Addiction Biology

SSA SOCIETY FOR THE STUDY OF ADDICTION

ORIGINAL ARTICLE

doi:10.1111/adb.12514

Noradrenergic signaling in the VTA modulates cocaine craving

Wojciech Barnaba Solecki[1,2], Klaudia Szklarczyk[1], Kamil Pradel[2], Krystyna Kwiatkowska[2], Grzegorz Dobrzański[2] & Ryszard Przewłocki[2]

Department of Neurobiology and Neuropsychology, Institute of Applied Psychology, Jagiellonian University, Poland[1] and Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, Poland[2]

ABSTRACT

Exposure to drug-associated cues evokes drug-seeking behavior and is regarded as a major cause of relapse. Conditional stimulus upregulates noradrenaline (NA) system activity, but the drug-seeking behavior depends particularly on phasic dopamine signaling downstream from the ventral tegmental area (VTA). The VTA dopamine-ergic activity is regulated via the signaling of alpha$_1$-adrenergic and alpha$_2$-adrenergic receptors (α_1-ARs and α_2-ARs); thus, the impact of the conditional stimulus on drug-seeking behavior might involve NAergic signaling in the VTA. To date, the role of VTA ARs in regulating cocaine seeking was not studied. We found that cocaine seeking under extinction conditions in male Sprague–Dawley rats was attenuated by intra-VTA prazosin or terazosin—two selective α_1-AR antagonists. In contrast, cocaine seeking was facilitated by intra-VTA administration of the selective α_1-AR agonist phenylephrine as well as α_2-AR antagonist RX 821002, whereas the selective β-AR antagonist propranolol had no effects. In addition, blockade of α_1-AR in the VTA prevented α_2-AR antagonist-induced enhancement of cocaine seeking. Importantly, the potential non-specific effects of the VTA AR blockade on cocaine seeking could be excluded, because none of the AR antagonists influenced sucrose seeking under extinction conditions or locomotor activity in the open field test. These results demonstrate that NAergic signaling potently and selectively regulates cocaine seeking during early cocaine withdrawal via VTA α_1-AR and α_2-AR but not β-AR. Our findings provide new insight into the NAergic mechanisms that underlie cocaine craving.

Keywords Alpha$_1$-adrenergic receptor, cocaine seeking, craving, noradrenaline, prazosin, ventral tegmental area.

Correspondence to: Wojciech Barnaba Solecki, Department of Molecular Neuropharmacology, Institute of Pharmacology Polish Academy of Sciences, 12 Smetna Street, 31-343 Krakow, Poland. E-mail: wsolecki@if-pan.krakow.pl

INTRODUCTION

Prevention of relapse is a primary goal of substance use disorder (SUD) recovery. Exposure to drug-associated cues during abstinence can induce drug-craving and drug-seeking behavior and this exposure is a major precipitant of drug relapse (Shaham & Hope 2005). Understanding the neurobiological mechanisms that underlie cue-induced craving is critical to the development of effective treatment. It has been demonstrated that α_2-AR agonists decrease cue-induced drug craving in humans (Jobes *et al.* 2011; Sinha *et al.* 2007; Fox & Sinha 2014), pointing to a potential role of noradrenergic signaling in relapse prevention. In addition, noradrenergic signaling has been implicated in cocaine-evoked conditioned place preference (Jasmin *et al.* 2006), escalated cocaine intake by self-administration (Zhang & Kosten 2007; Wee *et al.* 2008), cocaine subjective effects (Newton *et al.* 2012) and reinstatement of cocaine seeking (Zhang & Kosten 2005; Smith & Aston-Jones 2011; Schroeder *et al.* 2013) in rats. These studies indicate the utility of pharmacological manipulation of the NAergic signaling in cocaine addiction, however lack clear evidence of its brain locus mechanisms.

Cue-induced drug seeking is a goal-directed behavior associated with the activity of the ventral tegmental area (VTA) and the nucleus accumbens (NAc) circuitry (Wolf 2016). Presentation of cocaine-associated conditional stimulus (CSs) evokes phasic DA release in the NAc (Phillips *et al.* 2003). Consistently, phasic stimulation of the VTA evokes cocaine seeking, whereas VTA inactivation eliminates cocaine seeking under extinction

conditions (Solecki *et al.* 2013). In addition dopaminergic D_1 receptor blockade in the NAc core abolishes cocaine seeking during early cocaine withdrawal (Solecki *et al.* 2013). These results suggest that phasic DA signaling in the VTA/NAc pathway underlies cocaine seeking during abstinence. Consequently, modulation of NAc activity via its functional inputs from the basolateral amygdala and the prelimbic cortex as well as NAc outputs to the dorsolateral ventral pallidum has been demonstrated to regulate cocaine craving (Stefanik & Kalivas 2013; Stefanik *et al.* 2013a; Stefanik *et al.* 2013b).

Several different brain structures, including the prefrontal cortex, the lateral habenula, the NAc and the caudal portion of the VTA, have been demonstrated to send functional inputs into the VTA (Bromberg-Martin *et al.* 2010; Stamatakis & Stuber 2012; Volkow & Baler 2014). The central nervous noradrenaline (NA) system is well positioned to control DA system activity at the levels of both midbrain DA cell bodies and limbic DA terminals (Geisler & Zahm 2005; Masana *et al.* 2011; for review see Mejías-Aponte 2016). Importantly, NAergic activity in the VTA has been suggested to regulate burst activity of DA neurons and phasic DA release in the NAc via the VTA α_1-AR (Goertz *et al.* 2015). The majority of NA-inputs in the VTA are from the area A1 and A2 (Mejías-Aponte *et al.* 2009; Robertson *et al.* 2013). Furthermore, the area A2, which receives excitatory visceral inputs that ascend from the periphery, is activated during times of heightened arousal (Allchin *et al.* 1994; King & Williams 2009), and its activity is associated with negative affective states experienced during abstinence in drug-dependent subjects (Smith & Aston-Jones 2008). Together, these reports indicate that NA signaling in the VTA might be implicated in the drug-craving and drug-seeking behavior during early withdrawal.

Despite established projections of the NA system to the VTA, the receptor mechanisms, behavioral consequences of the NA signaling in the VTA are rarely studied. Recently, it has been shown that intra-VTA blockade of α_1-AR attenuated both cocaine-induced phasic DA release in the rat NAc shell and cocaine-induced hyperlocomotion, suggesting its involvement in cocaine-related behaviors (Goertz *et al.* 2015); however, to date, there are no reports of the role of VTA NA signaling in cocaine craving. Our study aimed to examine the role of NA receptor signaling in the VTA in cocaine seeking under extinction conditions. Such behavioral paradigm enables capturing effects of cocaine-associated CSs during early withdrawal on cocaine seeking and has been previously used to demonstrate the role of cholinergic receptors in the VTA in phasic DA signaling and cocaine seeking (Solecki *et al.* 2013). In addition, in order to assess the potential clinical utility of AR antagonist in relapse prevention, it is necessary to test cocaine seeking following abstinence in the absence of extinction as it is in most cases not a condition experienced by human cocaine addicts. Finally, such behavioral paradigm has been extensively used to study incubation of cocaine craving—a phenomena in which cue-induced cocaine seeking increases over prolonged withdrawal periods (Lu *et al.* 2004).

