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Norepinephrine regulates cocaine-primed reinstatement via α1-adrenergic receptors in the medial prefrontal cortex

Karl T Schmidt, B.S., **Jason P Schroeder, Ph.D.**, **Stephanie L Foster, B.S.**, **Katherine Squires, B.S.**, **Brilee M Coleman, B.S.**, **Elizabeth G Pitts, B.S.**, **Michael P Epstein, Ph.D.**, and **David Weinshenker, Ph.D.***

Department of Human Genetics, Emory University, Atlanta, GA, USA 30322

Abstract

Drug-primed reinstatement of cocaine seeking in rats is thought to reflect relapse-like behavior and is mediated by the integration of signals from mesocorticolimbic dopaminergic projections and corticostriatal glutamatergic innervation. Cocaine-primed reinstatement can also be attenuated by systemic administration of dopamine β-hydroxylase (DBH) inhibitors, which prevent norepinephrine (NE) synthesis, or by α1-adrenergic receptor (α1AR) antagonists, indicating functional modulation by the noradrenergic system. In the present study, we sought to further discern the role of NE in cocaine-seeking behavior by determining whether α1AR activation can induce reinstatement on its own or is sufficient to permit cocaine-primed reinstatement in the absence of all other AR signaling, and identifying the neuroanatomical substrate within the mesocorticolimbic reward system harboring the critical α1ARs. We found that while intracerebroventricular infusion of the α1AR agonist phenylephrine did not induce reinstatement on its own, it did overcome the blockade of cocaine-primed reinstatement by the DBH inhibitor nepicastat. Furthermore, administration of the α1AR antagonist terazosin in the medial prefrontal cortex (mPFC), but not the ventral tegmental area (VTA) or nucleus accumbens (NAc) shell, attenuated cocaine-primed reinstatement. Combined, these data indicate that α1AR activation in the mPFC is required for cocaine-primed reinstatement, and suggest that α1AR antagonists merit further investigation as pharmacotherapies for cocaine dependence.

Keywords

alpha-1 adrenergic receptor; cocaine; norepinephrine; prefrontal cortex; rat; reinstatement

*Address correspondence to: David Weinshenker, Ph.D., Department of Human Genetics, Emory University School of Medicine, Whitehead 301, 615 Michael St., Atlanta, GA 30322, Phone: (404) 727-3106, Fax: (404) 727-3949, dweinshenker@genetics.emory.edu.

Author contribution

KTS, JPS, and DW were responsible for the study concept and design. KTS, JPS, KS, BMC, and EGP conducted the behavioral studies. KTS, MPE, and DW analyzed the data and wrote the manuscript. All authors reviewed the content and approved the final version for publication.

1. Introduction

Cocaine abuse has persisted as a major public health concern within the United States for several decades. A prominent and problematic feature of addiction is the repeated occurrence of relapse episodes, even after prolonged periods of abstinence (O'Brien and Gardner, 2005), and there are no generally accepted or FDA-approved pharmacotherapies for cocaine dependence. In the reinstatement model of relapse, non-contingent administration of the drug, cues previously associated with drug delivery, or stressful stimuli can induce the recurrence of extinguished drug-seeking behavior. This model has been useful for identifying neurotransmitters and neuroanatomical substrates involved in drug-seeking behavior that may represent targets for new addiction therapies.

Cocaine is a monoamine reuptake inhibitor that increases extracellular levels of dopamine (DA), NE, and serotonin. While DA is responsible for the primary reinforcing properties of cocaine, all efforts to develop DA-based medications for cocaine addiction have failed due to lack of efficacy, side effects, and/or abuse liability (Amato et al., 2011; Haile et al., 2012; Pierce et al., 2012; Shorter et al., 2011), suggesting that other systems might make better therapeutic targets. Compounds that block NE signaling, such as the NE synthesis inhibitors disulfiram and nepicastat, attenuate all three modalities of reinstatement in rats, although different adrenergic receptors (ARs) are involved in each type. Cocaine-primed reinstatement requires α1ARs, stress-induced reinstatement requires βARs, and both α1- and βARs play a role in cue-induced reinstatement (Devoto et al., 2016; Gaval-Cruz and Weinshenker, 2009; Leri et al. 2002; Schmidt and Weinshenker, 2014; Schroeder et al., 2010, 2013; Smith and Aston-Jones, 2011; Weinshenker and Schroeder, 2007; Zhang and Kosten, 2005).

While the noradrenergic neural circuitry underlying stress-induced reinstatement has been identified (Leri et al., 2002; Vranjkovic et al., 2014), very little is known about how NE promotes drug-primed or cue-induced reinstatement. Previous work has implicated multiple nodes of the mesocorticolimbic reward circuit in cocaine-primed reinstatement; the NAc integrates coordinated DA signaling from the VTA and glutamate transmission from the PFC to produce drug-seeking behavior (Anderson et al., 2008; McFarland and Kalivas, 2001). Each of these regions also receives noradrenergic innervation, contains α1ARs, and is influenced by α1AR signaling, making them ideal candidates for mediating the effects of NE transmission on cocaine-primed reinstatement.

