OXFORD

International Journal of Neuropsychopharmacology (2021) 24(3): 221–228

doi:10.1093/ijnp/pyaa079
Advance Access Publication: October 30, 2020
Regular Research Article

REGULAR RESEARCH ARTICLE

Functional Interplay of Type-2 Corticotrophin Releasing Factor and Dopamine Receptors in the Basolateral Amygdala-Medial Prefrontal Cortex Circuitry

H. E. Yarur, J. Zegers, I. Vega-Quiroga, J. Novoa, F. Ciruela, M. E. Andres, K. Gysling

Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile (Dr Yarur, Mr Zegers, Mr Vega-Quiroga, Mr Novoa, Dr Andres, Dr Gysling); Unitat de Farmacologia, Departament Patologia i Terapèutica Experimental, Facultat de Medicina, IDIBELL, Universitat de Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain (Dr Ciruela); Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain (Dr Ciruela).

Correspondence: Katia Gysling, PhD, Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, 8331150 Santiago, Chile (kgysling@bio.puc.cl).

Abstract

Background: Basolateral amygdala (BLA) excitatory projections to medial prefrontal cortex (PFC) play a key role controlling stress behavior, pain, and fear. Indeed, stressful events block synaptic plasticity at the BLA-PFC circuit. The stress responses involve the action of corticotrophin releasing factor (CRF) through type 1 and type 2 CRF receptors (CRF1 and CRF2). Interestingly, it has been described that dopamine receptor 1 (D1R) and CRF peptide have a modulatory role of BLA-PFC transmission. However, the participation of CRF1 and CRF2 receptors in BLA-PFC synaptic transmission still is unclear.
Methods: We used in vivo microdialysis to determine dopamine and glutamate (GLU) extracellular levels in PFC after BLA stimulation. Immunofluorescence anatomical studies in rat PFC synaptosomes devoid of postsynaptic elements were performed to determine the presence of D1R and CRF2 receptors in synaptical nerve endings.
Results: Here, we provide direct evidence of the opposite role that CRF receptors exert over dopamine extracellular levels in the PFC. We also show that D1R colocalizes with CRF2 receptors in PFC nerve terminals. Intra-PFC infusion of antisauvagine-30, a CRF2 receptor antagonist, increased PFC GLU extracellular levels induced by BLA activation. Interestingly, the increase in GLU release observed in the presence of antisauvagine-30 was significantly reduced by incubation with SCH23390, a D1R antagonist.
Conclusion: PFC CRF2 receptor unmasks D1R effect over glutamatergic transmission of the BLA-PFC circuit. Overall, CRF2 receptor emerges as a new modulator of BLA to PFC glutamatergic transmission, thus playing a potential role in emotional disorders.

Key Words: CRF2 receptor, D1 receptor, glutamatergic transmission, dopaminergic transmission, prefrontal cortex

Received: June 2, 2020; **Revised:** October 9, 2020; **Accepted:** October 26, 2020

Significance Statement

Corticotrophin-releasing factor (CRF), through its action on CRF type 1 and CRF type 2 receptors, and dopamine (DA), through DA type 1 and DA type 2 receptor, regulate adaptive responses to stressors. However, the mechanism by which CRF and DA receptors signaling modulates synaptic transmission remains elusive, especially within the corticolimbic circuitry. Here, we found that CRF type 2 receptor functionally modulates dopaminergic transmission in prefrontal cortex and is positioned to limit dopamine type 1 receptor transmission through inhibition of BLA-to-PFC glutamatergic transmission.

Introduction

The medial prefrontal cortex (PFC) is the main brain area that processes information about cognition, motivation, and emotion (Miller and Cohen, 2001). The basolateral nucleus of the amygdala (BLA) is a key component of the network that regulates PFC to process emotional information, such as stressful stimuli or fear (Sotres-Bayon and Quirk, 2010; Courtin et al., 2013). Stressful stimuli constitute an important factor in the animal behavior that modifies PFC function (Snyder et al., 2015; Urban and Valentino, 2017). Interestingly, it has been shown that acute stress exposure blocks BLA-to-PFC synaptic plasticity by an unknown mechanism (Maroun and Richter-Levin, 2003). The corticotrophin releasing factor (CRF) system integrates both the endocrine and behavioral responses to stress (Vale et al., 1981; Henckens et al., 2016). The CRF system has 2 G protein-coupled receptors, CRF1 and CRF2, which control the cellular excitability and synaptic plasticity in some brain areas (Liu et al., 2004, 2005; Orozco-Cabal et al., 2008), including PFC (Millan et al., 1986; Yan et al., 1998; Grammatopoulos et al., 2001; Guan et al., 2014). While the role of CRF1 receptor in PFC is well known (Uribe-Mariño et al., 2016), the function of CRF2 receptor in the PFC has been involved in the modulation of BLA glutamatergic input to PFC (Yarur et al., 2020b). On the other hand, the dopaminergic system mediates the relevance of reward-related stimuli (Berridge, 2007). Dopamine (DA) exerts a modulatory influence over BLA-PFC synaptic transmission (Floresco and Tse, 2007). Remarkably, DA modulates the activity of PFC neurons via different receptor subtypes (Gao et al., 2001; Seamans et al., 2001; Tseng and O'Donnell, 2004). It has also been shown that CRF potentiates the modulation exerted by DA on the excitatory transmission of the BLA-to-PFC pathway (Orozco-Cabal et al., 2008).

Together, this evidence prompted us to hypothesize that presynaptic CRF2 receptor modulates the BLA-PFC transmission. To address this question, we first assessed CRF2 and DA receptors co-distribution in PFC synaptic terminals and then measured the impact of CRF2 receptor function in DA and glutamate (GLU) release associated with the BLA-PFC transmission. Indeed, we demonstrated that CRF2 receptors co-distributed with D1Rs in PFC nerve terminals and that its blockade reduced DA and increases GLU release in PFC by BLA activation. Interestingly, PFC treatment with a D1R antagonist precluded the CRF2 receptor blockade-dependent GLU release. Overall, our results showed that CRF2 receptor in PFC nerve terminals play a key role in regulating the BLA-PFC glutamatergic transmission needed for connecting emotional stress and decision-making control.

