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# Effects of the Alpha-1 Antagonist Prazosin on KOR Agonist-Induced Reinstatement of Alcohol Seeking

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## Abstract

**Background:** Stress is associated with relapse to alcohol seeking during abstinence, but the processes underlying this relationship are poorly understood. Noradrenaline is a key transmitter in stress responses and in stress-induced drug seeking. The alpha-1 adrenoceptor antagonist prazosin has been investigated as a treatment for alcoholism and for chronic stress disorders that are frequently comorbid with alcoholism. In rats, we previously showed that prazosin blocks reinstatement of alcohol seeking induced by footshock and yohimbine stressors and reduces yohimbine-induced brain activation. The role of alpha-1 adrenoceptors in reinstatement induced by other stressors is not known. Our most recent work is on the role of kappa opioid receptors in stress-induced reinstatement of alcohol seeking and have reported that the selective kappa opioid receptor agonist U50,488 induces reinstatement and neuronal activation in stress- and relapse-related brain regions. Here we determine the involvement of alpha-1 receptors in reinstatement and brain activation induced by U50,488.
**Methods:** We trained male Long-Evans rats to self-administer alcohol (12% w/v), extinguished alcohol-reinforced responding, and then determined the effects of prazosin (1 mg/kg) on U50,488 (2.5 mg/kg)-induced reinstatement and regional Fos expression.
**Results:** Prazosin blocked U50,488-induced reinstatement and decreased U50,488-induced Fos expression in the orbitofrontal cortex, nucleus accumbens core, ventral bed nucleus of the stria terminalis, central and basolateral amygdalar nuclei and ventral tegmental area.
**Conclusions:** These findings suggest that prazosin may reduce U50,488-induced relapse by inhibiting activity in 1 or more of these brain areas.

**Keywords:** kappa opioid, alpha-1 adrenoceptor, noradrenaline, stress, Fos

## Introduction

In humans, stressful events are associated with increased alcohol intake and higher rates of relapse after periods of abstinence (Sinha and Li, 2007). The endogenous opioid dynorphin and its receptor, the kappa opioid receptor (KOR), have been established as important mediators of stress-induced reinstatement of drug and alcohol seeking in preclinical studies. Stressors evoke the release of dynorphin (Morley et al., 1982; Nabeshima et al., 1992; Shirayama et al., 2004). KOR antagonists block stress-induced reinstatement, while KOR agonists, such as U50,488, induce reinstatement of drug and alcohol seeking and produce neuronal activation in stress- and relapse-related brain regions (Beardsley et al., 2005; Funk et al., 2014; Grella et al., 2014; Chavkin and Koob, 2016; Mantsch et al., 2016; Lê et al., 2018).

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## Significance Statement

Exposure to stress is associated with greater rates of relapse to alcohol seeking. Two neurotransmitters released in response to stress, dynorphin and noradrenaline, participate in stress-induced relapse to alcohol seeking. Dynorphin acts through the kappa opioid receptor (KOR), while noradrenaline acts via adrenoceptors, including the alpha-1 receptor. In this study we examined the interaction of these 2 neurotransmitters in stress-induced relapse to alcohol seeking in rats. We found that the alpha-1 receptor blocker prazosin, which has received recent attention as a treatment for stress disorders, reduced relapse to alcohol seeking caused by U50,488, a drug that stimulates KOR. We also found that the neural activation produced by U50,488 was blocked by prazosin in a number of brain regions involved in stress-induced relapse. Our results are relevant to the development of pharmacological treatments for stress-induced relapse and provide information on the underlying brain mechanisms.

The central noradrenaline (NA) systems are also integral to stress responses and relapse. Exposure to stress strongly activates the firing of NA neurons and increases the release of NA from their terminals. This release of NA in the forebrain underlies stress-induced reinstatement of drug seeking (Erb et al., 2000; Shaham et al., 2000b; Mantsch et al., 2016). The alpha-1 adrenoceptor plays an important role in stress-induced relapse. Prazosin, a selective alpha-1 adrenoceptor antagonist, blocks reinstatement induced by the stressors footshock and the alpha-2 antagonist yohimbine (Lê et al., 2011; Funk et al., 2016). Prazosin also reduces yohimbine-induced brain activation in areas implicated in stress-induced reinstatement (Bing et al., 1992; Stone and Zhang, 1995; Funk et al., 2016). Consistent with this, alpha-1 adrenoceptor antagonists including prazosin have received attention as treatments for alcohol use disorders and chronic stress conditions such as post-traumatic stress disorder, which frequently co-occur with alcoholism (Walker et al., 2008; Rasmussen et al., 2009; Green, 2014; Simpson et al., 2015; Haass-Koffler et al., 2018).

We speculate that NA, acting at alpha-1 adrenoceptors, may be involved in U50,488-induced reinstatement. U50,488, like other stressors, activates NA neurons in the locus coeruleus and nucleus of the solitary tract, the sites of origin of the NA projections to forebrain nuclei involved in reinstatement (Laorden et al., 2003). There is significant overlap in Dyn/KOR, NA terminals, and alpha-1 adrenoceptors in these areas (Khachaturian et al., 1982; Vincent et al., 1982; Fallon and Leslie, 1986; Mansour et al., 1994b). This potential role of alpha-1 adrenoceptors in the effects of U50,488 in reinstatement has not been examined.

To help resolve this issue, we will determine the effects of the selective alpha-1 antagonist prazosin on U50,488-induced reinstatement of alcohol seeking and regional brain activation, as indexed by Fos expression, in male rats. We hypothesize that prazosin will block U50,488-induced reinstatement of alcohol seeking, as previously shown for footshock and yohimbine, and this will be accompanied by decreases in U50,488-induced Fos expression in brain areas implicated in stress-induced reinstatement. These data will help to better understand the role of KOR and its interaction with NA in stress-induced reinstatement and will help to further evaluate prazosin as a potential medication for relapse prevention.