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats (280–350 g) were acquired from the Institute of Pharmacology PAS breeding facility (Krakow, Poland). Animals were housed five per cage in a temperature-controlled and humidity-controlled room (20–22°C, 40–50 percent humidity), on a 12-hour light/dark cycle (lights on at 7 AM), with ad libitum access to food and water, unless specified otherwise. Before any surgical procedures, rats were allowed to acclimate to the facility for 1 week. After surgery, all animals were housed singly. Each rat was semi-randomly assigned to a specific drug administration group. All behavioral tests were performed during the light phase of the cycle. All experimental procedures were conducted according to the EU Guide for the Care and Use of Laboratory Animals and were approved by the Committee on the Ethics of Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences (Krakow, Poland) as well as the Committee on the Ethics of Animal Experiments at the Jagiellonian University.

Drugs

Prazosin hydrochloride (Praz; 0.1–1 μg, Sigma-Aldrich, Germany)—a selective α_1-AR antagonist—was dissolved in PBS and sonicated before microinjections. Terazosin hydrochloride (Teraz; 1 μg, Sigma-Aldrich)—another selective α_1-AR antagonist with better solubility, as well as the selective α_2-AR antagonist RX 821002 (RX; 1.35–13.5 μg, Sigma-Aldrich), selective β-AR antagonist propranolol (Prop; 0.3–0.6 μg, Sigma-Aldrich) and selective α_1-AR agonist phenylephrine hydrochloride (Phenyl; 1 μg, Sigma-Aldrich)—was dissolved in PBS. In behavioral experiments, control groups were treated with PBS (vehicle; Veh). All drugs were infused into the VTA in a 0.5-μl volume at a rate of 0.5 μl/min, using a Hamilton 25 gauge syringe (Praz: 1.19–2.38 nmol/side; Teraz: 2.36 nmol/side; Phenyl: 4.91 nmol/side; RX: 4.99–49,86 nmol/side; RX + Teraz: 49,86 + 2.36 nmol/side and Prop: 1.01–2.02 nmol/side). After infusion, the internal cannula was left in place for one additional minute to allow adequate absorption of the drug. The

doses for all experiments were calculated based on previous work from our laboratory and others' demonstrating their ability to modulate behavior after local administration (Selken & Nichols 2007; Azami *et al.* 2010; Do-Monte *et al.* 2010; Ecke *et al.* 2012; Goertz *et al.* 2015).

Intra-ventral tegmental area cannula implantation

All rats were habituated to handling for at least five consecutive days prior to surgery. Rats were anesthetized with ketamine HCl (100 mg/kg, i.m., Biowet-Pulawy, Poland) and xylazine (10 mg/kg, i.m., Biowet-Pulawy, Poland) and placed in a stereotaxic frame (Stoelting Europe, Ireland) for cranial implantation of the cannula. All coordinates were obtained from the rat brain atlas (Paxinos & Watson 2007) with anteroposterior, mediolateral and dorsoventral positions referenced from Bregma. Bilateral guide cannulae (Plastics One, Roanoke, VA, USA) were placed dorsal to the VTA (anteroposterior −5.2 mm, mediolateral ±0.5 mm, dorsoventral −7 mm). Next, 4 anchor screws (Agnthos, Sweden) were mounted in the skull, and dental cement (Duracryl, SpofaDental, Czech Republic) was used to ensure stability of the cannula. Guide cannula patency was ensured by inserting a matching dummy infusion cannula and a dust cap. After the surgery, animals were given an anti-inflammatory and analgesic drug (Tolfdine 4 percent, i.p., Vetoquinol Biowet, Poland) and glucose (5 ml) to prevent dehydration. For the first 3 days after the surgery, animals were treated with antibiotics added to drinking water (Sul-Tridin 24 percent, Biowet-Pulawy, Poland). Rats were given at least a week to recover after intra-VTA cannula implantation.

Cocaine self-administration

Cocaine self-administration was performed as previously described (Solecki *et al.* 2013). Rats were anesthetized with ketamine HCl (100 mg/kg, i.p., Sigma-Aldrich) and xylazine (10 mg/kg, i.p., Sigma-Aldrich) and implanted with a silastic catheter in the external jugular vein, as described by others (McFarland & Kalivas 2001). Following catheter implantation, bilateral cannulae (Plastics One) were stereotaxically implanted above the VTA, as described earlier. Cocaine self-administration training started after 7 days of surgical recovery and was preceded by 2–3 days of food restriction to ~90 percent of free feeding levels. Rats were trained under a fixed ratio 1 schedule of reinforcement during which each active lever depression led to intravenous cocaine infusion (0.18 mg over 6 seconds, ~0.5 mg/kg) and conditioned stimulus (CS) cue presentation (tone + stimulus light for 6 seconds) in standard operant chambers illuminated by house light (Med Associate, St. Albans, USA). Each active lever response was followed by a 20-second time out during which lever pressing had no programmed consequences. Similarly, inactive lever depressions had no programmed consequences. Each rat received 2 hours daily training sessions for 9–11 consecutive days. During first 2–3 days of cocaine self-administration training, food restriction in home cages was continued to increase Skinner cage exploration and enhance operant responding training. Acquisition of stable self-administration behavior was defined as less than 15 percent variability in total active lever pressing over three consecutive days. Animals that did not acquire stable self-administration behavior (20 or more active lever depressions during three consecutive sessions) were excluded from the study ($n = 27$) and were not tested for cocaine seeking under extinction conditions.

Cocaine seeking under extinction conditions

Cocaine seeking under extinction conditions was performed as previously described (Solecki *et al.* 2013). After cocaine self-administration training, rats underwent 3 days of forced abstinence in their home cages (Fig. 1a). On withdrawal day 3 (WD3), animals were placed in operant chambers for 2 hours during which active lever depression led to the CS presentation alone with no cocaine delivery. Inactive lever depressions had no programmed consequences. Cocaine-seeking tests were performed on WD3 to avoid confounds from a potential extinction burst on WD1. Intra-VTA infusion of Praz, Teraz, Phenyl, RX, RX + Teraz, Prop or PBS (Veh) was performed immediately prior to the cocaine-seeking test (Fig. 1a; Table S1). In a separate experiment on WD5–6, the effects of intra-VTA Praz, Teraz, Phenyl, RX, RX + Teraz, Prop or PBS (Veh) administration on locomotion were studied in the open field test.