Materials and Methods

Animals

Male Sprague-Dawley rats (270–300 g) with ad libitum food and water were used throughout this study. Rats were obtained from the UC CINBIOT Animal Facility of Pontificia Universidad Católica de Chile. The experimental protocols were approved by the Institutional Bioethical Committee of Pontificia Universidad Católica de Chile.

Preparation of PFC Synaptosomes

Purified synaptosomes of PFC, devoid of postsynaptic density, were prepared on a discontinuous Percoll gradient as described (Rodrigues et al., 2005; Ciruela et al., 2006; Yarur et al., 2020b). The PFC was dissected out of coronal slices of 4 animals following the Atlas of Paxinos and Watson (1986). The extracted tissue was placed in a glass Potter homogenizer with 10 mM HEPES, 320 mM sucrose, and 3 mM EDTA, pH 7.4, and centrifuged at 1000 × g for 10 minutes at 4°C. The supernatant was centrifuged at 17 000 × g for 20 minutes at 4°C. The obtained pellet was resuspended and centrifuged in a Percoll gradient (PVP-silica colloid, Sigma Aldrich, St Louis, MO) at 15 000 × g for 20 minutes at 4°C. The synaptosomal fraction was dissolved (in an equal volume to the fraction obtained) in 320 mM sucrose solution for immunofluorescence or radioimmunoprecipitation assay lysis buffer (Millipore) solution for western blotting. The synaptosomal protein concentration was determined by Micro BCA Protein Assay Kit (Thermo Fisher).

Immunofluorescence in PFC Synaptosomes

Immunofluorescence in synaptosomes was performed as previously described (Ciruela et al., 2006; Yarur et al., 2020b). Synaptosomes from PFC (15 µg of synaptosomal protein) were seeded on coverslips coated with poly-L-lysine (Sigma Aldrich) and fixed with 4% paraformaldehyde/10 % sucrose for 15 minutes, permeabilized with 0.2% Triton X-100, and incubated for 1 hour with blocking solution (4% bovine serum albumin in phosphate buffered saline). The synaptosomes were incubated 1 hour at room temperature with the primary antibodies rabbit anti-D1R (1:500; ab20066, Abcam), goat anti-CRF2 receptor (1:500; SC-1826; Santa Cruz Biotechnology), and mouse anti-D2R (1:500; Santa Cruz Biotechnology, Dallas, TX), and thereafter for 1 hour with the corresponding secondary antibodies (donkey anti-goat Alexa fluor-488, donkey anti-mouse Alexa fluor-594, and donkey anti-rabbit Alexa fluor-555; 1:200; Invitrogen). The images were captured with a 100× objective in a confocal microscope (Olympus, Fluoview 1000) and analyzed with FLUOVIEW v6.0 software. Each synaptosomal preparation was obtained from 4 animals, and photographs for quantification were taken with 60× from 8 different subareas in each coverslip and processed by ImageJ software (rsb.info.nih.gov/ij).

In Vivo Microdialysis

Animals were anesthetized with 8% chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus. Body temperature was maintained at 37°C by an electrical blanket, and chloral hydrate

was maintained at 0.8 µL/min by an electrical infusion pump (Bioanalytical systems inc). A microdialysis probe (2 mm length; MAB 2.14.2, Microbiotech) was implanted in PFC (AP=3.2 mm, ML=0.7 mm, and DV=5.0 mm from Bregma), and a second microdialysis probe (CMA 11, CMA Microdialysis AB) was implanted in the BLA (AP=2.8 mm, ML=4.8 mm, and DV=8.2 mm from Bregma). The microdialysis protocol used was previously described (Vega et al., 2018). Artificial cerebrospinal fluid (aCSF) was perfused through the microdialysis probes at 2 µL/min. After the stabilization period (90 minutes), samples were collected every 10 minutes from the PFC. At the time indicated, 70 mM K^+-aCSF was perfused through the microdialysis probe in the BLA for 10 minutes. Antisauvagine-30 (1 µM, Tocris), CP154526 (1 µM, Tocris), Raclopride (0.1 µM, Tocris), or SCH23390 (1 µM, Tocris) was perfused intra-PFC, as indicated in the figures, and the drug concentrations used were as previously described (Orozco-Cabal et al., 2008; Yarur et al., 2020b). All drugs were diluted in aCSF containing 0.1 mg/mL bovine serum albumin. After the experiments, the brains were removed and stored in 4% paraformaldehyde for verification of the microdialysis probe placements. DQuantification of GLU and DA was performed using high performance liquid chromatography-fluorometric determination for GLU and high performance liquid chromatography-electrochemical determination for DA, as previously described (Sotomayor-Zarate et al., 2010).

Statistical Analyses

Statistical analyses were performed with the statistical software GraphPad Prism 6 (GraphPad Software). The data are expressed as the mean ± SEM. Microdialysis experiments were analyzed with 2-way ANOVA followed by Bonferroni's multiple comparisons test. The percentage of change was calculated using the average values from time point 70 minutes (K^+ stimulation period) with respect to the 10 minutes before K^+ stimulation onset and were analyzed with 1-way ANOVA followed by Dunnett's multiple comparison test.