Noradrenergic neurons in the locus coeruleus (LC), A1, and A2 brainstem nuclei innervate the VTA (Jones at al 1977; Mejias-Aponte et al., 2009; Simon et al 1979). We have shown that α1ARs are enriched on presynaptic GABAergic and glutamatergic elements in this brain region (Mitrano et al 2012; Rommelfanger et al 2009), which is consistent with electrophysiological studies showing that α1AR activation modulates GABA and glutamate release onto VTA DA neurons (Velasquez-Martinez et al 2012; 2015). α1AR agonists can also directly depolarize these DA neurons and facilitate burst firing (Grenhoff and Svensson, 1989, 1993; Grenhoff et al 1993, 1995; Paladini et al 2001), and intra-VTA administration of α1AR antagonists decreases DA release in the NAc following cocaine administration (Goertz et al 2015). Therefore, it appears that α1ARs in the VTA fine-tune DA neuron

activity via both direct and indirect mechanisms. The behavioral consequences of α1AR activation in the VTA are uncertain; we failed to detect an effect of local α1AR antagonist administration on cocaine-induced locomotion (Mitrano et al., 2012).

The NAc is primarily innervated by the A2 noradrenergic cell group (Delfs et al., 1998), and α1ARs are enriched on presynaptic glutamatergic elements in this brain region, although they can also be found on GABAergic and dopaminergic terminals (Mitrano et al., 2012). Local blockade of α1ARs in the NAc attenuates cocaine-induced DA overflow and locomotor activity (Mitrano et al 2012; Sommermeyer et al 1995).

NE transmission in the mPFC originates from the LC (Florin-Lechner et al 1996; Morrison et al 1981; Swanson and Hartman 1975), and α1ARs are found mainly on glutamatergic elements. Selective lesions of NE terminals in the mPFC or local α1AR blockade prevent psychostimulant-induced locomotor activity, reward, and DA overflow (Auclair et al., 2002, 2004; Blanc et al 1994; Darracq et al 1998; Pan et al 2004; Ventura et al 2003, 2005, 2007). Intra-mPFC infusion of the α1AR antagonist terazosin has no effect on the maintenance phase of cocaine self-administration (Ecke et al., 2012), but the influence of this manipulation on reinstatement has not been tested.

To further define the role of α1ARs in cocaine-primed reinstatement, we first determined whether activation of α1ARs alone can trigger drug-seeking behavior, and whether α1AR signaling is permissive for cocaine-primed reinstatement in the absence of all other NE transmission. Next, we tested whether infusion of terazosin into the VTA, NAc shell, or mPFC could prevent cocaine-primed reinstatement.

2. Materials and Methods

2.1 Subjects

Adult male Sprague Dawley rats (Charles River; Wilmington MA) weighing 250–300 g prior to surgery were used. Rats were housed individually in a temperature-controlled environment on a reverse light/dark cycle with lights off at 8 am and lights on at 8 pm. Unless otherwise noted, all rats had *ad libitum* access to food and water. Rats were acclimated for 1 week prior to experimentation. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Emory IACUC.

2.2 Operant Training

To facilitate operant behavior, animals used in self-administration studies were trained to lever press for food prior to surgery, as described previously (Fuchs et al 2006; Schroeder et al 2010, 2013). Briefly, rats were placed in rat operant chambers (Med Associates, St Albans, VT). Each chamber was housed within a sound-attenuating box and had a house light, two levers that extended during operant testing, and a light above each lever. Presses on the lever designated as active (right/left counterbalanced) were reinforced by a 45-mg food pellet (BioServ) on a FR1 schedule until 100 pellets were earned or 6 h elapsed. The criterion was 70% selectivity for active lever over inactive lever. Most rats met the criterion in 1 day, and all met the criterion within 3 days.

2.3 Jugular Catheter and Intracranial Cannula Surgery

Rats underwent surgery to implant jugular catheters and intracranial cannulae, as described previously (Schroeder et al 2010, 2013). Following isoflurane anesthesia and meloxicam (1 mg/kg, s.c.) analgesia, a catheter was inserted into the right jugular vein and threaded subcutaneously over the shoulder and exited via a mount placed between the shoulder blades. To maintain patency and prevent infections, catheters were flushed daily with 0.1 ml heparin solution (30 U/ml in saline) and 0.05 ml gentamicin (4 mg/ml). Immediately after catheter implantation, rats were placed in a stereotaxic apparatus, and cannulae targeting the lateral ventricle, VTA, NAc shell, or mPFC were implanted. Coordinates in mm from bregma (Paxinos and Watson, 1998) were: lateral ventrical (unilateral) A/P −1.0; M/L −1.4; D/V −2.6; VTA (bilateral), A/P −5.8; M/L ± 0.7; D/V: −7.0; NAc shell (bilateral), A/P + 1.3; M/L ± 2.5; D/V −7.1, 10° angle; and PFC (bilateral) A/P + 4.0; M/L ± 0.7 D/V −4.0. Animals were allowed 5−7 days to recover before behavioral testing.

2.4 Cocaine Self-Administration

Rats were allowed to self-administer cocaine on an FR1 schedule during 2-h sessions, as described previously (Schroeder et al, 2010, 2013). Each press on the active lever was reinforced by a cocaine infusion (0.5 mg/kg, i.v.), accompanied by illumination of a light above the active lever. For 20 s beginning with the infusion, the house light was extinguished to indicate a timeout period during which active lever presses were counted but did not lead to drug infusion. The maximum number of infusions per session was set at 40. Responses on the inactive lever were counted, but had no programmed consequences.

Rats were maintained on cocaine for at least 5 days with at least 20 infusions per session. Three consecutive days with less than 20% variability and at least 80% of response allocation on the active lever were required to progress to extinction. The average of these final three sessions was used to indicate the level of maintenance responding for each rat.