## Methods

### Subjects

Thirty-four male Long-Evans rats from Charles River (Kingston, NY) weighing 225–250 g at the start of the experiments were used. They were housed individually under a 12:12 reversed light-dark cycle (light on from 7:00 PM to 7:00 AM; 21°C ±1°C) and were fed 5 food pellets (25 g) 2 hours after experimental sessions. Rats weighed 454.5±3.09 g at the end of the experiment. Experimental procedures followed the NIH "Principles of Laboratory Animal Care" (Eighth edition, 2011) and were approved by the local animal care committee. We excluded 2 rats from analysis due to low alcohol intake (<0.4 g/kg).

### Drugs

Alcohol solution was prepared by diluting 95% alcohol in tap water. Prazosin (Sigma Aldrich, Oakville, ON) and U50,488 hydrochloride (Vibrant Pharma, Brantford, ON) were dissolved in distilled water. We selected prazosin and U50,488 doses (free base) and pretreatment times based on our previously published data on the effects of U50,488 on reinstatement and Fos expression and the effects of prazosin on yohimbine-induced reinstatement and Fos expression (Lê et al., 2011; Funk et al., 2014, 2016, 2019; Grella et al., 2014; Lê et al., 2018).

### Apparatus

Alcohol self-administration chambers (30 × 21 × 21 cm) were equipped with 2 levers. Responding on 1 lever (active lever) activated an infusion pump (Razel Scientific), while responding on the other (inactive lever) was recorded but did not activate the pump. Pump activation delivered 0.19 mL of 12% (w/v) alcohol into a drinking receptacle between the levers and initiated a 5-second timeout, during which the houselight was turned off and a compound tone + light cue was turned on. Houselight illumination signaled the start of the session. Alcohol self-administration training, extinction of operant responding, and reinstatement testing were conducted in the same operant chambers.

### Alcohol Self-Administration Training and Extinction of Alcohol-Reinforced Responding

Rats were trained to drink alcohol using an intermittent access procedure with access to 12% w/v alcohol in the home cage every 3rd day for 24 hours for 2 weeks. This was followed by further training using a limited access procedure with the alcohol presented for 30 min/d for 9 days. They were then trained to self-administer alcohol (12% w/v) in 1-hour daily sessions on a fixed ratio-1 (FR-1) 5-second timeout reinforcement schedule for 4 days, FR-2 for 5 days, and then FR-3 for 11 days, until they demonstrated 3 days of stable alcohol self-administration (variability <20%). The procedures used during the extinction sessions were similar to those during self-administration, except no alcohol or cues were delivered.

### Reinstatement Testing

The reinstatement test was done after 18 extinction sessions, when rats reached the extinction criterion (<12 active presses

per 1 hour). During the last 3 sessions prior to the reinstatement test, we gave the rats vehicle injections (distilled water, i.p. and s.c.) to habituate them to the injection procedures. Rats were injected with vehicle or prazosin (1 mg/kg i.p.) and 15 minutes later with vehicle or U50,488 (2.5 mg/kg s.c.) and 30 minutes later were placed in the chambers for a 60-minute reinstatement test. The reinstatement tests were conducted under extinction conditions with no alcohol or cues available.

### Brain Collection and Fos Immunohistochemistry

At the end of the test, 90 minutes after the vehicle or U50,488 injections, rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and perfused transcardially with 100 mL of phosphate-buffered saline (pH 7.4) followed by 300 mL of chilled 4% paraformaldehyde in phosphate buffer. Brains were removed, stored in buffered 4% paraformaldehyde for 1 hour, transferred to 30% sucrose in phosphate buffer for 48 hours, and then frozen in dry ice and stored at –70°C. Brains were cut at 40 microns on a cryostat and sections were collected into 0.1% Tween 20/PBS, and stored in cryoprotectant (30% glycerol/30% ethylene glycol in phosphate buffer) at –20°C. Brain sections were collected from dorsal and ventral medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), nucleus accumbens (NAC) core and shell, dorsal and ventral bed nucleus of the stria terminalis (BNST), central and basolateral amygdala (CeA, BLA), lateral hypothalamus (LH), dorsal and median raphe nuclei (DRN, MRN), ventral tegmental area (VTA), locus coeruleus (LC), and nucleus of the solitary tract (NTS) based on a brain atlas (Paxinos and Watson, 2005). These regions were selected based on previous work from our laboratory and elsewhere indicating their role in footshock-, yohimbine-, or U50,488-induced reinstatement and the inhibitory effects of prazosin on yohimbine-induced brain activation (McFarland et al., 2004; Funk et al., 2006, 2016, 2019; Koob and Volkow, 2016; Lê et al., 2018).