Results

CRF Receptors Modulate Extracellular DA Levels in PFC

Considering the modulatory role of CRF over the DA tone on the BLA-PFC synapses (Orozco-Cabal et al., 2008), we evaluated how CRF receptor blockade modulates DA release in PFC. To this end, we assessed PFC DA extracellular levels by microdialysis on BLA stimulation in the absence or presence of local probe infusion of CRF receptor antagonists (aSvg30 for CRF2 receptor and CP154,526 for CRF1 receptor) (Fig. 1). Interestingly, the infusion of CP154,526 in PFC significantly increased PFC DA extracellular levels (Fig. 1B). On the other hand, aSvg30 decreased PFC DA extracellular levels (Fig. 1B). Two-way ANOVA revealed a main effect of treatment ($F_{2,6}=74.16$, $P<.0001$), time ($F_{9,27}=102.4$, $P<.0001$), and a treatment×time interaction ($F_{18,54}=16.84$, $P<.0001$); (****$P<.00001$, ***$P<.0001$, **$P<.001$, *$P<.05$ vs aCSF, Dunnett post-hoc test). BLA stimulation induced PFC DA release in the presence of both CRF receptor antagonists (Fig. 1B). Interestingly, we found that the magnitude of change in DA levels between the stimulation and pre-stimulation time was affected only in the presence of aSvg30 (Fig. 1C). One-way ANOVA revealed a main effect in the reduction induced by BLA stimulation in the aSvg30 group (***$P<.0001$ vs aCSF, Dunnett post-hoc test; $F_{2,9}=17.49$, $P=.0008$), suggesting a modulation by CRF2 receptor of PFC DA extracellular levels induced by BLA stimulation. Overall, these results suggest that CRF1 and CRF2 receptors modulate PFC DA extracellular levels, but only CRF2 receptor modulates DA extracellular levels induced by the BLA activity.

CRF2 and DA Receptors Co-distribute in PFC Synaptic Terminals

Considering that CRF2 receptor modulates BLA-dependent PFC DA release, we interrogated whether CRF2 and DA receptors co-distribute in a synaptosomal preparation devoid of

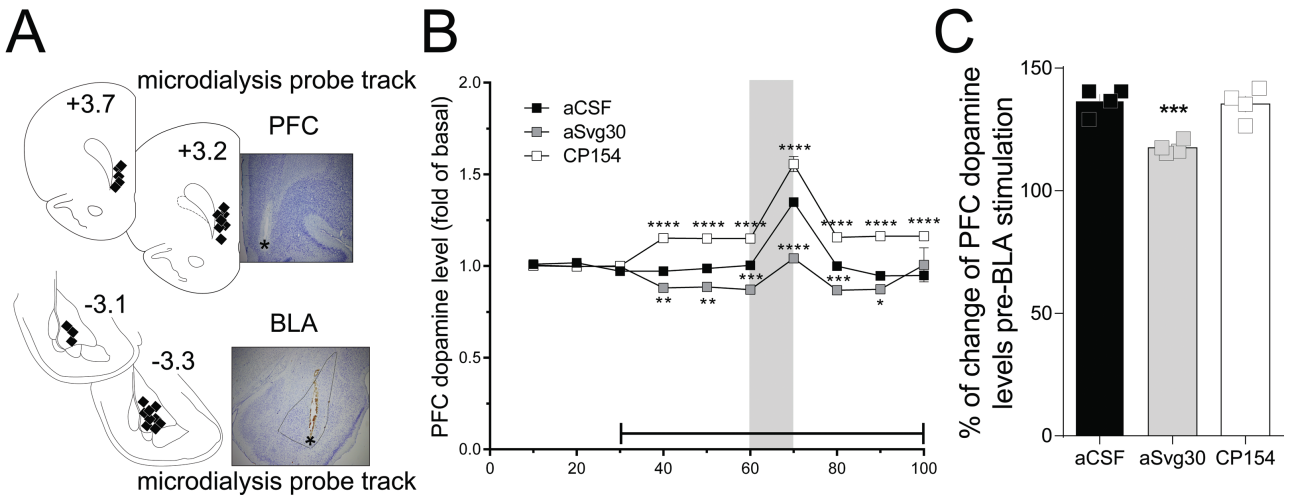

Figure 1. PFC infusion of the CRF antagonists modulates the extracellular DA levels in PFC. (A) Brain coronal sections showing the placement of the microdialysis probes in BLA (–3.1 mm to –3.3 mm from bregma) (bottom) and PFC (3.7 mm to 3.2 mm from bregma) (top). (B) In vivo measurement of PFC extracellular DA levels using in vivo microdialysis. The vertical gray bar indicates the time of BLA perfusion with 70 mM K^+-aCSF, and the horizontal black line indicates the time of intra-PFC infusion of the antagonists. Intra-PFC extracellular DA levels in the presence of aCSF (n=4), 1 µM aSvg30, CRF2 antagonist (n=4), and 1 µM CP154,526, CRF1 receptor antagonist (CP154, n=4). Values correspond to the -fold of the mean of the first 3 basal values for each condition. (C) Effects of aSvg30 and CP154 infusion on BLA-induced PFC extracellular dopamine levels. The average values were calculated from time point 70 minutes (K+ stimulation period) with respect to 20 minutes before K+ stimulation onset.

postsynaptic elements (Rodrigues et al., 2005). To this end, we performed CRF2 receptor immunofluorescence detection in PFC synaptosomes enriched in presynaptic elements (Fig. 2). Interestingly, CRF2 receptor was found in presynaptic PFC nerve terminals co-distributing with D1R (Fig. 2A, C–D). Overall, 79.11% ± 1.57% of PFC synaptosomes bearing CRF2 receptor were also positive for D1R and 23.98% ± 4.45% of PFC synaptosomes positive for D1R co-expressed CRF2 receptor. At the same time, CRF2 receptor co-distributed with D2R (Fig. 2B–D). The 64.5% ± 6.72% of PFC synaptosomes bearing CRF2 receptor were positive for D2R and 73.56% ± 3.4% of PFC synaptosomes positive for D2R co-expressed CRF2 receptor. Thus, these results showed that CRF2 receptors coexist with D1R and D2R in PFC nerve terminals.