2.5 Extinction

During extinction, animals were run daily during 2-h sessions where responses on either lever had no programmed consequences. To meet extinction criteria, animals were required to respond at less than 30% of maintenance responding levels for 3 consecutive days.

2.6 Cocaine-Primed Reinstatement

Once extinction criteria were met, rats underwent cocaine-primed reinstatement sessions. For the nepicastat and phenylephrine experiments, rats were injected with nepicastat (50 mg/kg, i.p.) or vehicle 2 h prior to the test session, and then immediately before testing they were infused with phenylephrine (10 μg/0.5 μl, i.c.v.) or artificial cerebrospinal fluid (aCSF) and injected with cocaine (10 mg/kg, i.p.). Drug doses were chosen as follows. Cocaine (10 mg/kg) is a standard priming dose in the field that reliably reinstates cocaine seeking and is blocked by nepicastat (50 mg/kg), a dose that does not impair food seeking (Schroeder et al., 2010). Phenylephrine (10 μg) is in the range of behaviorally active doses when administered i.c.v. (Alsene et al., 2006; Stone et al., 2003) and does not have adverse interactions with cocaine we observed with higher doses (i.e. 30 μg and above; our unpublished data). Treatments were administered in a counterbalanced order, and reinstatement sessions were

separated by extinction sessions to ensure extinction criteria were reached prior to each test. Due to the occasional clogged cannula, lost head cap, etc, not all rats received all treatments. For the α1AR antagonist experiments, rats were infused bilaterally with terazosin (3 μg/0.5 μl/side in the mPFC, VTA, or NAc shell) or aCSF and injected with cocaine (15 mg/kg, i.p.). Terazosin dose was chosen based on our previous studies showing that it can attenuate cocaine-induced locomotor activity when injected into the NAc shell (Mitrano et al., 2012). We selected a slightly higher dose of cocaine than in the phenylephrine experiment above because we have noted that cocaine-primed reinstatement is less effective when rats have implanted cannula and receive infusions in some brain regions, most notably the NAc (Fig. 2; our unpublished data). Each rat was subjected to a single treatment and reinstatement session. Following drug treatments, rats were placed in the testing chambers under extinction conditions. Active lever responses represented drug-seeking behavior, and data were analyzed in a between-subjects manner.

2.7 Food Self-Administration

Food self-administration occurred in the same operant chambers used for cocaine self-administration sessions. Responses on the active lever were reinforced on an FR1 schedule by 45-mg grain pellets. Responses on the inactive lever were counted, but had no programmed consequences. Sessions terminated with a maximum number of 60 reinforcers or after 1 h. Rats undergoing food self-administration were restricted to 19 g of chow per day.

2.8 Food-Primed Extinction and Reinstatement

Criteria to progress through food maintenance and extinction were identical to those described above for cocaine. After meeting extinction criteria, rats were infused with terazosin (3 μg/0.5 μl/side in the mPFC) or aCSF, as described above. For food-primed reinstatement, sessions began with a non-contingent delivery of 3 grain pellets and continued with 1 pellet delivered non-contingently every min thereafter, as described (Schroeder et al., 2010). Responses on either lever had no programed consequences. Active lever responses represented food-seeking behavior, and data were analyzed in a between-subjects manner.

2.9 Drugs

Phenylephrine and terazosin were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in aCSF. Nepicastat (SYN-117) was generously provided by Synosia Therapeutics (South San Francisco, CA) and injected as a suspension in sterile 0.9% saline containing 1.5% dimethyl sulfoxide (Sigma-Aldrich) and 1.5% Cremophor EL (Sigma-Aldrich). Cocaine HCl was generously provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD) and dissolved in sterile 0.9% saline.

2.10 Data Analysis

For the phenylephrine experiments, which employed a partial within-subjects design but not all rats received all treatments, we performed association testing using a generalized estimating equation (GEE) models that allow for repeated observations per subject and is robust to model misspecification of the within-subject correlation structure in the lever-

pressing data (Zeger and Liang, 1986). Prior to GEE analysis, we first transformed the data to approximate normality using the natural-log function (p=0.22, Shapiro-Wilk normality test), since the untransformed data was decidedly non-normal (p<0.0001, Shapiro-Wilk normality test). We then implemented GEE models for the transformed data assuming a normal distribution and subsequently constructed Wald tests to examine whether outcome significantly differed between extinction and each set of reinstatement conditions. We assumed the within-subject correlation structure in the GEE model followed an exchangeable model, but results using other correlation models yielded similar results (data not shown). We established significance of our Wald tests using a permutation procedure that preserved within-subject correlation structure in the sample. We performed all analysis using the R programming language, with GEE models being fit using R library *geepack* (Halekoh et al., 2006). For the standard between-subject terazosin experiments, data were analyzed by ANOVA, followed by Sidak's post hoc tests, where appropriate, using Prism 6.0 for Macintosh.

