novel object was measured for an additional 15 minutes. On test day 2, the animals received the alternative pharmacological treatment to what they received on day 1, were allowed to habituate again the open field and then exposed to a second novel object. Both pharmacological treatment and novel objects were counter-balanced across test days. Identically to the place conditioning experiments, one group of mice were exposed to swim stress 7 days prior to test day 1.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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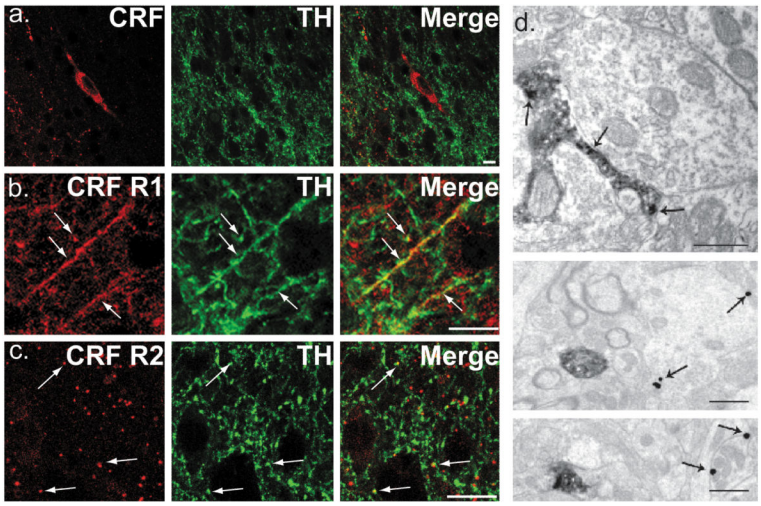


**Figure 1. Cellular localization of CRF peptide, CRF R1 and CRF R2 in the nucleus accumbens**

**a**, Immunoreactivity for CRF peptide (top), CRF R1 (middle) or CRF R2 (bottom) is shown in red and for tyrosine hydroxylase (TH) is shown in green. The arrows highlight examples of co-localization (yellow in the merged images). Scale bar = 10 μm. **b**, Transmission electron microscopy photomicrographs demonstrating CRF receptors (labeled with immunogold particles; arrows) present on both TH positive (immunoperoxidase labeled) and TH negative profiles. Top scale bar = 0.5 μm; bottom scale bars = 1 μm.

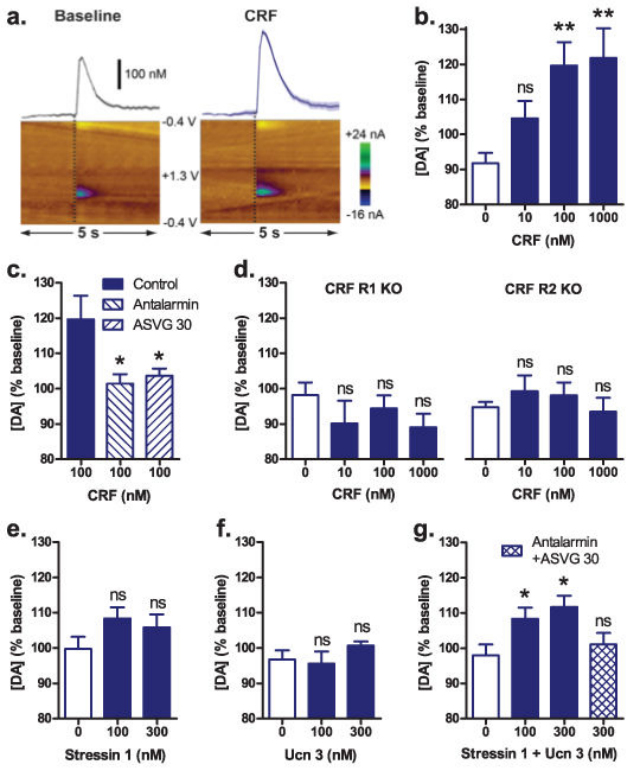


**Figure 2. CRF increases dopamine release in the nucleus accumbens through co-activation of CRF R1 and R2**

**a**, Representative dopamine release evoked by electrical stimulation (dashed lines) before (left) and after (right) application of 100-nM CRF (mean ± s.e.m. for 5 consecutive stimulations, top) and corresponding two-dimensional plots depicting changes in peak dopamine oxidation current (pseudocolor) with time as the abscissa and applied potential as the ordinate (bottom). **b**, Concentration response to CRF, n = 11-18. **c**, Effect of antagonists for CRF R1 (antalarmin, 1000 nM) or CRF R2 (anti-sauvagine 30, 250 nM; ASVG 30), n = 18-20. **d**, CRF in mice lacking gene encoding the CRF R1 (left) or CRF R2 (right) receptor, n = 7-13. **e-g**, Effect of CRF R1 agonist, stressin 1, n = 9-15 (**e**), CRF R2 agonist, urocortin 3 (100 or 300 nM), n = 5-8 (**f**) or their co-application, n = 8-15 (**g**). Data on bar graphs are mean + s.e.m.; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$ vs vehicle.