CRF2 and DA Receptors Modulate PFC GLU Extracellular Levels

It has been shown that CRF and DA receptors modulate BLA-evoked excitatory postsynaptic current in PFC (Orozco-Cabal et al. 2008). Thus, we evaluated whether CRF2 and DA receptors may modulate GLU content in PFC. To this end, we determined GLU levels in PFC on infusion of CRF2 and DA receptor antagonists (Fig. 3). Two-way ANOVA revealed a main effect of treatment ($F_{3,9}=29.98$, $P<.0001$), time ($F_{9,27}=75.51$, $P<.0001$), and a treatment × time interaction ($F_{27,81}=30.83$, $P<.0001$) (****$P<.00001$, ***$P<.0001$, **$P<.001$, *$P<.05$ vs aCSF, Dunnett post-hoc test). Interestingly, the infusion of aSvg30 in PFC through the microdialysis probe significantly increased BLA-induced PFC GLU release (Fig. 3B–C). One-way ANOVA revealed a main effect in the facilitation induced by BLA stimulation in the aSvg30 group (**$P<.0001$ vs aCSF, Dunnett post-hoc test; $F_{3,12}=30.57$, $P<.0001$). These results suggested that CRF2 receptor negatively regulates BLA-induced PFC GLU release. Next, we assessed the effect of PFC infusion of SCH23390 (1 μM), a D1R selective antagonist, in BLA-induced PFC GLU release. The infusion of SCH23390 alone did not affect basal and BLA-induced PFC GLU release (Fig. 4). Interestingly, the co-infusion of SCH23390 with aSvg30 significantly blocked the increase in BLA-induced PFC GLU release (Fig. 3B–C). Next, we analyzed the effect of PFC infusion of raclopride (0.1 μM), a D2R selective antagonist, in BLA-induced PFC GLU release. Two-way ANOVA revealed a main effect of treatment ($F_{2,6}=4.68$, $P=.0594$), time ($F_{9,27}=13.88$, $P<.0001$), and a treatment × time interaction ($F_{18,54}=7.51$, $P<.0001$) (****$P<.00001$, **$P<.001$ vs aCSF, Dunnett post-hoc test). Thus, the infusion of raclopride alone did not affect basal and BLA-induced PFC GLU release (Fig. 4). Interestingly, the co-infusion of raclopride with aSvg30 blocked the effect of the aSvg30 in the increase of the BLA-induced PFC GLU release (Fig. 3B–C). Together, it shows us that CRF2 and DA receptors significantly modulate GLU levels in the BLA-PFC synapse.

Discussion

In the present study, we addressed the role of CRF receptors controlling extracellular DA levels in the PFC. Indeed, CRF2 receptors were able to modulate DA levels in the PFC depending on BLA-PFC circuit activity. In addition, we revealed the coexpression of CRF2 receptor with D2R and D1R in PFC synaptic terminals and its functional interaction regulating GLU release in the BLA-PFC glutamatergic transmission.

The electrical stimulation of BLA prompts DA release from ventral tegmental area (VTA) DAergic projections to PFC (Jackson and Moghaddam, 2001). Indeed, we were able to recap increments of PFC DA extracellular levels on BLA stimulation with a depolarizing solution (Fig. 1). It was previously shown that electrical stimulation of the BLA increases PFC DA efflux (Jackson and Moghaddam, 2001). The available information