All immunohistochemical solutions were made in Tris-buffered saline with 0.2% Triton X-100. Sections were rinsed 3 times, heated to 80°C in 10 mM Na citrate antigen retrieval solution (pH 9) for 30 minutes (Jiao et al., 1999), rinsed 3 times, incubated in 0.12% $H_2O_2$ in for 30 minutes, rinsed 3 times, and blocked in 3% normal goat serum for 1 hour. Sections were then incubated with rabbit anti-Fos (1:5000, #2250, Lot #9, Ref. 08/2017; Cell Signaling) with 3% normal goat serum at room temperature overnight on a shaker, rinsed 3 times, and incubated with biotinylated anti-rabbit secondary antibody for 1 hour (1:200, Vector Labs, BA1000) with 1 % normal goat serum. Sections were then treated with avidin/biotin horseradish peroxidase (Vectastain Elite, Lot #ZD0224) for 1 hour, received 3 rinses, and then were reacted with 0.5% diaminobenzidine solution (with 0.035% $H_2O_2$ and 0.032% $NiCl_2$, pH=7.8) until the desired staining intensity was achieved (approximately 4 minutes). The reaction was terminated with 3 rinses and sections were mounted on gelatin-coated slides, dried overnight, and then dehydrated and coverslipped. Guided by a brain atlas (Paxinos and Watson, 2005), images of the brain sites were digitized with a video system attached to a light microscope, and Fos-positive neurons in each of the images were counted in a blind manner using particle analysis software (ImageJ, NIH). The mean counts per brain area per square millimeter across images within each rat were calculated.

### Statistical Analyses

The effects of prazosin on U50,488-induced lever-pressing during the reinstatement test were analyzed with between-groups ANOVAs using the factors of U50,488 dose (0, 2.5 mg/kg) and prazosin dose (0, 1 mg/kg) separately for active and inactive lever presses. The Fos expression data in each brain region were analyzed using the same design. We followed significant effects ($P < .05$) from the ANOVAs using Bonferroni post-hoc tests (control vs treatment).

## Results

### Self-Administration and Extinction

See Figure 1A for experimental timeline. As in our previous publication using similar training conditions (Lê et al., 2018; Funk et al., 2019), rats demonstrated reliable alcohol self-administration (approximately 0.7 g/kg at the end of training) and comparable extinction of the alcohol-reinforced responding (Figure 1B–C).

### Effects of Prazosin on U50,488-Induced Reinstatement

Systemic administration of 1 mg/kg prazosin blocked reinstatement of alcohol seeking induced by 2.5 mg/kg of U50,488, which was reflected in a significant U50,488 dose × prazosin dose interaction ($F_{1,28}=4.48$, $P=.043$) in the analysis of active lever press data (Figure 1D). Post-hoc tests showed that rats treated with prazosin prior to U50,488 had lower active lever pressing than those treated with vehicle prior to U50,488 ($P=.008$). Prazosin on its own did not affect active lever pressing, and inactive lever pressing was not affected by U50,488 or prazosin ($Ps > 0.05$).

### Effects of Prazosin on U50,488-Induced Regional Fos Expression

Figure 2 shows the effects of prazosin on U50,488-induced Fos expression in the brain regions studied, and Table 1 shows the results of the ANOVAs conducted on these data. Prazosin blocked U50,488-induced Fos expression in the OFC, NAC core, ventral BNST, CeA, BLA, and VTA, which was reflected in significant U50,488 dose × prazosin dose interactions (Table 1). Post-hoc tests showed that rats that received prazosin prior to U50,488 had lower Fos expression in these regions compared with rats that received U50,488 alone. There were significant main effects of U50,488 dose on Fos expression in the dorsal and ventral mPFC, OFC, NAC core and shell, dorsal and ventral BNST, CeA, BLA, LH, DRN, MRN, LC, and NTS because, compared with vehicle-treated rats, those treated with U50,488 showed significantly higher numbers of Fos immunoreactive neurons (Table 1). There were significant main effects of prazosin dose in the dorsal and ventral mPFC, OFC, ventral BNST, CeA, and BLA, as overall, rats treated with prazosin had fewer Fos-labeled neurons than did vehicle-treated rats (Table 1). Figure 3 shows representative brain sections from the regions where U50,488-induced increases in Fos expression were blocked by prazosin. Figure 4 shows the brain regions in which Fos immunoreactivity was sampled, drawn on brain atlas plates (Paxinos and Watson, 2005).

## Discussion

The major findings of our study are that the alpha-1 antagonist prazosin blocked U50,488-induced reinstatement of alcohol seeking, which was associated with regionally specific reductions in U50,488-induced Fos expression in the OFC, NAC core, ventral BNST, CeA, BLA, and VTA, regions that contain KOR and