Locomotor activity

Rats were tested for locomotor activity after intra-VTA Praz, Teraz, Phenyl, RX, RX + Teraz, Prop or PBS (Veh) micro-infusion 48–52 hours after cocaine-seeking testing on WD3 (Fig. 1a; Table S1). Immediately after drug micro-infusion, rats were placed in the center of an open field apparatus (black Plexiglas arena of 80 cm× 80 cm arena with black Plexiglass walls 60-cm high) and left inside for 30 minutes. The apparatus was illuminated with a light intensity of 20 lux, providing just enough illumination for the videotracking software to work correctly. Distance was tracked by video camera and analyzed with ANY-MAZE software (Stoetling Europe, Ireland) and was used as a measure of locomotor activity.

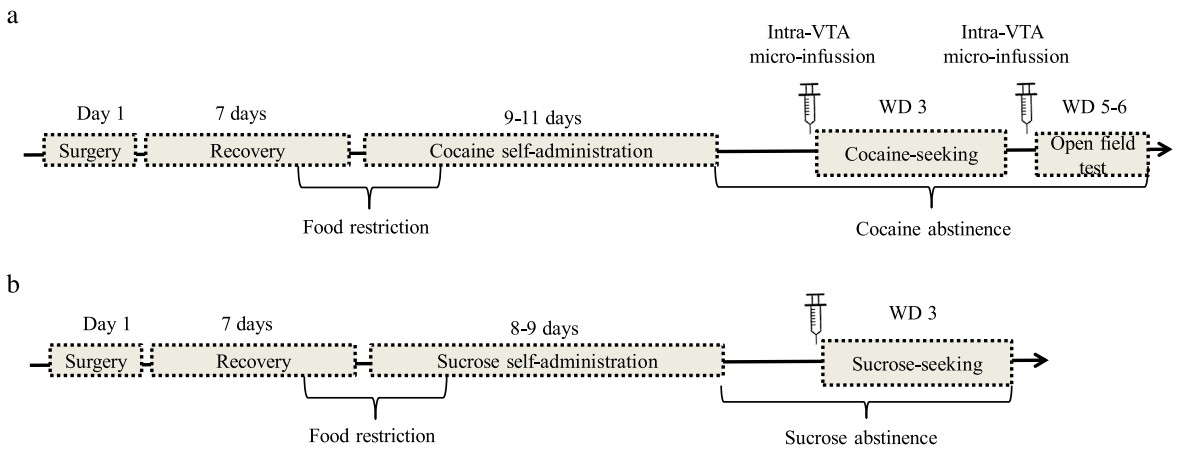

Figure 1 The experimental time-line and schedule of intra-ventral tegmental area (VTA) micro-infusions during cocaine seeking and sucrose seeking under extinction conditions. (a) Rats, after intravenous catheter and intra-VTA cannula implantation surgeries and recovery were trained to self-administer cocaine (~0.5 mg/kg/inf) for 9–11 days, after which they underwent forced abstinence for 3 days. On withdrawal day 3 (WD3), rats were tested for cocaine seeking during which active lever depression led to presentation of the cocaine-paired cue alone with no cocaine delivery. Next, locomotor activity in the open field test was measured on WD5–6. Intra-VTA drug micro-infusions were performed immediately prior to behavioral testing (cocaine seeking and open field test). (b) Rats after intra-VTA cannula implantation surgery and recovery were trained to self-administer sucrose pellets (45 mg) for 8–9 days, followed by sucrose abstinence for 3 days. On WD3, rats were tested for sucrose seeking during which active lever depression led to presentation of the sucrose-paired cue alone with no sucrose delivery

The apparatus was cleaned using 70 percent ethanol and dried with a cleaning cloth after each rat.

Sucrose self-administration

Sucrose self-administration was performed as previously described (Addy *et al.* 2015). Following 6 days of surgical recovery, rats were restricted to 90 percent of their free feeding levels for 2–3 days. One day prior to training, 20–30 sucrose pellets (45 mg; BioServ, Flemington, NJ, USA) were placed into the home cage to introduce the rats to sucrose. Briefly, rats were trained in the same operant chambers used in the cocaine self-administration experiment, described earlier (Med Associates). Each rat was placed in the chamber with a fixed ratio 1 schedule of sucrose reinforcement, where active lever depression led to the delivery of a 45-mg sucrose pellet and the simultaneous presentation of a 6-second audio-visual cue (tone + cue light presentation), followed by a 10-second timeout during which the lever was retracted. Inactive lever depression had no programmed consequence. Rats received 1-hour training sessions over 8–10 consecutive days and then underwent a 3-day period of forced abstinence, during which they had no exposure to sucrose pellets, the operant chamber, or sucrose-associated contextual or discrete cues. Rats that did not acquire stable sucrose self-administration (>20 active lever depressions during three consecutive sessions) were excluded from the study (n = 4).

Sucrose seeking under extinction conditions

Sucrose seeking under extinction conditions was performed as previously described (Addy *et al.* 2015) and similarly to the cocaine seeking experiment. After sucrose self-administration training, rats underwent 3 days of forced abstinence in their home cages (Fig. 1b). On WD3, animals were placed in operant chambers for 1 hour during which active lever depression led to the CS presentation alone with no sucrose delivery. Inactive lever depressions had no programmed consequences. Intra-VTA infusion of Praz, RX, Prop or PBS (Veh) was performed immediately prior to the WD3 test (Fig. 1b; Table S1).

Histological verification of cannula placement

Animals were anesthetized with pentobarbital (150 mg/kg i.p., Biowet-Puławy, Poland), after which 0.5-µl Chicago Sky-Blue dye (Sigma-Aldrich) was bilaterally microinjected into the VTA. Immediately after dye micro-infusion, animals were decapitated; their brains were removed and placed in 4 percent solution of paraformaldehyde for 72 hours. Brains were sliced (200 µm) with a vibratome (model VT1000S, Leica Biosystems, Germany), and the diffusion of the dye was analyzed to verify accuracy of cannula placement. All data from subjects with misplaced cannulae were removed from the analysis of intra-VTA AR antagonist effects (Table S2).

Statistics

Data were analyzed for normal distribution using the Kolmogorov–Smirnov test (Statistica 12.5, Stat-Soft, Poland). The effects of intra-VTA drug administration on total lever presses during the 2-hour cocaine-seeking test were analyzed using two-way ANOVA. For analysis of lever responding during cocaine self-administration or locomotor activity over time, a two-way repeated-measures ANOVA was performed. For analysis of lever responding during cocaine-seeking test over time, a three-way repeated-measures MANOVA was performed. If there was a significant main effect or a significant interaction, a subsequent Newman Keuls *post hoc* analysis was performed. For locomotor activity, time-dependent analysis was measured in a 5-minute bins over the 30-minute session. For all cocaine-seeking tests, there were no significant differences between treatment groups after the initial 60 minutes of the session; therefore, time course data are reported only for the first hour. In addition, during cocaine-seeking testing subjects stopped responding after 60 minutes and during sucrose-seeking testing after 30 minutes; therefore, statistical analysis was performed for the initial 60 and 30 minutes only, respectively. Table S3 presents the factors and levels of ANOVA according to the drug micro-infusions and performed behavioral tests. The $P < 0.05$ was considered statistically significant for all tests. All data values are presented as the means + SEM.