3. Results

3.1 Central α1AR Stimulation Alone Does Not Induce Reinstatement, but Restores Cocaine-Primed Reinstatement to NE-Depleted Animals

Blockade of α1ARs attenuates cocaine-primed reinstatement (Zhang and Kosten, 2005), but it is not known whether α1AR stimulation alone is sufficient to elicit cocaine-seeking behavior. When rats received phenylephrine (10 μg/0.5 μl, i.c.v.) following cocaine self-administration and extinction and just prior to a saline-primed reinstatement session, active lever responding did not rise above extinction levels (Fig. 1). DBH inhibition, which reduces NE production, blocks signaling from all AR subtypes and prevents cocaine-primed reinstatement (Devoto et al., 2016; Schroeder et al., 2010, 2013). We next tested whether α1AR activation is sufficient to restore cocaine-primed reinstatement in the absence of all other AR signaling. Rats received vehicle or the DBH inhibitor nepicastat (50 mg/kg, i.p.), followed 2 h later by aCSF or phenylephrine (10 μg/0.5 μl, i.c.v.). Rats were then given a priming injection of cocaine (10 mg/kg, i.p.) and placed in the operant chambers. As reported previously, cocaine elicited significant cocaine-seeking behavior in rats pretreated with vehicle, but not nepicastat. Phenylephrine did not significantly alter cocaine-primed reinstatement in vehicle-pretreated rats, but restored cocaine-primed reinstatement to nepicastat-treated animals (Fig. 1). GEE analysis comparing each treatment combination to extinction levels showed significant reinstatement of active lever pressing for the vehicle + aCSF + cocaine (Wald test=59.77, permutation p-value <0.0001), vehicle + phenylephrine + cocaine (Wald test=23.56, permutation p-value <0.001), and the nepicastat + phenylephrine + cocaine (Wald test=29.01, permutation p-value < 0.0001) groups, but not the nepicastat + aCSF + cocaine group (Wald test=0.78, permutation p-value=0.43). Although there was a trend for an association between increased noradrenergic transmission and increased reinstatement responding (vehicle + phenylephrine + cocaine > vehicle + aCSF + cocaine > npicastat + phenylephrine + cocaine), no significant differences were observed between these 3 groups that significantly reinstated over extinction. In addition, no differences in inactive lever responses were observed across treatments (data not shown). Combined, these

data indicate that α1AR activation cannot elicit cocaine-seeking behavior on its own, but permits cocaine-primed reinstatement when endogenous NE transmission is quiescent.

3.2 α1AR Blockade in mPFC, but Not the VTA or NAc Shell, Attenuates Cocaine-Primed Reinstatement

Systemic administration of an α1AR antagonist attenuates cocaine-primed reinstatement (Zhang and Kosten, 2005). To identify the underlying neuroanatomical substrates, aCSF or the α1AR antagonist terazosin (3 μg/0.5 μl/side) was infused into the NAc shell, VTA, or mPFC just prior to a cocaine-primed (15 mg/kg, i.p.) reinstatement session following cocaine self-administration and extinction. We found that α1AR blockade in the mPFC (Fig. 2a), but not the VTA (Fig. 2b) or NAc shell (Fig. 2c), attenuated cocaine-primed reinstatement. Two-way repeated-measures (by phase) ANOVAs were run for each brain region tested. For the VTA, there was a main effect of phase ($F_{2,20}$=17.89, p<0.001) but not treatment ($F_{1,10}$=0.017, p>0.05) or phase x treatment interaction ($F_{2,20}$=0.006, p>0.05). For the NAc shell, there was a main effect of phase ($F_{2,20}$=8.648, p<0.02), but no treatment ($F_{1,10}$=0.5524, p>0.05) or phase × treatment interaction ($F_{2,20}$=0.3172, p>0.05). For the mPFC, there was a main effect of phase ($F_{2,18}$=12.13, p=0.0002), treatment ($F_{1,9=}$8.294, p=0.0182), and phase x treatment interaction ($F_{2,18}$=7.506, p=0.0043). Post hoc tests revealed a significant difference between the aCSF and terazosin groups on active lever presses during cocaine-primed reinstatement (t=4.82, p<0.001). No significant differences in inactive lever responses were observed (data not shown). These data indicate that α1AR activation in the mPFC, but not the VTA or NAc shell, is required for cocaine-primed reinstatement.

3.3 α1AR Blockade in the mPFC Has No Effect on Food-Primed Reinstatement of Food Seeking

To determine whether α1ARs in the mPFC modulate general reward seeking, rats were trained to self-administer food pellets. Following extinction, aCSF or terazosin (3 μg/0.5 μl/side) was infused into the mPFC just prior to a food-primed reinstatement test. α1AR blockade had no effect on food-seeking behavior (Fig. 3). A two-way repeated measures (by phase) ANOVA revealed a main effect of phase ($F_{2,14}$=20.37, p<0.001), but not treatment ($F_{1,7=}$4.366, p>0.05) or a phase × treatment interaction ($F_{2,14}$=0.6993, p>0.05). These results suggest that mPFC α1ARs are important for relapse-like behavior associated with drugs, but not natural rewards.

4. Discussion

α1ARs, but not βARs, are known to be required for cocaine-primed reinstatement (Leri et al., 2002; Zhang and Kosten, 2005), but it was not clear was whether stimulation of α1ARs would be sufficient for reinstatement. Furthermore, the neuroanatomical substrate(s) governing α1AR control of cocaine-primed reinstatement had yet to be identified. The results of our study indicate that stimulation of α1ARs alone is not sufficient to induce reinstatement, but these receptors are permissive for cocaine-primed reinstatement in the absence of all other noradrenergic signaling. Moreover, α1AR activation is necessary in the

mPFC, but not the VTA or NAc shell, for cocaine-primed reinstatement. In the following paragraphs, we will discuss each of these findings in greater detail.