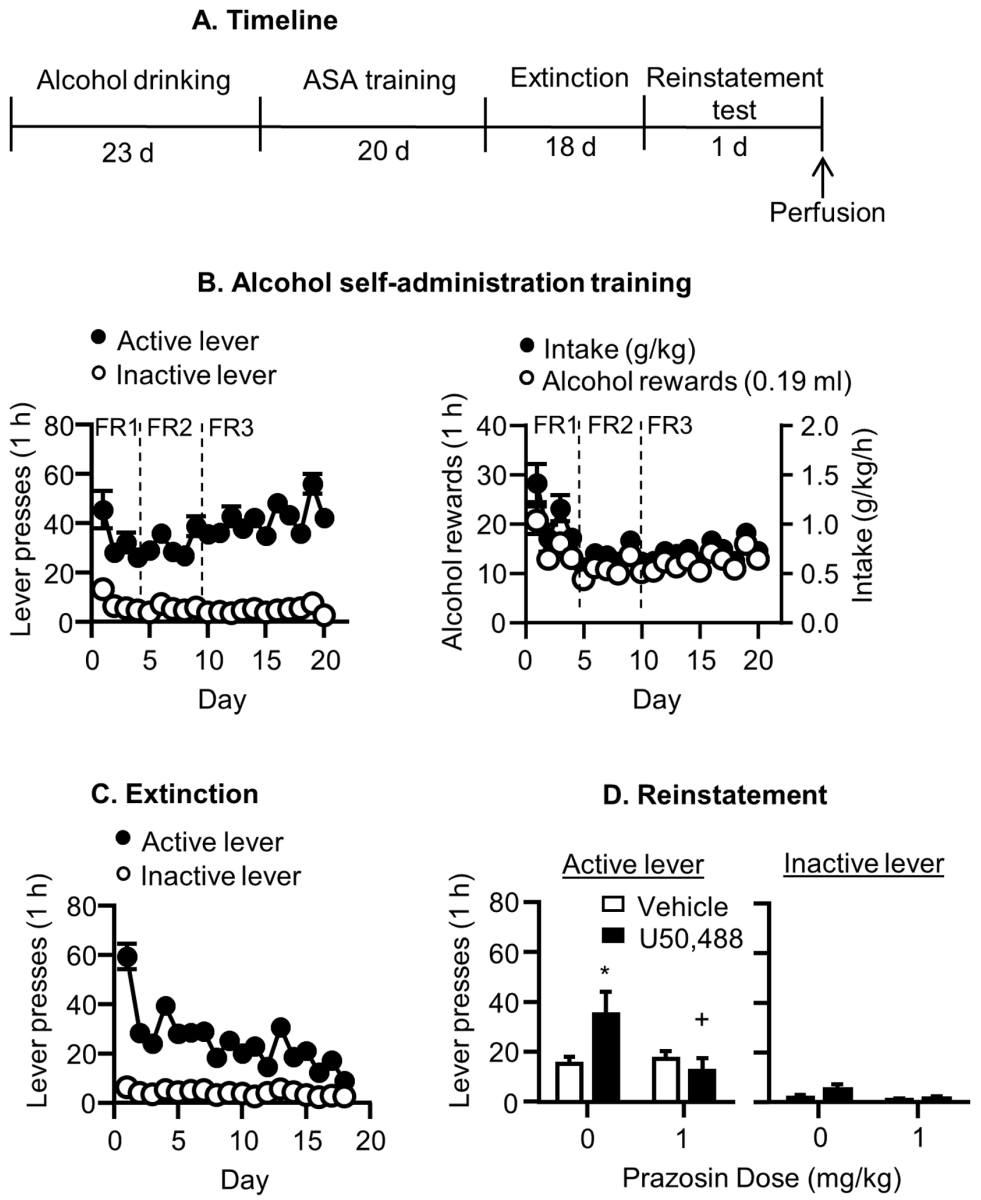


**Figure 1.** Prazosin blocks U50,488-induced reinstatement of alcohol seeking. (A) Timeline of the experiment. (B) Self-administration training: mean ± SEM number of active and inactive lever presses (left) and alcohol rewards and g/kg intake (right) during the 20 training sessions. (C) Extinction: mean ± SEM number of nonreinforced presses on the previously active lever and on the inactive lever during the 20 extinction days. (D) Reinstatement: mean ± SEM number of nonreinforced presses on the previously active lever (left) and on the inactive lever (right) during the 1-hour reinstatement test in rats injected with vehicle or prazosin (1.0 mg/kg i.p.) followed by vehicle or U50,488 (2.5 mg/kg, s.c.). *Different from U50,488 vehicle, + different from prazosin vehicle (0 dose), $P < .05$, n = 7–9/group. FR, fixed ratio.

alpha-1 receptors and that have been previously implicated in stress-induced reinstatement of drug and alcohol seeking. These results provide new information on the brain circuitry through which prazosin acts to reduce stress-induced reinstatement, suggesting that prazosin may act in 1 or more of these regions. Our results also indicate that NA has an important role in U50,488-induced reinstatement.

### Alpha-1 Adrenoceptors and U50,488-Induced Reinstatement

We found that U50,488-induced reinstatement of alcohol seeking was blocked by prazosin. This is consistent with our previous reports that yohimbine- and footshock stress-induced reinstatement of alcohol seeking was blocked by prazosin (Lê et al., 2011; Funk et al., 2016). Our present data extend these effects of prazosin to another stressor that induces reinstatement, the KOR agonist U50,488. Our results also provide the first clear evidence, to our knowledge, for the interaction of alpha-1 adrenoceptors with KOR in drug seeking, as the previous work examining this interaction dealt with feeding, water intake, and locomotor and autonomic responses to KOR activation (Saunders and Thornhill, 1987; Leighton et al., 1988; Thornhill et al., 1989; Nencini et al., 1991).

Preclinical work investigating the use of prazosin as a treatment for alcoholism has primarily examined its effects on alcohol intake. The majority of these studies show that prazosin or other selective alpha-1 antagonists reduce alcohol drinking or self-administration (Walker et al., 2008; Rasmussen et al., 2009; Froehlich et al., 2013; O'Neil et al., 2013). The literature on