RESULTS

Alpha$_1$-adrenergic signaling in the ventral tegmental area regulates cocaine seeking under extinction conditions

Intra-VTA micro-infusion of prazosin attenuated cocaine seeking in a dose-dependent manner as evidenced by decreased operant responding during testing on WD3 (Fig. 2c; dose: $F_{(2,\ 60)} = 4.05$, $p < 0.05$, *post hoc* test $p < 0.05$ for Praz 1 versus Veh.; lever: $F_{(1,\ 60)} = 90.79$, $p < 0.001$, *post hoc* test $P < 0.001$; dose × lever: $F_{(2,\ 60)} = 1.52$, n.s.). Time-dependent analysis of cocaine seeking revealed that 1 μg Praz attenuated active and inactive lever responding during the initial 5 minutes, whereas 0.5 μg Praz had no effects (Fig. 2d and e; dose × time: $F_{(22,\ 616)} = 1.61$, $p < 0.01$, *post hoc* test $p < 0.01$; dose × lever: $F_{(2,\ 56)} = 1.89$, n.s.; dose × time × lever: $F_{(22,\ 616)} = 1.35$, n.s.). The observed effects were not due to previous differences during training, as 'to be Veh' and 'to be Praz' subjects showed no between-group differences during cocaine self-administration training (Fig. 2a and b; n.s. and Fig. S1a; n.s.; detailed statistics are presented in the Table S4 and Fig. S1 legend).

Similarly, intra-VTA administration of terazosin at 1-μg/side attenuated cocaine seeking (Fig. 2h; dose × lever: $F_{(1,\ 28)} = 5.97$, $p < 0.05$, *post hoc* test $p < 0.001$ for Teraz 1 versus Veh active lever responses). Time-dependent analysis of cocaine seeking revealed that Teraz attenuated active but not inactive lever responding during the session regardless of time (Fig. 2i and j; dose × lever: $F_{(1,\ 28)} = 4.85$, $p < 0.001$, *post hoc* test $p < 0.001$ for active lever Teraz 1 versus Veh, n.s. for inactive lever Teraz 1 versus Veh; time × dose $F_{(11,\ 308)} = 1.53$, n.s.; time × dose × lever $F_{(11,\ 308)} = 1.76$, n.s.). The observed effects were not due to previous differences during training, as 'to be Veh' and 'to be Teraz' subjects showed no significant between-group differences during cocaine self-administration training (Fig. 2f and g; n.s. and Fig. S1b; n.s.; detailed statistics are presented in the Table S4 and Fig. S1 legend). In contrast, intra-VTA micro-infusion of phenylephrine increased active but not inactive lever responding during testing on WD3 (Fig. 2m; dose × lever: $F_{(1,\ 26)} = 5.98$, $p < 0.05$, *post hoc* test $p < 0.01$ for active lever in Phenyl 1 versus Veh). Time-dependent analysis of cocaine seeking revealed that 1-μg Phenyl increased active but not inactive lever responding during the initial 15 minutes, as well as during 55-minute time period (Fig. 2n and o; dose × lever × time: $F_{(11,\ 286)} = 2.31$, $p < 0.01$, *post hoc* test $p < 0.05$). The observed effects were not due to previous differences during training, as 'to be Veh' and 'to be Phenyl' subjects showed no significant between-group differences during cocaine self-administration training (Fig. 2k and l; n.s. and Fig. S1c; n.s.; detailed statistics are presented in the Table S4 and Fig. S1 legend).

Alpha$_2$-adrenergic receptors blockade in the ventral tegmental area facilitates cocaine seeking under extinction conditions

Intra-VTA administration of RX 821002 facilitated cocaine seeking in a dose-dependent manner (Fig. 3c; dose: $F_{(2,\ 58)} = 6.09$, $p < 0.01$, lever: $F_{(1,\ 58)} = 49.15$, $p < 0.001$; dose × lever: $F_{(2,\ 58)} = 3.36$, $p < 0.05$), with the highest RX dose (13.5 μg/side) effectively increasing cocaine seeking (*post hoc* test $p < 0.001$). Examination of the effects of RX over time revealed increased operant responding on the active but not the inactive lever during the first 15 minutes after 13.5 μg RX (Fig. 3d and e; dose × time × lever: $F_{(22,\ 649)} = 1.94$, $p < 0.01$, *post hoc* test $p < 0.001$ for RX versus Veh in 5, 10 and 15-minutes time point). Importantly, during cocaine self-administration training, the number of lever presses or cocaine intake in 'to be Veh' subjects did not differ from that in 'to be RX' subjects (Fig. 3a and b and Fig. S1d;