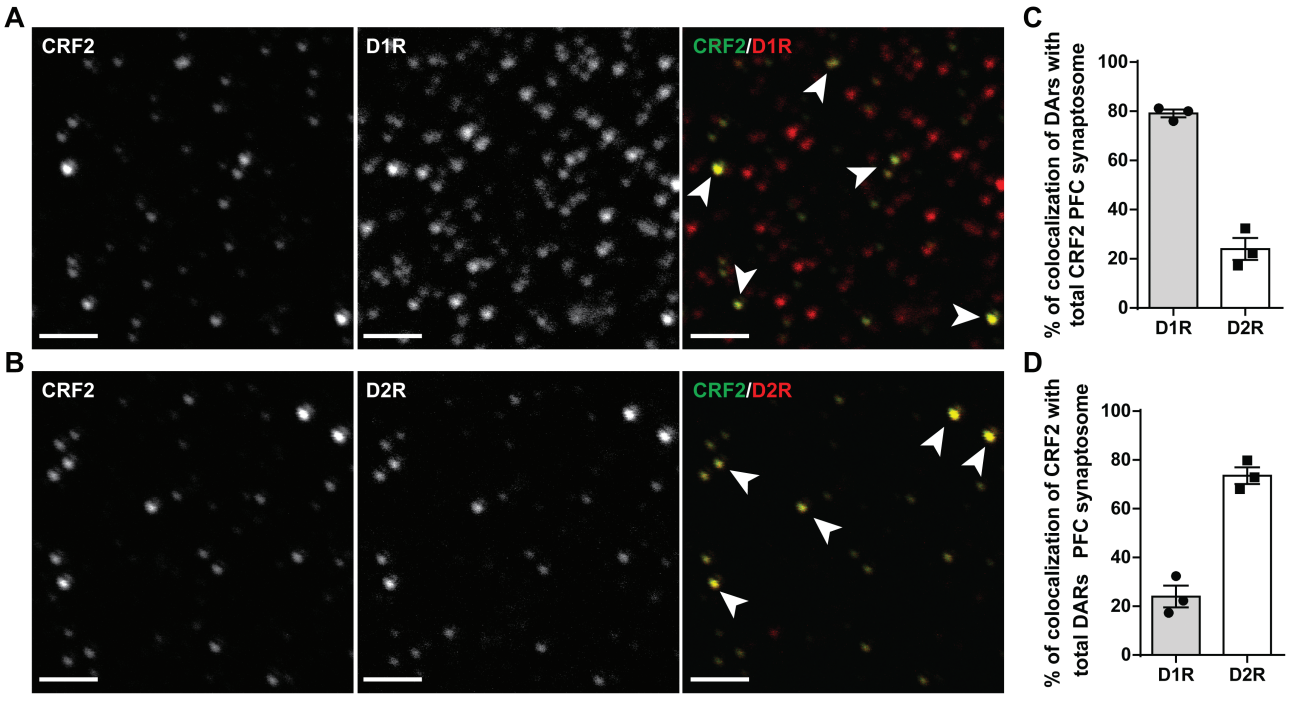

Figure 2. CRF2 receptor co-distributes with DA receptors in PFC presynaptic terminals. (A–B) Confocal immunodetection of CRF2 receptor in preparation of PFC synaptosomes, devoid of presynaptic elements. (A) Immunofluorescence processed for CRF2 receptor (green) and D1R (red) (scale bar = 2 μm). Arrows depict synaptosomes colocalizing D1R and CRF2 receptor. (B) Immunofluorescence processed for CRF2 receptor (green) and D2R (red) (scale bar = 2 μm). Arrows depict synaptosomes colocalizing D2R and CRF2 receptor. (C) Percentage of colocalization of positive CRF2 synaptosomes with total D1R or D2R positive PFC synaptosomes. (D) Percentage of colocalization of positive D1R or D2R synaptosomes with total CRF2 positive PFC synaptosomes.

also shows that afferent projections from PFC to VTA make direct connection with dopaminergic neurons, which in turn project back to the PFC (Carr and Sesack, 2000; Lammel et al., 2011, 2012). Thus, BLA can increase PFC DA extracellular levels through a reciprocal interaction with BLA-PFC-VTA (Jackson and Moghaddam, 2004).

CRF receptors are differentially expressed in the brain (Henckens et al., 2016). Thus, while CRF1 receptors are expressed and function in PFC (Van Pett et al., 2000; Uribe-Mariño et al., 2016), CRF2 receptor expression has been described in glutamatergic nerve terminals of the lateral septum (Liu et al., 2004, 2005) and in VTA GABAergic interneurons (Williams et al., 2014). We recently described the expression of CRF2 receptors in PFC synaptic terminals originated in BLA (Yarur et al., 2020b) where they co-distribute with both D1R and D2R (Fig. 2) yet show the highest co-distribution with D2R compared with D1R (74% vs 24%, respectively). Interestingly, our results suggest an additional mechanism of functional interplay between DA and CRF2 receptors in PFC synaptic terminals.

CRF1 and CRF2 receptors control neurotransmitter release, for instance DA, in different brain areas (Isogawa et al., 2000; Orozco-Cabal et al., 2006; Wang et al., 2007; Lemos et al., 2012; Boyson et al., 2014). Here, we unravel the differential contribution of each CRF receptor controlling DA levels in PFC. Herein, CRF1 receptors have a local inhibitory role over PFC extracellular DA levels that does not depend on BLA stimulation (Fig. 1B–C) in contrast to what was previously reported (Isogawa et al., 2000). Importantly, while these authors assessed the role of CRF1 receptor on systemic (i.p.) antagonist administration, we locally administered the CRF1 receptor antagonist. Conversely, local infusion of a CRF2 receptor antagonist significantly reduces PFC DA extracellular levels depending on BLA stimulation (Fig. 1B–C). Overall, our data show that CRF1 and CRF2 receptors exert opposite effects on basal PFC DA extracellular levels, whereas CRF2 receptor may also exert additional effects on PFC DA extracellular levels that depend on BLA stimulation.

BLA projections into PFC are predominantly glutamatergic (Gabbott et al., 2006) with CRF and DA modulating BLA-to-PFC

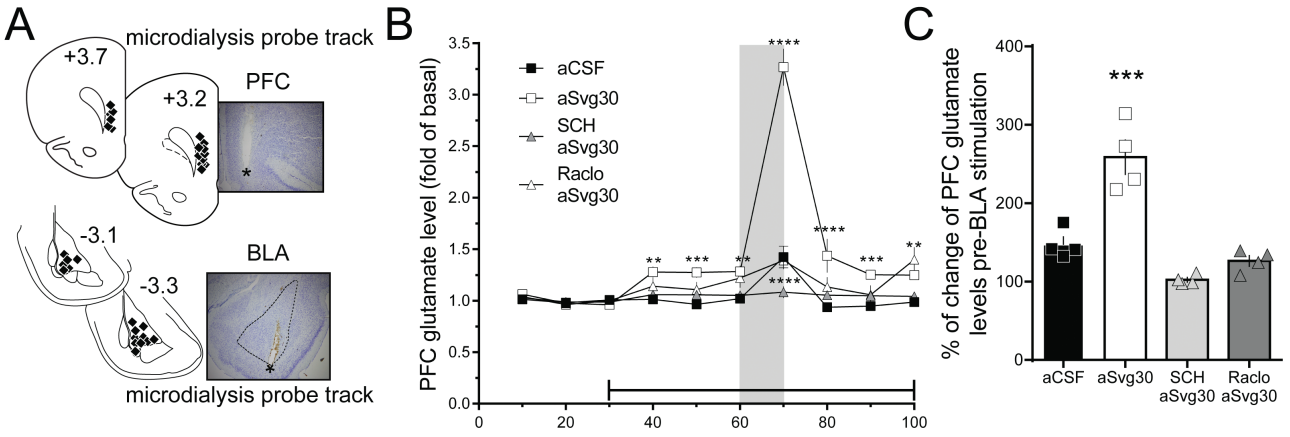

Figure 3. PFC infusion of the CRF2 receptor antagonist aSvg-30 (aSvg30) enhanced BLA-induced extracellular GLU increase in PFC but depends of DA receptors. (A) Brain coronal sections showing the placement of the microdialysis probes in BLA (–3.1 mm to –3.3 mm from bregma) (bottom) and PFC (3.7 mm to 3.2 mm from bregma) (top). (B) In vivo measurement of PFC extracellular GLU levels using in vivo microdialysis. The vertical gray bar indicates the time of BLA perfusion with 70 mM K^+-aCSF, and the horizontal black line indicates the time of intra-PFC infusion of the antagonists. Intra-PFC extracellular GLU levels in the presence of aCSF (n = 4), 1 μM aSvg30 (n = 4), 1 μM SCH23390 (SCH, n = 4), and 0.1 μM raclopride (Raclo, n = 4). Values correspond to the -fold of the mean of the first 3 basal values for each condition. For the effects of DA receptor antagonist and aSvg30 infusion on BLA-induced PFC extracellular GLU levels, the average values were calculated from time point 70 minutes (K^+ stimulation period) with respect to 10 minutes before K^+ stimulation onset.