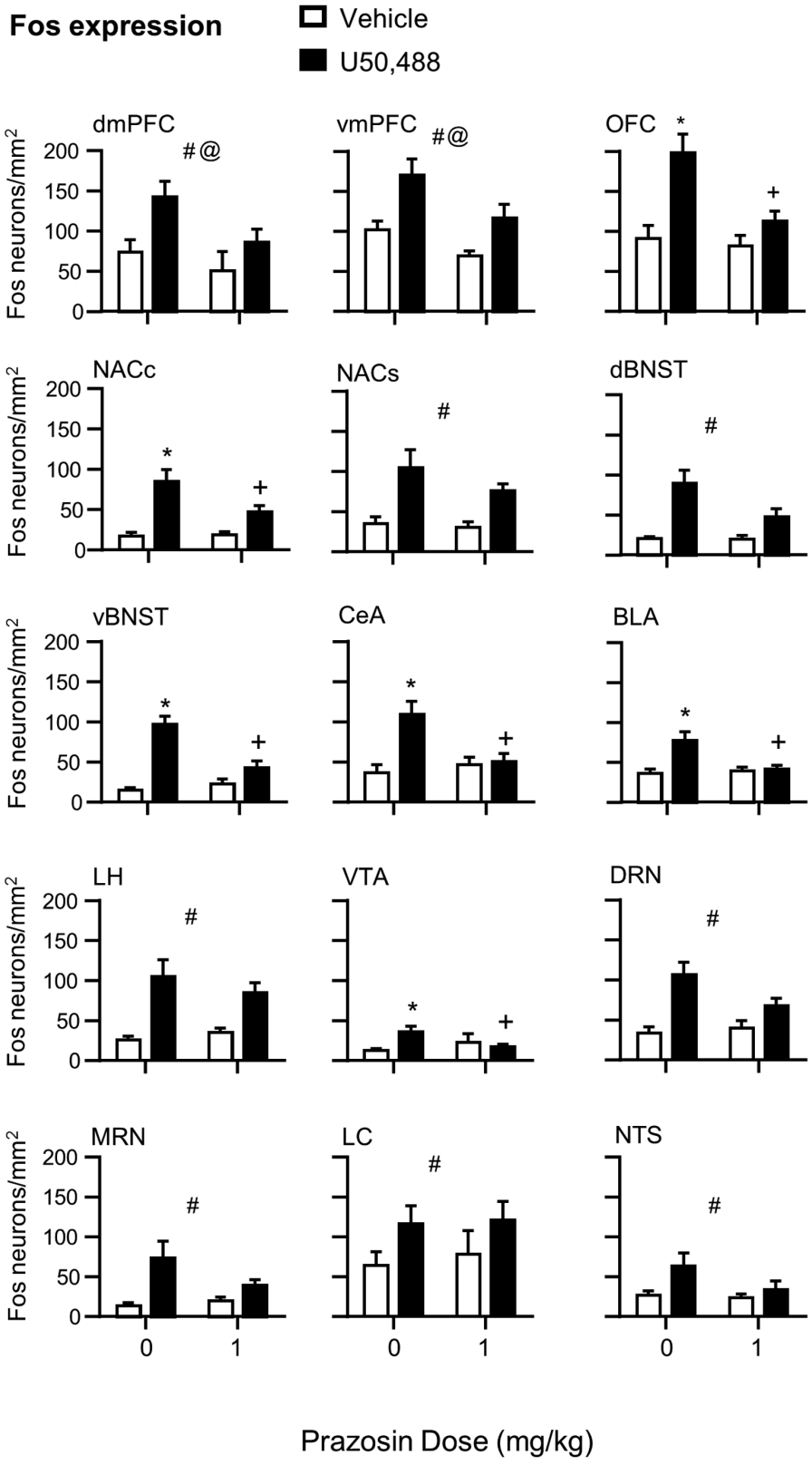


Figure 2. Prazosin blocks U50 488-induced regional Fos expression accompanying reinstatement of alcohol seeking. Mean ± SEM number of Fos-labeled neurons in the dorsal and ventral medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), nucleus accumbens (NAC) core and shell, central nucleus of the amygdala (CeA), basolateral nucleus of the amygdala (BLA), lateral hypothalamus (LH), dorsal raphe nucleus (DRN), median raphe nucleus (MRN), ventral tegmental area (VTA), locus coeruleus (LC) and nucleus of the solitary tract (NTS) in rats injected with vehicle or prazosin (1.0 mg/kg i.p.) followed by vehicle or U50,488 (2.5 mg/kg, s.c.). *Different from U50,488 vehicle; +different from prazosin vehicle. # and @ denote significant main effects of, respectively, U50,488 dose or prazosin dose in the absence of a U50,488 dose × prazosin dose interaction. *Ps* < .05, n as in Figure 1.

**Table 1.** Results (*P* values) from the 2-way ANOVAs on Fos expression in individual brain regions

| Site | | U50,488 | | Prazosin | | U50,488 × Prazosin | |
|---|---|---|---|---|---|---|---|
| | *Df* | *F* | *P* | *F* | *P* | *F* | *P* |
| dmPFC | 1,28 | 7.72 | .01 | 4.39 | .045 | 0.78 | .39 |
| vmPFC | 1,28 | 13.97 | .001 | 7.66 | .01 | 0.45 | .51 |
| OFC | 1,28 | 14.18 | .001 | 6.77 | .015 | 4.27 | .048[a] |
| NACc | 1,28 | 27.74 | .001 | 3.93 | .057 | 4.47 | .043[a] |
| NACs | 1,28 | 18.18 | .001 | 1.50 | .23 | 0.79 | .38 |
| dBNST | 1,28 | 20.3 | .001 | 3.94 | .057 | 3.61 | .068 |
| vBNST | 1,27 | 44.31 | .001 | 8.76 | .006 | 16.57 | .001[a] |
| CeA | 1,28 | 10.54 | .003 | 4.30 | .047 | 8.39 | .007[a] |
| BLA | 1,28 | 10.12 | .004 | 6.19 | .019 | 7.75 | .004[a] |
| LH | 1,28 | 23.92 | .001 | 0.18 | .67 | 1.34 | .256 |
| VTA | 1,28 | 1.99 | .17 | 0.49 | .488 | 5.16 | .031[a] |
| DRN | 1,28 | 20.48 | .001 | 2.12 | .157 | 4.15 | .052 |
| MRN | 1,28 | 12.04 | .002 | 1.56 | .222 | 3.09 | .090 |
| LC | 1,26 | 4.24 | .05 | 0.17 | .687 | 0.032 | .859 |
| NTS | 1,28 | 4.42 | .045 | 2.25 | .145 | 1.62 | .214 |

Abbreviations: ***
[a]Significant U50,488 dose × prazosin dose interaction.