Intracerebroventricular infusion of NE can trigger drug-seeking behavior in rats with a history of cocaine self-administration (Brown et al., 2009), although the receptor subtype mediating this effect was unknown. We found that i.c.v. infusion of the α1AR agonist phenylephrine, alone, failed to elicit cocaine seeking, suggesting that a different receptor was responsible for triggering NE-induced reinstatement. As mentioned in the Introduction, the ventral noradrenergic bundle, originating from the A1/A2 brainstem nuclei and projecting to the central nucleus of the amygdala and bed nucleus of the stria terminalis (BNST), is required for stress-induced reinstatement of cocaine seeking (Leri et al., 2002; Vranjkovic et al., 2014). This pathway mediates these effects via βARs; administration of βAR antagonists into the amygdala or BNST block footshock-induced reinstatement, while intra-BNST infusion of βAR agonists can provoke drug-seeking behavior in cocaine-experienced rats (Leri et al., 2002; Vranjkovic et al., 2014). Furthermore, i.c.v. administration of NE induces expression of the immediate early gene c-fos in the amygdala and BNST (Brown et al., 2011). Thus, it seems likely that the ability of i.c.v. NE to promote reinstatement depends on βARs, not α1ARs, and engages stress circuitry rather than primary nodes of the mesocorticolimbic reward system directly.

For over a decade, we have known that activation of α1ARs is necessary for the full expression of cocaine-primed reinstatement (Zhang and Kosten, 2005). Although insufficient to induce reinstatement on its own or enhance cocaine-primed reinstatement in NE-competent animals, we found that α1AR stimulation restored cocaine-primed reinstatement to NE-depleted rats, further implicating this AR subtype. Thus, we next aimed to identify the brain region harboring the critical α1ARs. There were good reasons to suspect the 3 main nodes of the mesocorticolimbic reward system (VTA, NAc, and mPFC); all express α1ARs, and blockade of α1AR transmission in each of these regions attenuates several behavioral and/or neurochemical effects of cocaine (see Introduction). Our results clearly show that α1ARs in the mPFC, but not the VTA or NAc shell, are required for cocaine-primed reinstatement. Both positive and negative controls for the terazosin experiments support this conclusion. The same dose of terazosin in the NAc shell can block cocaine-induced locomotor activity (Mitrano et al., 2012), and local blockade of α1ARs in the mPFC had no effect on food-primed reinstatement of food seeking (Fig. 3), indicating specificity of mPFC α1ARs for drug-induced relapse-like behavior. We note that overall magnitude of responding during reinstatement varied between experiments; cocaine-primed reinstatement was the lowest when aCSF was infused into the NAc, and highest when infusions were aimed at the mPFC. The reasons for these differences are not clear, but we have observed it before in other experiments (our unpublished data), and it is possible that NAc function is particularly sensitive to mechanical damage from the cannula combined with an endogenous neurotransmitter diluting effect of aCSF infusion. It is also important to recognize that because we used i.c.v. administration for the phenylephrine experiments, we do not know the neuroanatomical substrate underlying the ability of α1AR stimulation to restore cocaine-primed reinstatement to nepicastat-treated rats. Based on the antagonist results, we suspect the mPFC, but further experiments will be necessary to obtain a definitive answer.

Cocaine-primed reinstatement requires coordinated DA transmission from the VTA and glutamatergic transmission from mPFC projection neurons in the NAc (Knackstedt and Kalivas, 2009; McFarland et al., 2003). Because cocaine does not act on glutamate directly, a monoamine must mediate cocaine-induced activation of mPFC pyramidal neurons, and our results implicate NE- α1AR transmission. We have shown that α1ARs are expressed on both presynaptic and postsynaptic glutamatergic elements in the mPFC (Mitrano et al., 2012), and previous studies indicate that activation of these receptors increases local glutamate transmission and excitation of pyramidal neurons (Luo et al., 2014, 2015; Marek and Aghajanian, 1999). Thus, we propose that a cocaine prime blocks NE reuptake in the mPFC, which acts on presynaptic α1ARs to facilitate local glutamate release that, together with direct α1AR-mediated depolarization of pyramidal neurons, drives glutamate transmission in the NAc. The NAc then integrates the PFC-derived glutamate and VTA-derived DA signals to trigger cocaine-seeking behavior (Fig. 4). In future experiments, it will be important to confirm this model by comparing glutamate release in the mPFC and NAc, as well as pyramidal neuron excitation, during reinstatement testing in the presence and absence of α1AR antagonists.

Devoto and colleagues have published a series of articles suggesting that increased DA transmission via the D1 receptor (D1R) in the mPFC from noradrenergic neurons, rather than decreased NE transmission, underlies the ability of DBH inhibitors to attenuate cocaine-primed reinstatement (Devoto et al., 2012, 2014, 2016). Our findings that phenylephrine can restore cocaine-primed reinstatement to nepicastat-treated animals and that α1AR blockade in the mPFC prevents cocaine-primed reinstatement clearly implicate NE- α1AR transmission, as well. These two models are not necessarily mutually exclusive. We have shown that α1ARs and D1Rs colocalize on glutamatergic neuronal elements in the mPFC (Mitrano et al., 2014), and previous studies suggest that α1AR signaling restrains D1 signaling in mPFC pyramidal neurons (Trovero et al., 1994). Thus, supranormal D1 activation in the mPFC following DBH inhibition may result from a combination of increased DA release from noradrenergic terminals and decreased NE-α1AR transmission, thereby allowing D1 signaling to proceed unchecked. How excessive D1 activation might inhibit NAc-projecting pyramidal neuron excitability is not yet clear.