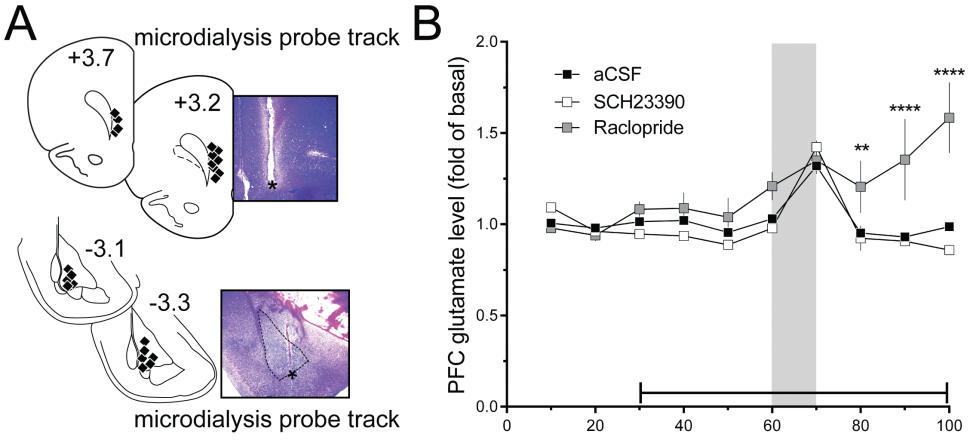

Figure 4. PFC infusion of the DA antagonists did not modulate the extracellular GLU levels in PFC dependent of the BLA stimulation. (A) Brain coronal sections showing the placement of the microdialysis probes in BLA (–3.1 mm to –3.3 mm from bregma) (bottom) and PFC (3.7 mm to 3.2 mm from bregma) (top). (B) In vivo measurement of PFC extracellular GLU levels using in vivo microdialysis. The vertical gray bar indicates the time of BLA perfusion with 70 mM K^+-aCSF, and the horizontal black line indicates the time of intra-PFC infusion of the antagonists. Intra-PFC extracellular DA levels in the presence of aCSF (n = 4), 1 μM SCH23390 (n = 4), and 0.1 μM raclopride (n = 4). Values correspond to the -fold of the mean of the first 3 basal values for each condition.

synapses (Orozco-Cabal et al., 2008). Under basal conditions, BLA stimulation did not significantly increase PFC GLU levels (Fig. 3). It has been described that GLU transmission is more tightly regulated by reuptake or feedforward inhibition in the PFC by BLA synaptic terminals (Tejeda et al., 2013; McGarry and Carter, 2016), precluding detection with microdialysis. Herein, we described that CRF2 receptor modulation of PFC GLU levels depends on BLA stimulation (Fig. 3). It should be noted that the microdialysis probe placement includes all mPFC regions (prelimbic and infralimbic regions), which suggest that CRF2 regulation of DA and GLU levels is throughout the mPFC regions. Thus, this BLA-dependent regulation of GLU content by CRF2 receptor is modulated by D1R and D2R locally in the PFC (Fig. 3). Interestingly, DA antagonists alone did not modify PFC GLU extracellular levels that depend on BLA stimulation (Fig. 4). Hence, the modulatory role of DA receptors was apparent only in the presence of the CRF2 receptor antagonist (Fig. 3). Interestingly, it appears that the functional interaction between CRF2 receptor and D1R or D2R has different levels. First, the co-infusion of the CRF2 receptor plus the D2R antagonist in the PFC precluded the CRF2 receptor antagonist effects over BLA-dependent GLU increase. Second, the co-infusion of the CRF2 receptor and D1R antagonist in the PFC totally occludes the BLA-dependent GLU increase observed in PFC of control animals (Fig. 3B–C). Our results suggested that both dopamine receptors negatively interact to block CRF2 modulation over PFC GLU release after BLA activation. Interestingly, we have described a negative interaction between CRF2 and D1R in PFC synaptosomes (Yarur et al., 2020a). Further studies should address synaptic mechanism of this possible negative interaction between CRF2 and D2R.

The interaction between CRF receptors and DA receptors in the PFC has been previously described (Radulovic et al., 2000; Orozco-Cabal et al., 2008; Yarur et al., 2020b). It has been shown that a D1R tone in the PFC attenuates excitatory synaptic transmission in layer V pyramidal neurons (Gao et al., 2001) and that PFC stimulation recruits interneurons that inhibit BLA projecting neurons (Rosenkranz and Grace, 2001). Consistently, we did not see BLA-dependent GLU increase in PFC because of the inhibitory modulation of PFC over BLA by the reduced D1R tone on the PFC by the infusion of the D1R antagonist. It has been shown that in the PFC, DA release is regulated at the presynaptic site (Tejeda et al., 2013) and that dopamine release in PFC can be modulated at the postsynaptic site as well (Takahata and Moghaddam, 1998). Based on the observed co-distribution levels of CRF2 and D2R in PFC synaptic terminals, we can postulate the existence of a precise CRF2-D2R functional interplay in these GLU terminals. On the other hand, it has been described that D2R agonist in the PFC attenuates BLA-evoked inhibition of PFC neurons (Floresco and Tse, 2007). This could suggest that the D2R effect over CRF2-induced increase in PFC GLU after stimulation of BLA could be due to the recruitment of an inhibitory component in PFC. We observed an increase of GLU post-BLA stimulation induced by D2R antagonist. It has been suggested that PFC D2R has an inhibitory action over NMDA-induced responses in the PFC (Tseng and O'Donnell, 2004; Floresco and Tse, 2007), which could explain why post-BLA stimulation PFC GLU extracellular levels were increased. One remaining question to be addressed is whether the presence of a D1R antagonist in the PFC would inhibit the BLA and if the recruitment of inhibitory components by D2R in the PFC modulates the CRF2 effect in the BLA-dependent GLU increase in the PFC.