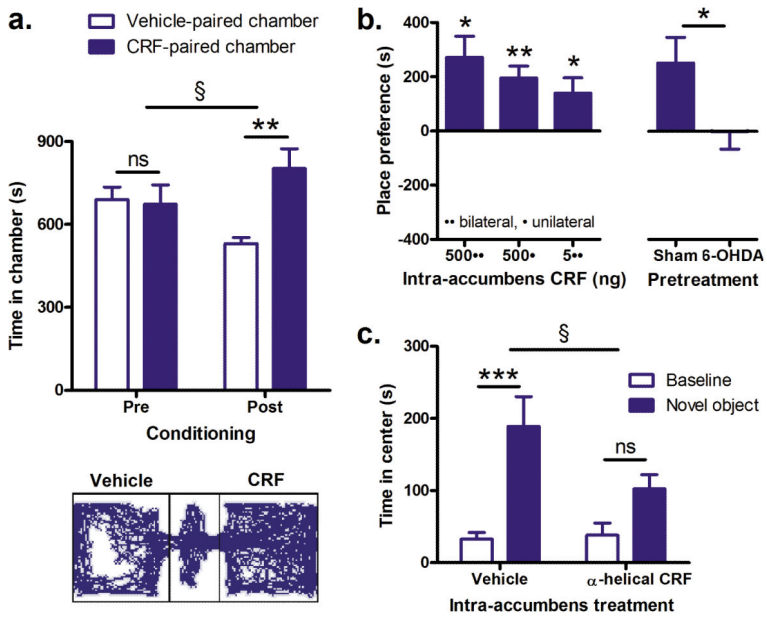


**Figure 3. CRF in the nucleus accumbens promotes appetitive behavior**

**a**, Mean (+ s.e.m.) mean times spent in CRF-paired chamber than the vehicle paired chambers before and after conditioning, n = 7 (top) and representative post-conditioning activity trace (bottom). **b**, Place preference (time in CRF-paired chamber – time in vehicle paired chamber post conditioning) for intra-nucleus accumbens injections of 500 ng CRF bilateral, 500 ng unilateral or 5 ng bilateral, n = 7-10 (left). Place preference for 500 ng CRF (unilateral) in sham or 6-OHDA treated mice, n = 10 (right). **c**, Time spent in the center of an open field before and during presentation of a novel object (placed in center of field) following bilateral intra-accumbens infusion of the CRF-receptor antagonist α-helical CRF (500 ng) or its vehicle, n = 10. Data on bar graphs are mean + s.e.m.; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; § $p < 0.05$ for interaction.

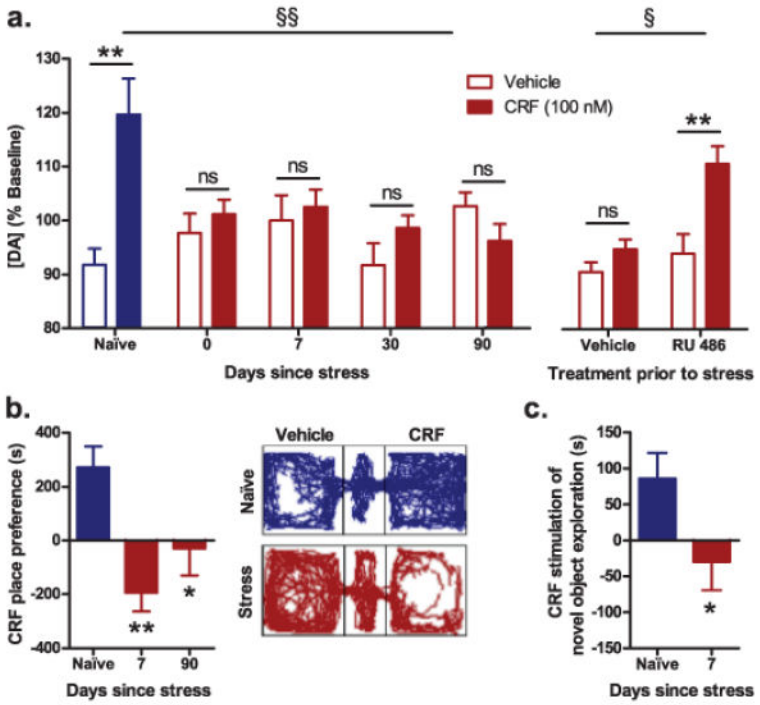


**Figure 4. Stress exposure abolishes the CRF mediated increase in evoked dopamine release and subsequent appetitive behaviors**

**a**, Effect of CRF on dopamine release in naïve mice (blue) and following swim stress (red), n = 8-18 (left), and in animals that were pretreated with the glucocorticoid-receptor antagonist, RU 486 (30 mg/kg, i.p.) or its vehicle prior to stress, n= 6-10 (right). **b**, Mean (+ s.e.m) place preferences for intra-accumbensCRF in naïve (blue) and stress exposed mice (red), n = 6-8 (left) and representative activity traces (right). **c**, Difference in the increased center time during presentation of novel object between vehicle and CRF-receptor antagonism in naïve (blue) and 7-day post-stress (red) animals, n = 9-10. 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.