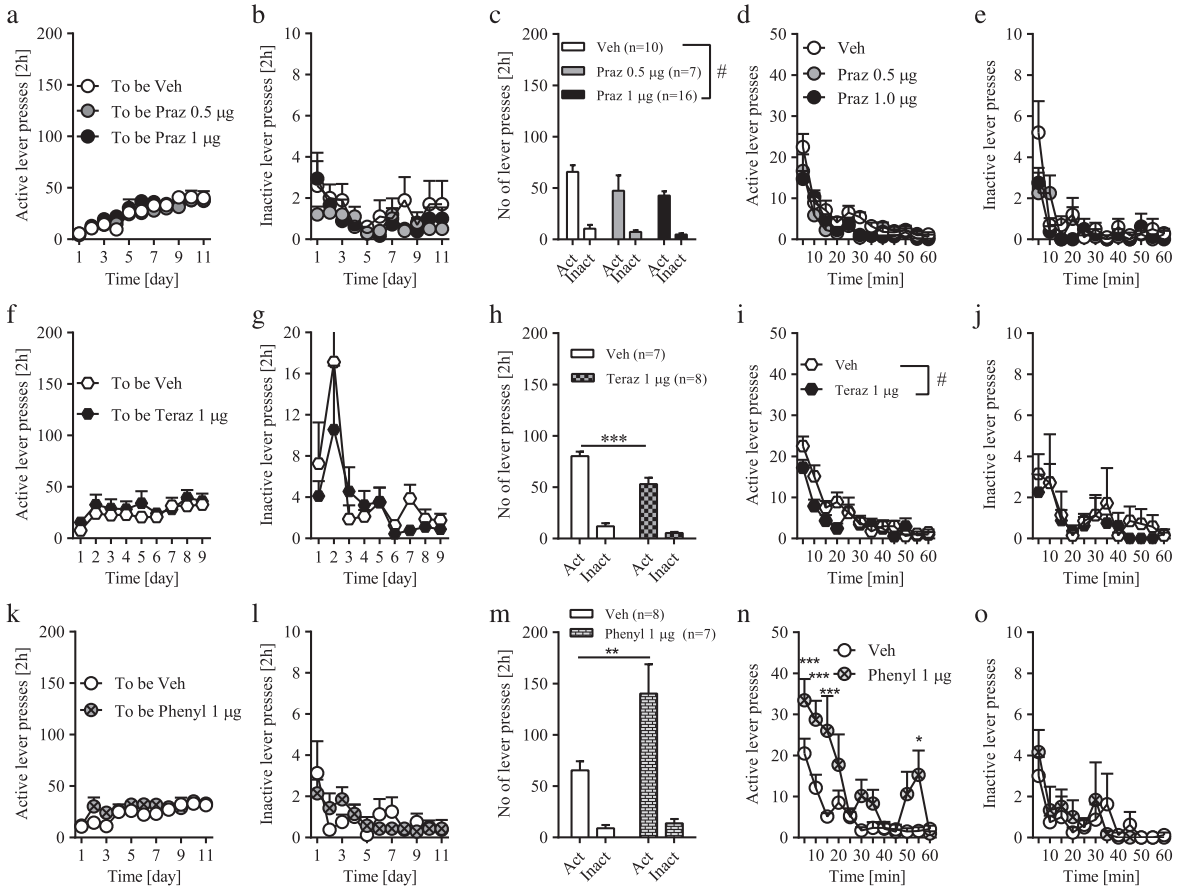

Figure 2 Intra-ventral tegmental area (VTA) administration of the α_1-AR antagonists attenuates, whereas α_1-AR agonist facilitates cocaine seeking under extinction conditions. (a, b, f, g, l, l) There were no differences in the lever responding during cocaine self-administration across drug treatments. (c) Intra-VTA micro-infusion of the α_1-AR antagonist prazosin (Praz; 0.5, 1 µg/side) attenuated both active and inactive lever responding in comparison to the PBS (Veh) treated control (dose: $F_{(2,\ 60)} = 4.05$, $p < 0.05$, *post hoc* test $p < 0.05$ for Praz 1 versus Veh; dose × lever: n.s.). Analysis of lever responding across time showed that prazosin attenuated active (d) and inactive (e) lever responding during the initial 5 minutes of cocaine-seeking testing (1 µg/side Praz; dose × time: $p < 0.001$ followed by *post hoc* test $p < 0.05$, asterisk not shown). (h) Intra-VTA micro-infusion of another α_1-AR selective antagonist, terazosin (Teraz; 1 µg/side) attenuated active but not inactive lever responding. Analysis of lever responding across time (i, j) confirmed those effects (Teraz; dose × lever: $p < 0.001$ followed by the Newman Keuls *post hoc* test $p < 0.05$). In contrast, intra-VTA micro-infusion of (m) the α_1-AR agonist phenylephrine selectively facilitated active lever responding. Analysis of lever responding across time (n, o) confirmed those effects (Phenyl; dose × lever × time: $F_{(11,\ 286)} = 2.31$, $p < 0.01$ followed by the Newman Keuls *post hoc* test $p < 0.05$). Subjects in all treatment groups discriminated between the active and inactive levers during cocaine seeking (c, h, m; prazosin: lever factor: $F_{(1,\ 60)} = 90.79$, $p < 0.001$, *post hoc* test $p < 0.001$, terazosin: $F_{(1,\ 28)} = 188.9$, $p < 0.001$, *post hoc* test $p < 0.001$, phenylephrine: $F_{(1,\ 26)} = 40.68$, $p < 0.001$, *post hoc* test $p < 0.001$ for active versus inactive lever responding; asterisks not shown). Intra-VTA canulae placements are shown in Fig. 5. Data are presented as the mean + SEM. Act—active lever; Inact—inactive lever. #$P < 0.05$ for given treatment group versus Veh. ***$p < 0.001$, * $p < 0.05$ versus active lever in Veh-treated group, Newman Keuls *post hoc* test

n.s.; detailed statistics are presented in the Table S4 and Fig. S1 legend).

Facilitation of cocaine seeking induced by local α_2-AR blockade is attenuated by intra-ventral tegmental area α_1-AR antagonist

Intra-VTA administration of terazosin at 1-µg/side attenuated effects of intra-VTA α_2-AR blockade on cocaine seeking as evidenced by similar level of responding in RX + Teraz and Veh-treated subjects (Fig. 3h; dose × lever: $F_{(1,\ 28)} = 2.3$, n.s.; dose: $F_{(1,\ 28)} = 1.33$, n.s.; lever: $F_{(1,\ 28)} = 26.84$, $p < 0.001$). Further examination of the effects of intra-VTA RX + Teraz administration over time revealed no differences between control and experimental groups in the lever responding (Fig. 3i and j; dose: $F_{(1,\ 28)} = 2.68$, n.s.; dose × lever: $F_{(1,\ 28)} = 1.57$, n.s.; dose × time: $F_{(11,\ 308)} = 0.84$, n.s.; dose × time × lever: $F_{(11,\ 308)} = 0.70$, n.s.). Importantly, during cocaine self-administration training, the number of lever presses or cocaine intake in 'to be Veh' subjects did not differ from that in 'to be RX + Teraz'