In summary, we show that CRF receptors modulate PFC DA content and that CRF2 receptor–mediated modulation is dependent on BLA stimulation. CRF2 receptor colocalizes with D1R and D2R in PFC synaptic terminals, but D2R apparently colocalizes more with CRF2 receptor than D1R. Finally, we show that PFC GLU levels dependent on BLA stimulation are significantly modulated by CRF2 and DA receptors in the PFC. Overall, our findings should contribute to a better understanding of how CRFergic and dopaminergic systems modulate the BLA-PFC synapses and expand the understanding of CRF-dopamine interactions in PFC, which has been shown to be relevant in some pathologies associated with stress (Moghaddam, 2016; Sun et al., 2019). Further studies should address whether the observed interaction between CRF2 and DA receptors in the PFC-BLA synapses, an important pathway and target involved in stress response, is involved in the complexity of the role of PFC in the interaction between emotional stress and decision-making.

Acknowledgments

The authors acknowledge the services provided by UC CINBIOT Animal Facility funded by PIA CONICYT* ECM-07 and the Unidad de Microscopía Avanzada UC (UMA UC).
This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT Grant 1191274). H.E.Y. was recipient of a doctoral fellowship from Comisión Nacional de Investigación Científica y Tecnológica (CONICYT), Chile.

Statement of Interest

None.

References

Berridge KC (2007) The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology (Berl) 191:391–431.

Boyson CO, Holly EN, Shimamoto A, Albrechet-Souza L, Weiner LA, DeBold JF, Miczek KA (2014) Social stress and CRF-dopamine interactions in the VTA: role in long-term escalation of cocaine self-administration. J Neurosci 34:6659–6667.

Carr DB, Sesack SR (2000) Dopamine terminals synapse on callosal projection neurons in the rat prefrontal cortex. J Comp Neurol 425:275–283.

Ciruela F, Casadó V, Rodrigues RJ, Luján R, Burgueño J, Canals M, Borycz J, Rebola N, Goldberg SR, Mallol J, Cortés A, Canela EI, López-Giménez JF, Milligan G, Lluis C, Cunha RA, Ferré S, Franco R (2006) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci 26:2080–2087.

Courtin J, Chaudun F, Rozeske RR, Karalis N, Gonzalez-Campo C, Wurtz H, Abdi A, Baufreton J, Bienvenu TC, Herry C (2013) Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression. Nature 505:92–96.

Floresco SB, Tse MT (2007) Dopaminergic regulation of inhibitory and excitatory transmission in the basolateral amygdala-prefrontal cortical pathway. J Neurosci 27:2045–2057.

Gabbott PL, Warner TA, Busby SJ (2006) Amygdala input monosynaptically innervates parvalbumin immunoreactive local circuit neurons in rat medial prefrontal cortex. Neuroscience 139:1039–1048.

Gao WJ, Krimer LS, Goldman-Rakic PS (2001) Presynaptic regulation of recurrent excitation by D1 receptors in prefrontal circuits. Proc Natl Acad Sci U S A 98:295–300.

Grammatopoulos DK, Randeva HS, Levine MA, Kanellopoulou KA, Hillhouse EW (2001) Rat cerebral cortex corticotropin-releasing hormone receptors: evidence for receptor coupling to multiple G-proteins. J Neurochem 76:509–519.

Guan X, Wan R, Zhu C, Li S (2014) Corticotropin-releasing factor receptor type-2 is involved in the cocaine-primed reinstatement of cocaine conditioned place preference in rats. Behav Brain Res 258:90–96.

Henckens MJ, Deussing JM, Chen A (2016) Region-specific roles of the corticotropin-releasing factor-urocortin system in stress. Nat Rev Neurosci 17:636–651.

Isogawa K, Akiyoshi J, Hikichi T, Yamamoto Y, Tsutsumi T, Nagayama H (2000) Effect of corticotropin releasing factor receptor 1 antagonist on extracellular norepinephrine, dopamine and serotonin in hippocampus and prefrontal cortex of rats in vivo. Neuropeptides 34:234–239.

Jackson ME, Moghaddam B (2001) Amygdala regulation of nucleus accumbens dopamine output is governed by the prefrontal cortex. J Neurosci 21:676–681.

Jackson ME, Moghaddam B (2004) Stimulus-specific plasticity of prefrontal cortex dopamine neurotransmission. J Neurochem 88:1327–1334.

Lammel S, Ion DI, Roeper J, Malenka RC (2011) Projection-specific modulation of dopamine neuron synapses by aversive and rewarding stimuli. Neuron 70:855–862.

Lammel S, Lim BK, Ran C, Huang KW, Betley MJ, Tye KM, Deisseroth K, Malenka RC (2012) Input-specific control of reward and aversion in the ventral tegmental area. Nature 491:212–217.

Lemos JC, Wanat MJ, Smith JS, Reyes BA, Hollon NG, Van Bockstaele EJ, Chavkin C, Phillips PE (2012) Severe stress switches CRF action in the nucleus accumbens from appetitive to aversive. Nature 490:402–406.

Liu J, Yu B, Neugebauer V, Grigoriadis DE, Rivier J, Vale WW, Shinnick-Gallagher P, Gallagher JP (2004) Corticotropin-releasing factor and Urocortin I modulate excitatory glutamatergic synaptic transmission. J Neurosci 24:4020–4029.

Liu J, Yu B, Orozco-Cabal L, Grigoriadis DE, Rivier J, Vale WW, Shinnick-Gallagher P, Gallagher JP (2005) Chronic cocaine administration switches corticotropin-releasing factor2 receptor-mediated depression to facilitation of glutamatergic transmission in the lateral septum. J Neurosci 25:577–583.