The present results have important clinical implications. A recent pilot study showed that the α1AR antagonist doxazosin decreased ratings of “like cocaine” and “likely to use cocaine if had access” in cocaine-dependent participants (Newton et al, 2012), and a larger follow-up study found that doxazosin significantly decreased cocaine use and increased the number of participants maintaining abstinence for at least two weeks (Shorter et al, 2013). α1AR blockade also lacked serious adverse effects that might be present with direct glutamatergic or dopaminergic approaches (Parson et al, 2005). Combined with the preclinical data linking NE and α1ARs to cocaine responses, these studies have led to several ongoing clinical trials testing α1AR antagonists for the treatment of cocaine dependence (NCT01953432, NCT01145183, NCT02538744, NCT01371851).

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Highlights

- Inhibition of NE synthesis blocks cocaine-primed reinstatement
- α1AR activation restores cocaine-primed reinstatement to NE-depleted animals
- Blockade of α1ARs in the mPFC attenuates cocaine-primed reinstatement
- Blockade of α1ARs in the VTA or NAc has no effect on cocaine-primed reinstatement

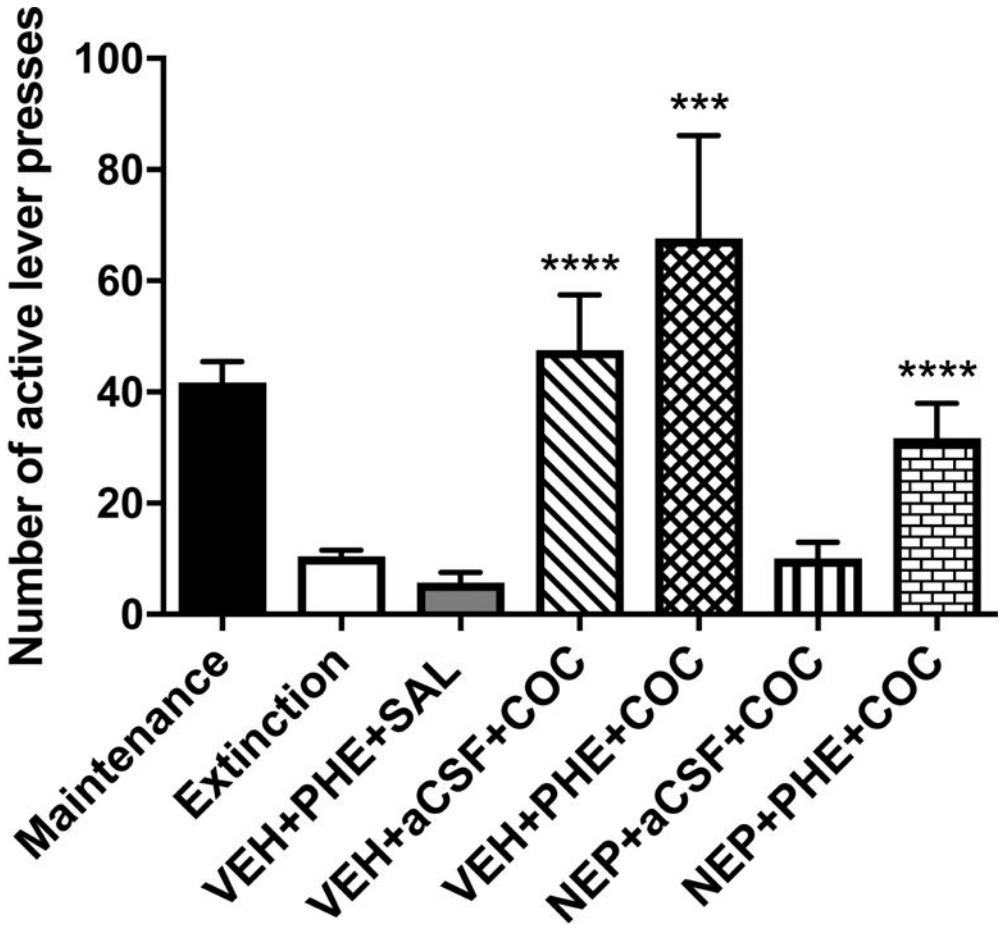

Figure 1. Stimulation of central α1ARs restores cocaine-primed reinstatement to NE-depleted rats

Once maintenance and extinction criteria for operant cocaine self-administration were met, rats (n=6–7 per group) were pretreated with vehicle (VEH) or nepicastat (NEP; 50 mg/kg, i.p.) 2 h prior to infusion of artificial CSF (aCSF) or the α1AR agonist phenylephrine (PHE; 10 μg/0.5 μl, i.c.v.). Rats were then primed with saline (SAL) or cocaine (COC; 10 mg/kg, i.p.), and a 2-h reinstatement session commenced. Shown are mean ± SEM active lever responses. Maintenance values reflect an average of the last 3 days of maintenance sessions, and extinction values reflect an average of the last 3 days of extinction. ****$p<0.0001$, ***$p<0.001$ compared with extinction.