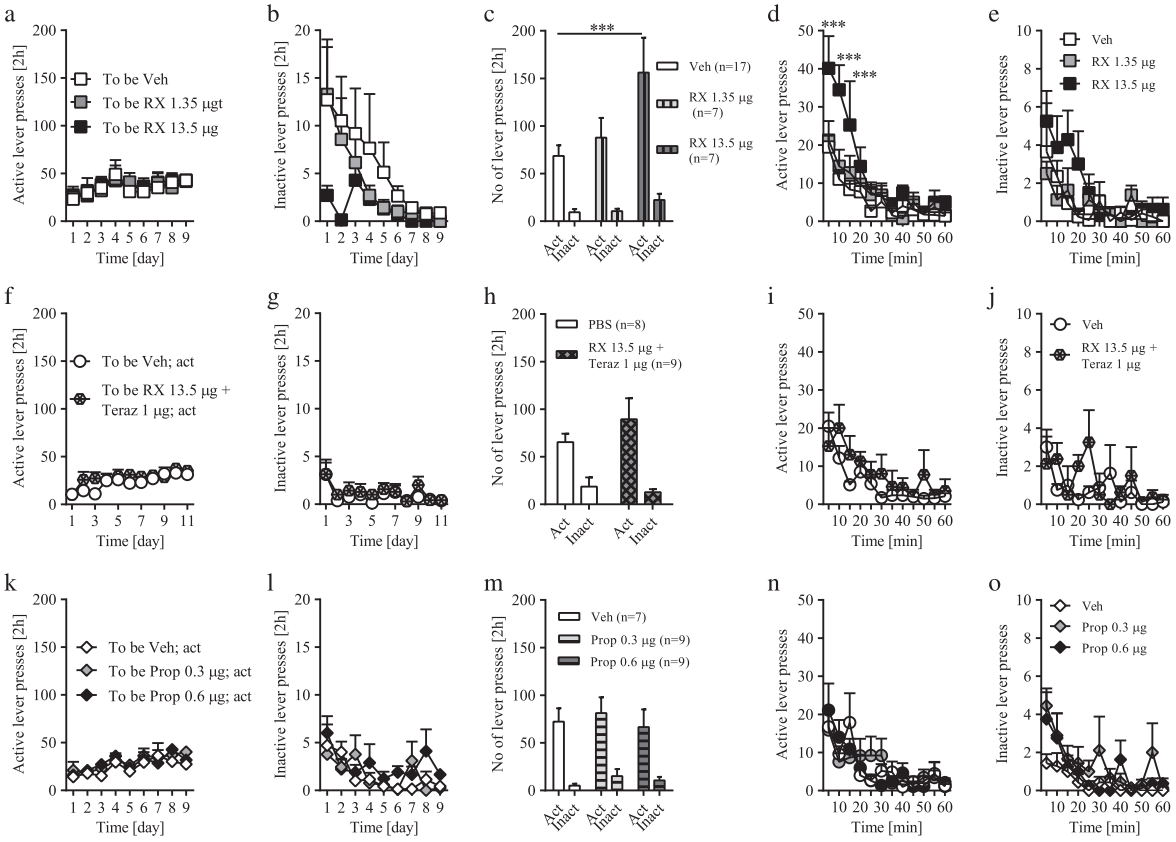

Figure 3 Ventral tegmental area (VTA) NAergic signaling regulates cocaine seeking under extinction conditions via α_1-AR and α_2-AR but not β-AR. (a, b, f, g, k, l) There were no differences in the acquisition of cocaine self-administration across drug treatments. (c) Intra-VTA micro-infusion of the α_2-AR antagonist RX 821002 selectively facilitated active lever responding. This effects was confirmed by analysis of lever responding across time (d, e: RX; dose × time × lever: $F_{(22, 649)} = 1.94$, $p < 0.01$ followed by the Newman Keuls *post hoc* test $p < 0.001$). In contrast, intra-VTA micro-infusion of RX 821002 and terazosin had no effect on cocaine seeking during entire 2-hour testing (h) or when analyzed across time (i, j). (m) The β-AR antagonist propranolol had no effects on responses during cocaine seeking with either lever (n, o). Subjects in all treatment groups discriminated between the active and inactive levers during cocaine seeking (RX 821002: $F_{(1, 58)} = 49.15$, $p < 0.001$, *post hoc* test $p < 0.001$, RX = Teraz: $F_{(1, 58)} = 49.15$, $p < 0.001$, *post hoc* test $p < 0.001$, propranolol: $F_{(2, 52)} = 7.9$, $p < 0.001$, *post hoc* test $p < 0.001$ for active versus inactive lever responding; asterisks not shown). Intra-VTA canulae placements are shown in Fig. 5. Data are presented as the mean + SEM. Act—active lever; Inact—inactive lever. ***$p < 0.001$ versus active lever in Veh-treated group, Newman Keuls *post hoc* test

subjects (Fig. 3f and g and Fig. S1e; n.s.; detailed statistics are presented in the Table S4 and Fig. S1 legend).

β-adrenergic receptors blockade in the ventral tegmental area has no effects on cocaine seeking under extinction conditions

Intra-VTA Prop administration at either dose had no effects on either active or inactive lever responding during cocaine-seeking testing on WD3 (Fig. 3m; dose: $F_{(2, 52)} = 7.9$, n.s.; dose × lever: $F_{(2, 52)} = 7.9$, n.s.; lever: $F_{(2, 52)} = 7.9$, $p < 0.001$) and did not impact lever discrimination ($p < 0.001$). Similarly, there were no effects of Prop administration when analyzed over a 5-minutes time epoch (Fig. 3n and o; dose: $F_{(2, 42)} = 0.14$, n.s.; dose × lever: $F_{(2, 42)} = 0.08$, n.s.; dose × time: $F_{(22, 462)} = 1.16$, n.s.; dose × time × lever: $F_{(22, 462)} = 0.83$, n.s.). During cocaine self-administration training, there were no significant differences between 'to be Veh' and 'to be Prop' groups (Fig. 3k and l and Fig. S1f; n.s.; detailed statistics are presented in the Table S4 and Fig. S1 legend).

Noradrenaline-ergic signaling in the ventral tegmental area does not modulate locomotor activity

To control for potential non-specific effects associated with VTA drug micro-infusion, locomotor activity was subsequently measured on WD5–6. Intra-VTA administration of Praz, Teraz, Phenyl, RX, RX + Teraz or Prop had no effects on distance traveled (Fig. 4a–f and Fig. S2a–f; n.s.) or velocity (Fig. S3a–f; n.s.) measured during a 30-minute open field test. Detailed statistics are presented in the figure legends.

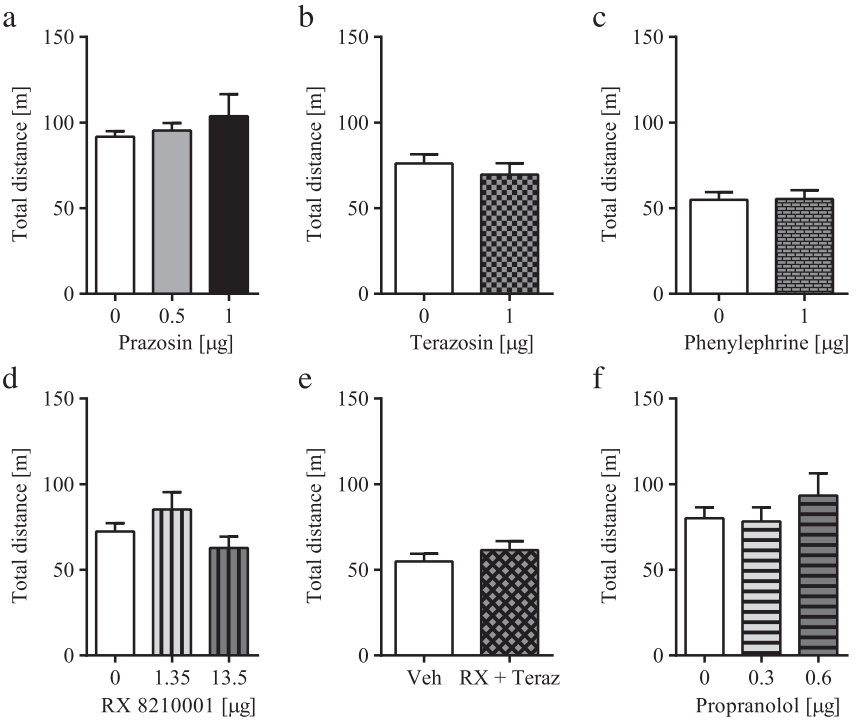

Figure 4 Ventral tegmental area (VTA) NAergic signaling does not modulate locomotor activity. Intra-VTA micro-infusion of prazosin (a; 0.5–1 µg/side; $F_{(2, 14)} = 0.57$, n.s.), terazosin (b; 1 µg/side; $t = 0.76$, df = 17, n.s.), phenylephrine (c; 1 µg/side; $t = 0.06$, df = 17, n.s.), RX 821001 (d; 1.35–13.5 µg/side; $F_{(2, 34)} = 2.23$, n.s.), RX 821001 + terazosine (e; RX 13.5 µg/side + Teraz 1 µg/side; $t = 0.09$, df = 15, n.s.), or propranolol (f; 0.3–0.6 µg/side; $F_{(2, 25)} = 0.76$, n.s.), similarly to vehicle (0) did not modulate total distance travelled by rats during open field test on WD 5–6. Intra-VTA canulae placements are shown in Fig. 6. Data are presented as the mean + SEM