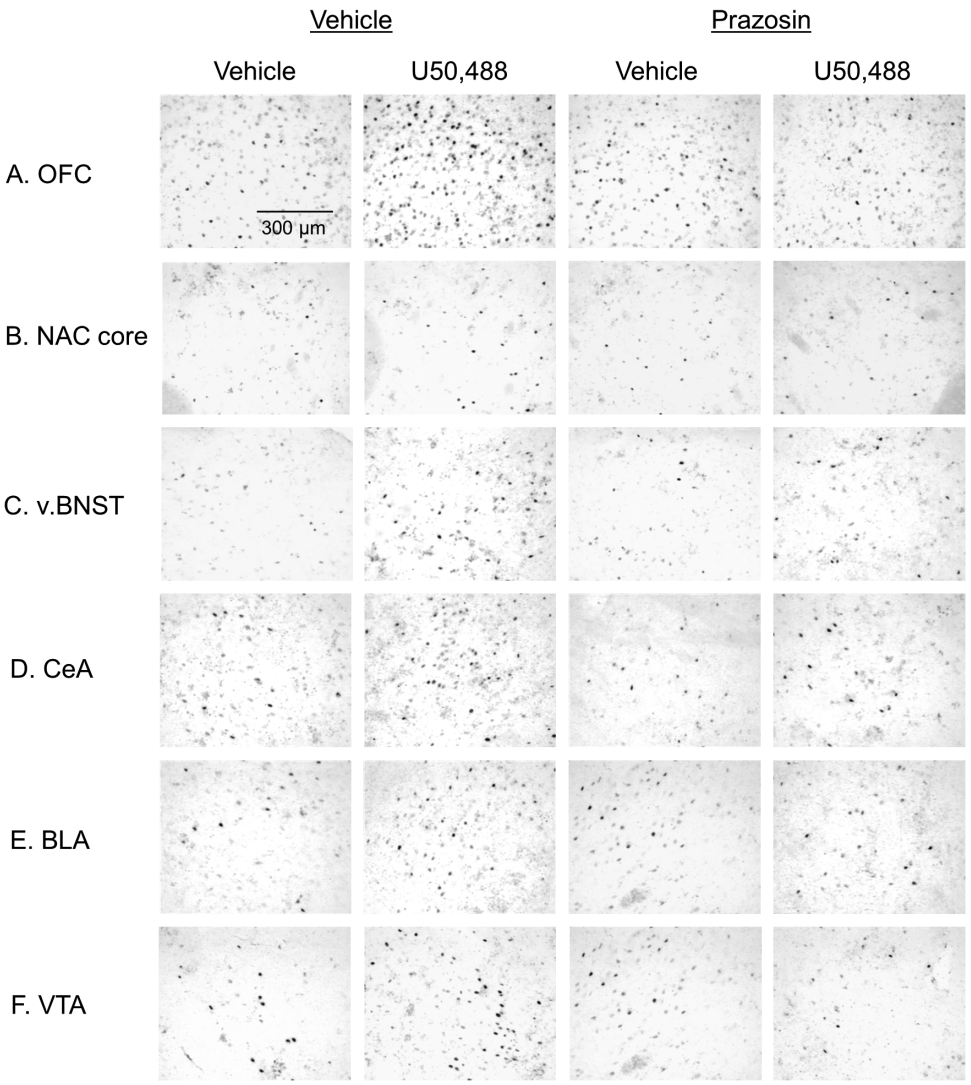


**Figure 3.** Photomicrographs of Fos expression. Prazosin blocks the U50,488-induced Fos expression accompanying reinstatement in the orbitofrontal cortex (OFC), nucleus accumbens (NAC) core, ventral bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), basolateral amygdala (BLA) and ventral tegmental area (VTA). Representative photomicrographs of Fos expression in the OFC (A), NAC core (B), ventral BNST (C), CeA (D), BLA (E), and VTA (F) from rats treated with vehicle or prazosin and vehicle or U50,488 (scale bar = 300 µm).

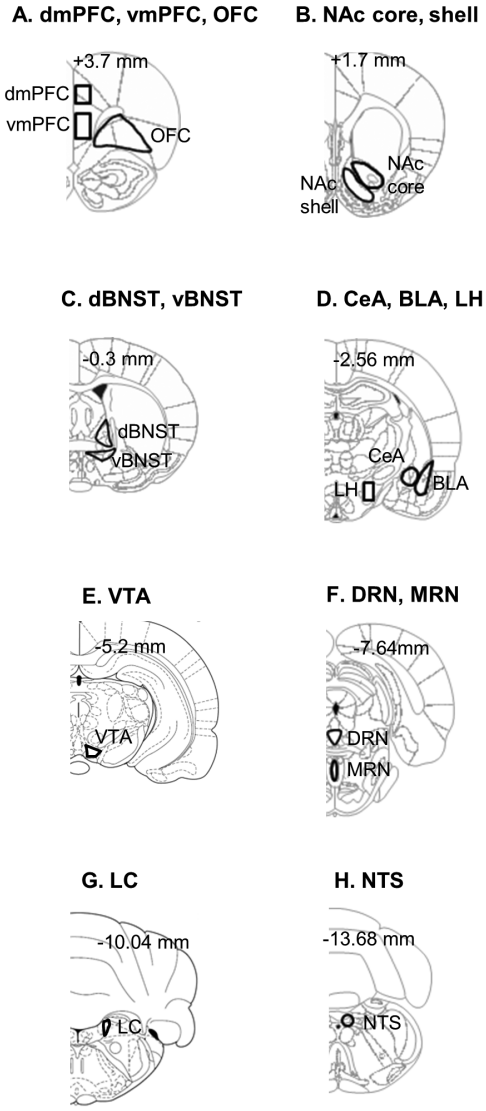


**Figure 4.** (A-H) Sampling regions analyzed. Distance from bregma in mm are indicated on each section (Paxinos and Watson, 2005). The rectangular microscope fields were aimed in the centre of the regions depicted. The field size used to capture images in all regions was 0.46 × 0.35 mm (100×).