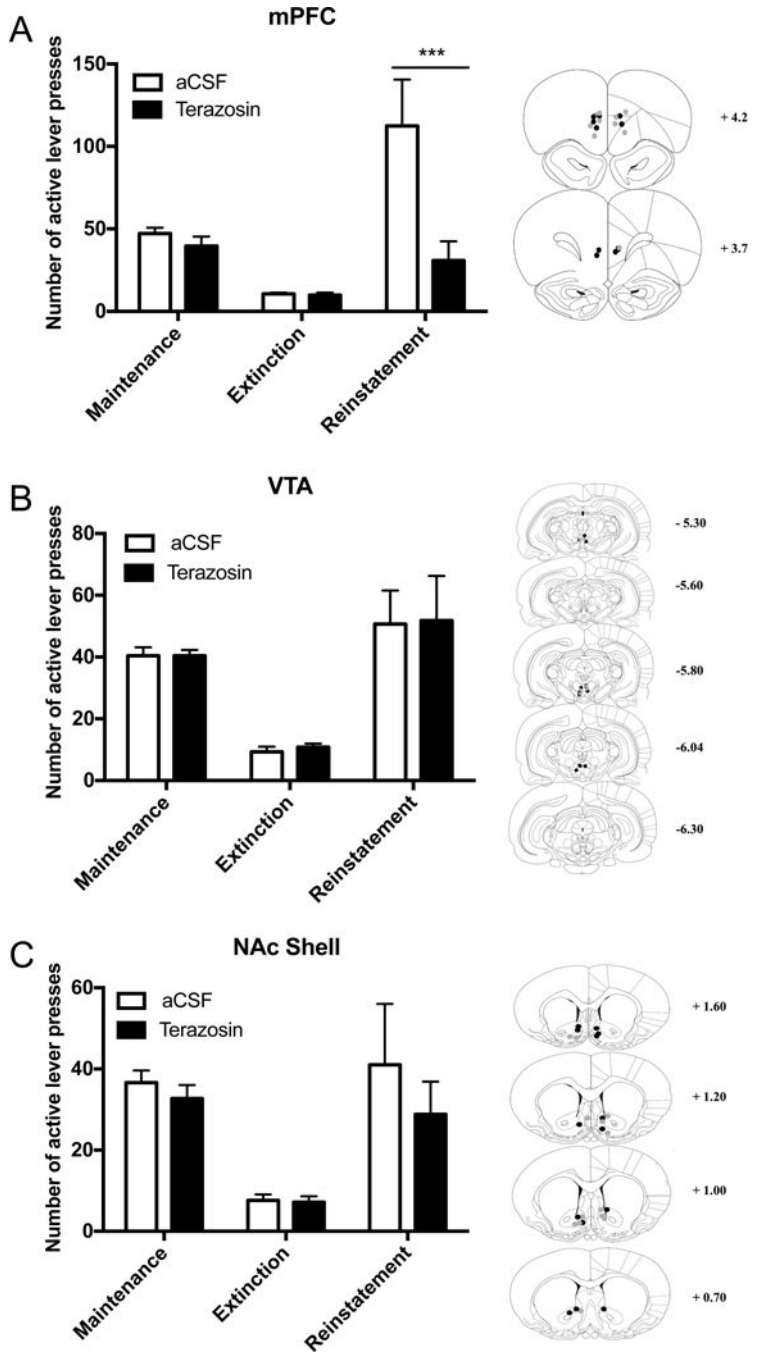

Figure 2. Blockade of α1ARs in the mPFC, but not the VTA or NAc shell, attenuates cocaine-primed reinstatement

Once maintenance and extinction criteria for operant cocaine self-administration were met, rats (n=5–7 per group) were infused with artificial CSF (aCSF) or the α1AR antagonist terazosin (3 μg/0.5 μl/side) into (**A**) the medial prefrontal cortex, (**B**) the ventral tegmental area, or (**C**) the nucleus accumbens shell. Rats were then primed with cocaine (15 mg/kg, i.p.), and a 2-h reinstatement session commenced. Shown are mean ± SEM active lever responses, with probe placements to the right of each graph (aCSF, gray circles; terazosin,

black circles). Maintenance values reflect an average of the last 3 days of maintenance sessions, and extinction values reflect an average of the last 3 days of extinction. ***$p<0.001$ compared with aCSF.