Noradrenaline-ergic signaling in the ventral tegmental area does not regulate sucrose seeking under extinction conditions

Micro-infusions of Praz, RX or Prop had no effects on sucrose seeking (Fig. 6c, h and m; n.s.) and did no impact lever discrimination ($p < 0.001$) as all treatment groups displayed more active than inactive lever presses. Analysis of ARs antagonists over time confirmed these results (Fig. 6d, e, i, j, n and o; n.s.). Importantly, during sucrose self-administration training in all experiments, the number of active or inactive lever presses by 'to be' Veh subjects did not differ from 'to be' drug subjects (Fig. 6a, b, f, g, k and l; n.s.). Detailed statistics are presented in the figure legends.

DISCUSSION

Here, our data reveals that NAergic signaling in the VTA regulates cocaine seeking under extinction conditions during early withdrawal via α_1-AR and α_2-AR but not β-AR. First, we showed that the selective α_1-AR antagonist prazosin attenuated responding on both the active and inactive levers during cocaine-seeking testing on WD3. We found that VTA prazosin infusion did not change locomotion measured as either distance or velocity in cocaine-abstinent rats. These results suggest that prazosin selectively attenuated arousal, manifested as decreased motivation to seek cocaine (either by engaging in previously cocaine-reinforced responding or by additional exploration of the operant box as indexed by inactive lever pressing) during abstinence but not general locomotion. To further delineate the effects of the VTA α_1-AR blockade, we used the selective α_1-AR antagonist terazosin. Terazosin treatment, in a dose similar to the effective dose of prazosin, attenuated active but not inactive lever pressing in cocaine seeking. Similarly to prazosin, VTA micro-infusion of terazosin had no effects on locomotor activity as measured in the open field test, further confirming that the effects of VTA α_1-AR blockade on cocaine seeking were not due to sedation. In contrast to the effects of α_1-AR antagonists, intra-VTA administration of the selective α_1-AR agonist phenylephrine facilitated active but not inactive lever pressing during cocaine seeking and had no effects on locomotion.

Next, we demonstrated that the selective α_2-AR antagonist RX 821002 facilitated active but not inactive lever responding during cocaine seeking and had no effects on locomotion in the open field. Importantly, such α_2-AR antagonist-evoked enhancement of cocaine seeking was blocked by intra-VTA administration of the α_1-AR antagonist terazosin. Additionally, the selective β-AR antagonist propranolol had no effects on cocaine craving or locomotion. Thus, the proposed functional role of NAergic signaling in the VTA is supported by observation that (i) the intra-VTA α_1-AR antagonists attenuated, whereas (ii) α_1-AR agonist or (iii) α_2-AR

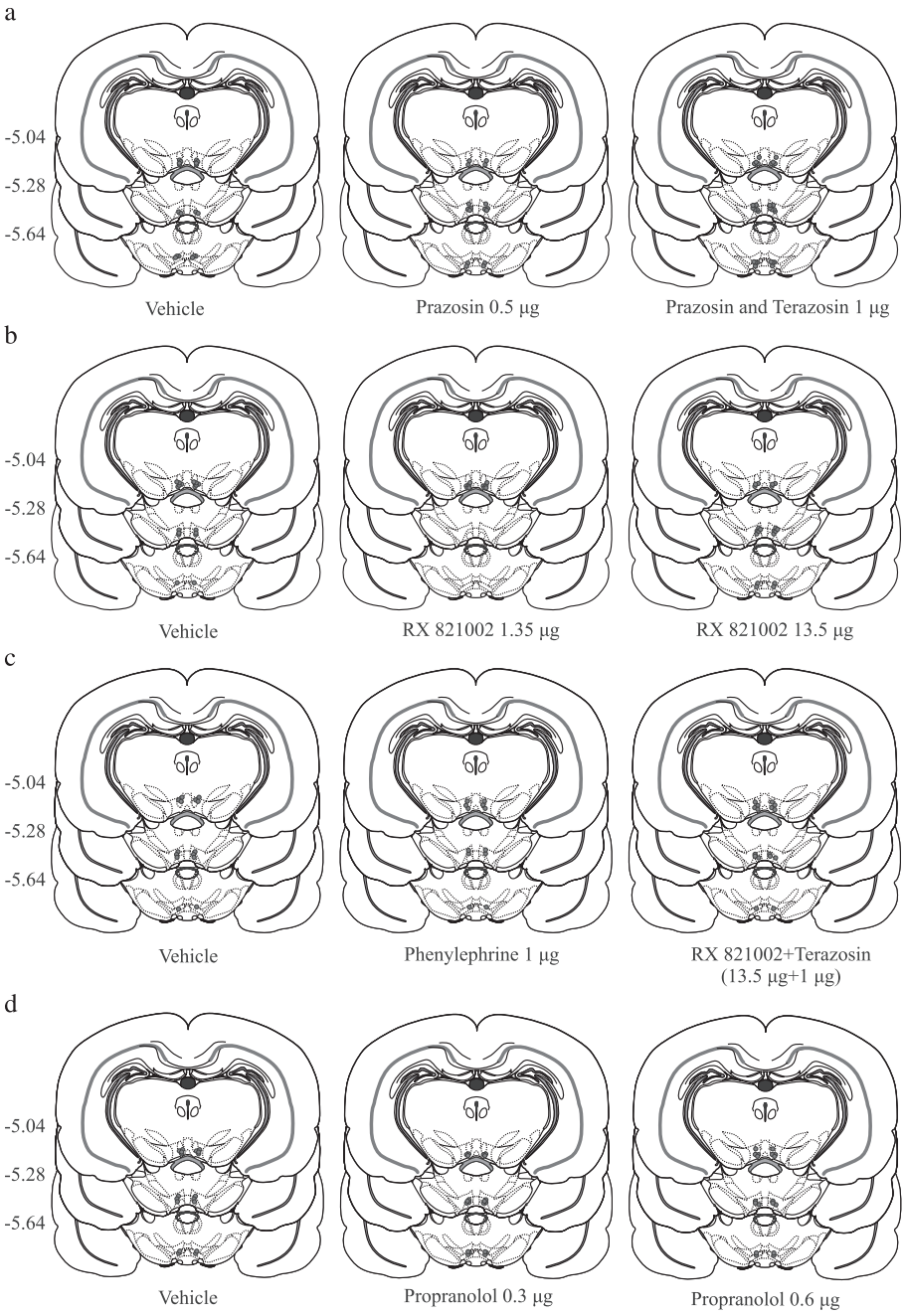

Figure 5 Representative ventral tegmental area (VTA) micro-infusion cannula placements in cocaine seeking under extinction conditions after treatment with (a–c) PBS (vehicle), (a) prazosin (0.5, 1 μg/side) or terazosin (1 μg/side), (b RX 821002 (1.35, 13.5 μg/side), (c) phenylephrine and RX 821002 + terazosin (1 and 13.5 μg/side +1 μg/side) and (d) propranolol (0.3, 0.6 μg/side). Drawings are adapted from (Paxinos & Watson 2007). All coordinates for the VTA were obtained from the rat brain atlas (Paxinos & Watson 2007)

antagonist increased cocaine seeking. Finally, (iv) α_1-AR antagonist attenuated α_2-AR antagonist-induced enhancement of cocaine seeking.