the effects of alpha-1 antagonists on relapse to alcohol seeking or other drugs in animal models of drug seeking is relatively sparse. Prazosin blocked priming-induced reinstatement of cocaine and nicotine seeking in rats (Zhang and Kosten, 2005; Forget et al., 2010) but did not affect priming-induced reinstatement of place preference to cocaine (Mantsch et al., 2010). With the exception of our present study with U50,488 and our previous work with yohimbine and footshock stressors showing that prazosin blocks stress-induced reinstatement of alcohol seeking (Lê et al., 2011; Funk et al., 2016), there are only a few studies on the effects of systemic prazosin on stress-induced reinstatement. In monkeys, reinstatement of cocaine seeking induced by the NA reuptake blocker nisoxetine, which, like stressors, increases synaptic levels of NA, was reduced by prazosin (Platt et al., 2007). In contrast, in rodents prazosin did not affect yohimbine- or swim stress-induced reinstatement of place preference to cocaine (Mantsch et al., 2010). These and our present data suggest that the effects of prazosin may depend on factors including the drug administered, species, stressor, and the paradigm employed.

## Alpha-1 Adrenoceptors, U50,488-Induced Brain Activation, and Reinstatement

We have shown that the alpha-1 antagonists prazosin and doxazosin are effective in a preclinical model of stress-induced alcohol seeking (Lê et al., 2011; Funk et al., 2016), and there are also promising clinical data in humans with alcohol use disorders (Fox et al., 2012; Haass-Koffler et al., 2018). The brain circuitry underlying these effects of alpha-1 antagonists is unclear, however. A primary purpose of our study was to help resolve this issue by determining the effects of prazosin on the U50,488-induced regional brain activation accompanying its blockade of U50,488-induced alcohol seeking. We found that prazosin blocked U50,488-induced Fos expression in the OFC, NAC core, ventral BNST, CeA, BLA, and VTA, regions previously implicated in stress effects and stress-induced drug seeking (McFarland and Kalivas, 2001; Koob and Volkow, 2016; Lê et al., 2018). Prazosin may therefore act to reduce U50,488-induced reinstatement by blocking neuronal activity in 1 or more of these regions. Although previous work supports the role of these structures in stress- or priming-induced reinstatement and implicates local KOR and alpha-1 receptors in the mediation of these effects, ours is the first to indicate their potential interaction in these areas in reinstatement.

Studies using local inactivation with baclofen/muscimol support the role of a number of these sites in reinstatement induced by footshock, including the OFC, NAC core, ventral BNST, CeA, and VTA (Capriles et al., 2003; McFarland et al., 2004). This earlier work using local inactivation and our present and previous data using Fos mapping are consistent with the importance of the extended amygdala in stress-induced reinstatement (Kalivas and McFarland, 2003; Funk et al., 2016, 2019; Koob and Volkow, 2016; Lê et al., 2018).

In contrast, local inactivation of the BLA, a site where we observed systemic prazosin to block U50,488- and yohimbine-induced Fos expression (Funk et al., 2016), does not affect footshock-induced reinstatement of cocaine seeking (McFarland et al., 2004). The lack of effect of BLA inactivation on footshock-induced reinstatement is consistent with data suggesting the BLA may play a greater role in reinstatement induced by conditioned cues than acute stressors (Kalivas and McFarland, 2003). A speculative interpretation of our present data is that prazosin-sensitive BLA activation may be involved in yohimbine- and U50,488-induced reinstatement, but not footshock. Studies assessing the effects of BLA prazosin injection on stress-induced alcohol seeking are necessary to resolve this issue.

We noted that prazosin significantly blocked U50,488-induced increases in Fos expression in the VTA, the site of origin of dopamine projections to the forebrain, suggesting the possible involvement of dopamine in U50,488-induced reinstatement and its blockade by prazosin. This is unlikely, however, because, in contrast to other common laboratory stressors that reliably increase dopamine release, U50,488 decreases basal and stimulated dopamine release (Cortez et al., 2010; Karkhanis et al., 2016), which may occur at the level of the dopaminergic cell bodies by decreasing dopamine cell firing or at the level of the terminals by inhibiting dopamine release (Margolis and Karkhanis, 2019). It is also possible that U50,488 activates non-dopaminergic neurons in the VTA; a double labeling approach would be necessary to verify this.

We did not observe a significant effect of prazosin on U50,488-induced Fos expression in the LC or NTS, the location of the NAergic cell bodies that innervate the forebrain. Both cell groups are activated by stressors, including U50,488 (Laorden et al., 2003), and it was reported that KOR in the LC are responsible for U50,488-induced reinstatement of place preference to cocaine (Al-Hasani et al., 2013). One possible interpretation of our present data is that alpha-1 receptors do not influence the NA systems at the level of the NAergic cell bodies.

There is little work specifically examining the role of alpha-1 receptors in discrete brain nuclei in reinstatement of drug seeking and only one report investigating their potential role in stress-induced reinstatement. Injections of the alpha-1 antagonist terazosin into vmPFC reduced priming-induced reinstatement of cocaine-seeking while VTA or NAC shell injections were ineffective (Schmidt et al., 2017). Other work using local injection on alpha-1 antagonists examined impulsivity, a construct related to relapse, and found that OFC injections of prazosin did not affect impulsive responding (Pardey et al., 2013; Adams et al., 2017).