Maroun M, Richter-Levin G (2003) Exposure to acute stress blocks the induction of long-term potentiation of the amygdala-prefrontal cortex pathway in vivo. J Neurosci 23:4406–4409.

McGarry LM, Carter AG (2016) Inhibitory gating of basolateral amygdala inputs to the prefrontal cortex. J Neurosci 36:9391–9406.

Millan MA, Jacobowitz DM, Hauger RL, Catt KJ, Aguilera G (1986) Distribution of corticotropin-releasing factor receptors in primate brain. Proc Natl Acad Sci U S A 83:1921–1925.

Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167–202.

Moghaddam B (2016) The complicated relationship of stress and prefrontal cortex. Biol Psychiatry 80:728–729.

Orozco-Cabal L, Pollandt S, Liu J, Shinnick-Gallagher P, Gallagher JP (2006) Regulation of synaptic transmission by CRF receptors. Rev Neurosci 17:279–307.

Orozco-Cabal L, Liu J, Pollandt S, Schmidt K, Shinnick-Gallagher P, Gallagher JP (2008) Dopamine and corticotropin-releasing factor synergistically alter basolateral amygdala-to-medial prefrontal cortex synaptic transmission: functional switch after chronic cocaine administration. J Neurosci 28:529–542.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. 2nd ed. Sydney, Australia: Academic Press.

Radulovic J, Fischer A, Katerkamp U, Spiess J (2000) Role of regional neurotransmitter receptors in corticotropin-releasing factor (CRF)-mediated modulation of fear conditioning. Neuropharmacology 39:707–710.

Rodrigues RJ, Alfaro TM, Rebola N, Oliveira CR, Cunha RA (2005) Co-localization and functional interaction between adenosine A(2A) and metabotropic group 5 receptors in glutamatergic nerve terminals of the rat striatum. J Neurochem 92:433–441.

Rosenkranz JA, Grace AA (2001) Dopamine attenuates prefrontal cortical suppression of sensory inputs to the basolateral amygdala of rats. J Neurosci 21:4090–4103.

Seamans JK, Durstewitz D, Christie BR, Stevens CF, Sejnowski TJ (2001) Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. Proc Natl Acad Sci U S A 98:301–306.

Snyder KP, Barry M, Valentino RJ (2015) Cognitive impact of social stress and coping strategy throughout development. Psychopharmacology (Berl) 232:185–195.

Sotomayor-Zárate R, Araya KA, Pereira P, Blanco E, Quiroz G, Pozo S, Carreño P, Andrés ME, Forray MI, Gysling K (2010) Activation of GABA-B receptors induced by systemic amphetamine abolishes dopamine release in the rat lateral septum. J Neurochem 114:1678–1686.

Sotres-Bayon F, Quirk GJ (2010) Prefrontal control of fear: more than just extinction. Curr Opin Neurobiol 20:231–235.

Sun T, et al. (2019) Basolateral amygdala input to the medial prefrontal cortex controls obsessive-compulsive disorder-like checking behavior. Proc Natl Acad Sci U S A 116:3799–3804.

Takahata R, Moghaddam B (1998) Glutamatergic regulation of basal and stimulus-activated dopamine release in the prefrontal cortex. J Neurochem 71:1443–1449.

Tejeda HA, Counotte DS, Oh E, Ramamoorthy S, Schultz-Kuszak KN, Bäckman CM, Chefer V, O'Donnell P, Shippenberg TS (2013) Prefrontal cortical kappa-opioid receptor modulation of local neurotransmission and conditioned place aversion. Neuropsychopharmacology 38:1770–1779.

Tseng KY, O'Donnell P (2004) Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. J Neurosci 24:5131–5139.

Urban KR, Valentino RJ (2017) Age- and sex-dependent impact of repeated social stress on intrinsic and synaptic excitability of the rat prefrontal cortex. Cereb Cortex 27:244–253.

Uribe-Mariño A, Gassen NC, Wiesbeck MF, Balsevich G, Santarelli S, Solfrank B, Dournes C, Fries GR, Masana M, Labermeier C, Wang XD, Hafner K, Schmid B, Rein T, Chen A, Deussing JM, Schmidt MV (2016) Prefrontal cortex corticotropin-releasing factor receptor 1 conveys acute stress-induced executive dysfunction. Biol Psychiatry 80:743–753.

Vale W, Spiess J, Rivier C, Rivier J (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394–1397.

Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE (2000) Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. J Comp Neurol 428:191–212.

Vega-Quiroga I, Yarur HE, Gysling K (2018) Lateral septum stimulation disinhibits dopaminergic neurons in the anteroventral region of the ventral tegmental area: role of GABA-A alpha 1 receptors. Neuropharmacology 128:76–85.

Wang B, You ZB, Rice KC, Wise RA (2007) Stress-induced relapse to cocaine seeking: roles for the CRF(2) receptor and CRF-binding protein in the ventral tegmental area of the rat. Psychopharmacology (Berl) 193:283–294.

Williams CL, Buchta WC, Riegel AC (2014) CRF-R2 and the heterosynaptic regulation of VTA glutamate during reinstatement of cocaine seeking. J Neurosci 34:10402–10414.

Yan XX, Baram TZ, Gerth A, Schultz L, Ribak CE (1998) Co-localization of corticotropin-releasing hormone with glutamate decarboxylase and calcium-binding proteins in infant rat neocortical interneurons. Exp Brain Res 123:334–340.

Yarur HE, González MP, Verbel-Vergara D, Andrés ME, Gysling K. (2020a) Cross-talk between dopamine D1 and corticotropin releasing factor type 2 receptors leads to occlusion of their ERK1/2 signaling. J Neurochem. 155:264–273.

Yarur HE, Vega-Quiroga I, González MP, Noches V, Thomases DR, Andrés ME, Ciruela F, Tseng KY, Gysling K (2020b) Inhibitory control of basolateral amygdalar transmission to the prefrontal cortex by local corticotrophin type 2 receptor. Int J Neuropsychopharmacol 23:108–116.