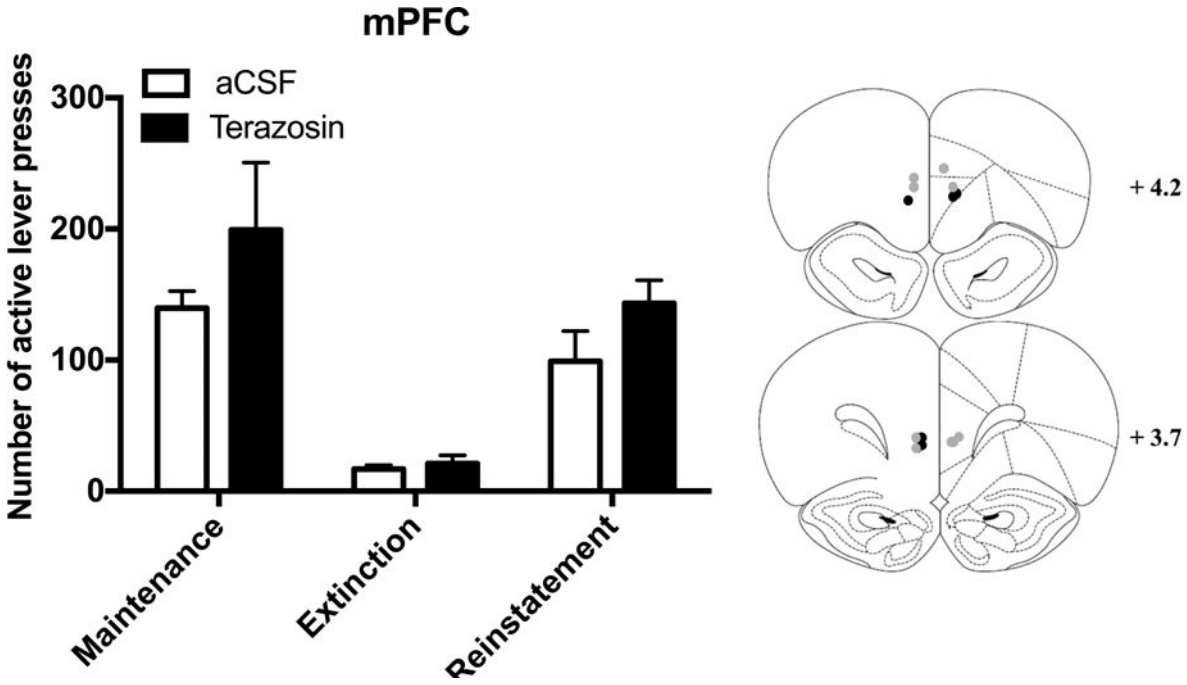

Figure 3. Blockade of α1ARs in the mPFC does not perturb food-primed reinstatement of food seeking

Once maintenance and extinction criteria for operant food self-administration were met, rats (n=5 per group) were infused with artificial CSF (aCSF) or the α1AR antagonist terazosin (3 μg/0.5 μl/side) into the medial prefrontal cortex, and a 2-h food-primed reinstatement session commenced. Shown are mean ± SEM active lever responses, with probe placements to the right (aCSF, gray circles; terazosin, black circles). Maintenance values reflect an average of the last 3 days of maintenance sessions, and extinction values reflect an average of the last 3 days of extinction.

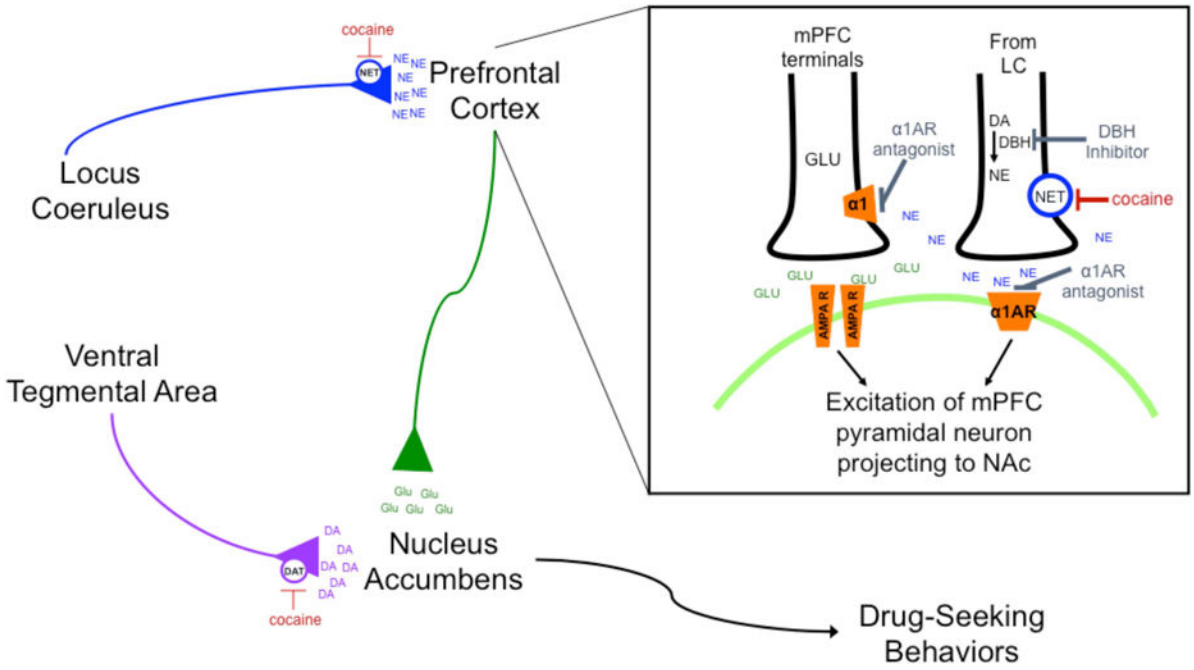

Figure 4. Hypothetical model for noradrenergic control of cocaine-primed reinstatement

Shown on the left is a wiring diagram summarizing the neural circuitry underlying cocaine-primed reinstatement. The nucleus accumbens integrates dopamine (DA) signaling from the ventral tegmental area and glutamate (GLU) signaling from the prefrontal cortex to drive drug-seeking behavior. In our model, cocaine activates the nucleus accumbens-projecting mPFC neurons via norepinephrine (NE) derived from locus coerueleus (LC) noradrenergic terminals. Shown on the right is a detailed picture of the mPFC environment. Cocaine increases extracellular NE by blocking its plasma membrane transporter (NET). The NE then activates mPFC glutamatergic neurons projecting to the NAc directly via postsynaptic α1ARs on these neurons, as well as indirectly via presynaptic α1ARs on mPFC glutamatergic elements that facilitate local glutamate release. Conditions of reduced NE transmission (DBH inhibition or α1AR blockade) prevent the activation of NAc-projecting pyramidal neurons and suppress cocaine-primed reinstatement.