The only study that examined the role of an individual brain nucleus in stress-induced reinstatement found that CeA injections of prazosin did not affect footshock-induced reinstatement of nicotine seeking (Yamada and Bruijnzeel, 2011). In contrast, in our present study, we found that prazosin blocked U50,488-induced CeA activation. This may suggest drug- or stressor- specific differences in the role of the CeA in reinstatement, but local injection studies are necessary to resolve this.

Together with previous work, our data suggest the ventral BNST may be one of the sites where prazosin acts to block U50,488-induced reinstatement. Prazosin blocked U50,488-induced Fos in this region, consistent with the blockade of yohimbine-induced Fos by prazosin we previously reported (Funk et al., 2016). The BNST is involved in stress-induced reinstatement of cocaine (McFarland et al., 2004) and heroin seeking (Shaham et al., 2000a). U50,488-induced reinstatement is blocked by local BNST infusion of a KOR antagonist; ventral BNST Fos expression is significantly correlated with U50,488-induced reinstatement, and local U50,488 injections elicit reinstatement (Lê et al., 2018). Furthermore, the ventral BNST contains high concentrations of NA terminals (Forray and Gysling, 2004), alpha-1 receptors and KOR (Mansour et al., 1994a; Day et al., 1997; Domyancic and Morilak, 1997; Poulin et al., 2009), and alpha-1 blockade in the BNST has anti-stress effects (Cecchi et al., 2002; Morilak et al., 2003). A key future study we will conduct will determine if intra-ventral BNST injections of prazosin block U50,488-induced reinstatement of alcohol seeking.

## Methodological and Interpretational Considerations

A limitation of the present study is that we tested only 1 dose of prazosin on U50,488-induced reinstatement of alcohol seeking and Fos expression. We selected this dose (1 mg/kg) from our previous studies analyzing the dose-dependent effects of prazosin on stress-induced reinstatement. We noted that the prazosin affected U50,488-induced reinstatement of active lever responding and Fos expression but was without effect on active or inactive lever pressing or basal levels of Fos in vehicle-treated rats. This is in agreement with our previous findings on lever pressing and Fos expression and with the lack of effect of prazosin on responding for food pellets (Lê et al., 2011; Funk et al., 2016).

The rats in our study were not dependent on alcohol. Dependent rats are more sensitive to the suppressive effects of prazosin on withdrawal-induced drinking (Walker et al., 2008), but there are no data on its effects on reinstatement under conditions of dependence. Since we previously reported that alcohol dependence leads to enhanced reinstatement induced by U50,488 (Funk et al., 2019), a key extension will be to determine if alcohol dependence modifies the effects of prazosin on U50,488-induced reinstatement.

Another limitation of the present work is that only male rats were employed. There are sex differences in alcohol self-administration, relapse, and response to stressors and drugs used to treat addiction to alcohol and other drugs (Chartoff and Mavrikaki, 2015; Becker and Koob, 2016; Jury et al., 2017; Priddy et al., 2017). Recent work also shows differences between males and females in responses to KOR agonists and antagonists (Chartoff and Mavrikaki, 2015; Conway et al., 2019). Therefore, a critical extension of the present work is to compare females with males in these effects of prazosin on alcohol seeking induced by U50,488 or other stressors.

The mechanism by which U50,488 induces the Fos expression that accompanies reinstatement and how prazosin reduces it is not clear. As described earlier, U50,488 may increase Fos by activating the noradrenaline systems, and blockade of the actions of the released noradrenaline on Fos-containing neurons by prazosin reduces Fos expression. Another possibility is that U50,488 induces Fos by stimulating KOR in the Fos-expressing brain regions, which is then modulated locally by prazosin blockade of alpha-1 receptors. KOR are G protein$_{Gi/Go}$ coupled receptors, and their stimulation can have activational or inhibitory effects on neuronal activity depending on the intracellular signaling pathway recruited (Maejima et al., 2013). KOR can inhibit neuronal activity by reducing cAMP (Konkoy and Childers, 1989, 1993; Durkee et al., 2019) or activate neurons via stimulation of MAPK intracellular pathways (Bruchas et al., 2006; Funk et al., 2019). U50,488 stimulation of KOR on Fos-containing neurons could therefore activate Fos by recruitment of MAPK. Another possibility is that U50,488 stimulates KOR on inhibitory interneurons afferent to the Fos-containing cells, and the reduced inhibition results in a net increase in Fos expression. Alpha-1 receptors are present in these brain regions and local injections of alpha-1 antagonists can block effects of stressors, but the mechanism by which prazosin blocks U50,488-induced Fos expression is not known. Future studies employing co-labeling methods with Fos, KOR, and alpha-1 adrenoceptor expression could help to determine the circuitry involved.

## Concluding Remarks

Our results show that the alpha-1 adrenoceptor antagonist prazosin robustly blocks reinstatement of alcohol seeking induced by the KOR agonist U50,488, and indicates that the OFC, NAC core, ventral BNST, CeA, BLA, and VTA may be involved in the effects of prazosin. Our previous and present data suggest that, of these regions, the ventral BNST may be a key site underlying the effects of prazosin on U50,488-induced reinstatement. Combined with our previous data on prazosin blockade of footshock- and yohimbine-induced reinstatement, our present results also highlight the importance of NA, acting via alpha-1 adrenoceptors in stress-induced reinstatement, and extends it to another stressor that also activates the NA systems: U50,488. These data supports the further investigation of prazosin and other alpha-1 antagonists as treatments for relapse to alcohol seeking in humans.

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## Statement of Interest

None.

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