The NA signaling in the VTA modulates neuronal activity via α_1-AR and α_2-AR, whereas β-adrenergic receptor protein expression has not been reported so far. The observed contrasting behavioral effects of α_1-AR and α_2-AR antagonists indicate that α_2-AR blockade may lead to increased NA release from α_2-AR-expressing NAergic terminals, potentially resulting in increased α_1-AR signaling. The majority of α_1-ARs are found on unmyelinated axons but are also distributed on both glutamatergic and GABAergic axons terminals as well as on the VTA neuron dendrites and glia (Rommelfanger *et al.* 2009; Mitrano *et al.* 2012). This pattern of α_1-ARs expression supports both pre-synaptic and postsynaptic effects of the VTA NA signaling. Indeed, pre-synaptic α_1-AR activation in the VTA enhances glutamate release

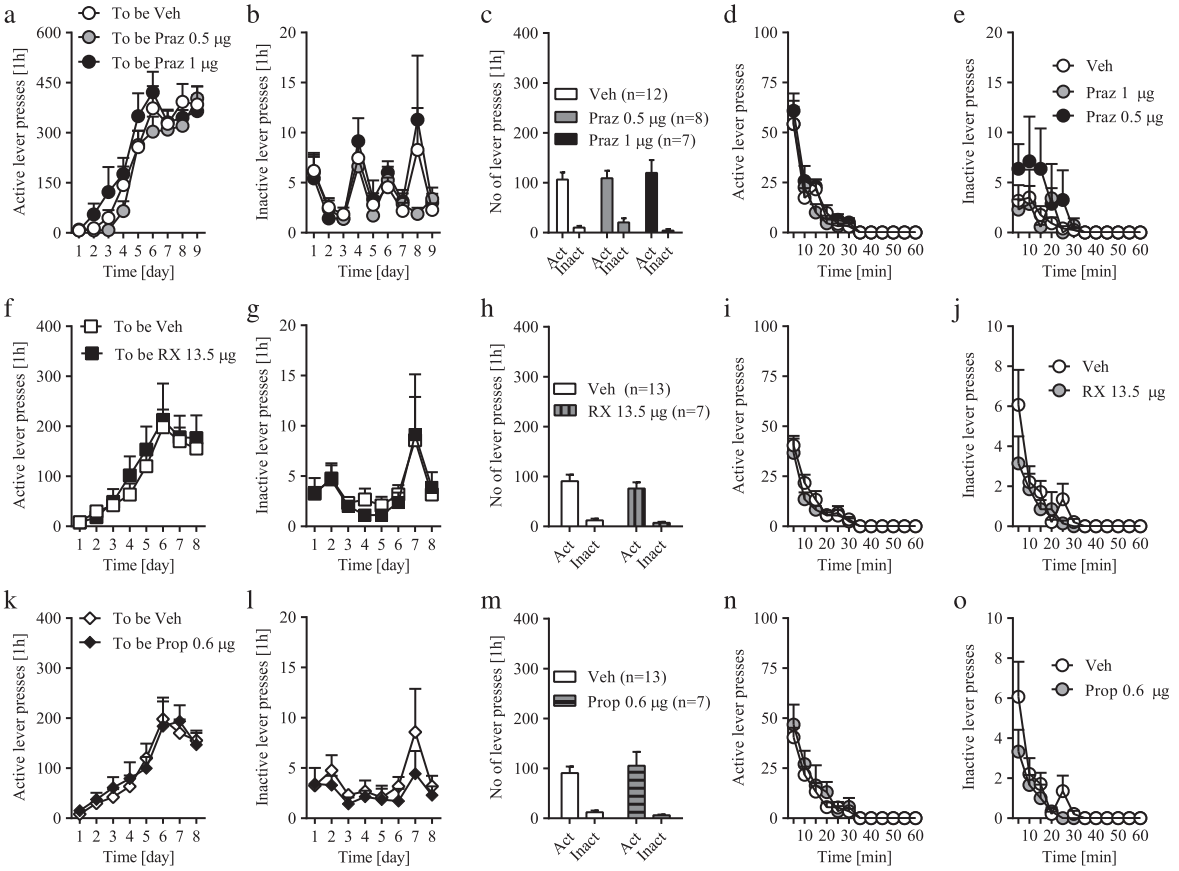

Figure 6 Ventral tegmental area (VTA) NAergic signaling does not regulate sucrose seeking under extinction conditions. (a, b, f, g and k, l) There were no pre-existing differences in the acquisition of sucrose self-administration behavior between rats in the 'to be Veh' and (a, b) 'to be Praz' groups (dose: $F_{(2, 24)} = 1.07$, n.s., time: $F_{(8, 192)} = 47.91$, $p < 0.001$; dose × time: $F_{(16, 192)} = 0.83$, n.s.), (f, g) 'to be RX' (dose: $F_{(1, 18)} = 0.32$, n.s., time: $F_{(7, 126)} = 15.80$, $p < 0.001$; dose × time: $F_{(7, 126)} = 0.19$, n.s.) or (k, l) 'to be Prop' subjects (dose: $F_{(1, 18)} = 0.06$, n.s., time: $F_{(7, 126)} = 20.60$, $p < 0.001$; dose × time: $F_{(7, 126)} = 1.5$, n.s.). (c) Intra-VTA micro-infusion of prazosin (Praz; 0.5, 1 µg/side) or PBS (Veh) had no effect on active and inactive lever pressing during entire 1-hour sucrose-seeking testing (dose: $F_{(2, 48)} = 0.13$, n.s.; lever: $F_{(1, 48)} = 84.97$, $p < 0.001$; dose × lever: $F_{(2, 48)} = 0.84$, n.s.) or when analyzed across time (d, e; dose: $F_{(2, 48)} = 0.75$, n.s.; dose × lever: $F_{(2, 48)} = 0.12$, n.s.; dose × time: $F_{(10, 240)} = 0.56$, n.s.; dose × time × lever: $F_{(10, 240)} = 0.72$, n.s.). (h) Similarly, intra-VTA micro-infusion of the α_2-AR antagonist RX 821002 (RX; 1.35, 13.5 µg/side; dose: $F_{(2, 50)} = 0.37$, n.s.; lever: $F_{(1, 50)} = 59.26$, $p < 0.001$; dose × lever: $F_{(2, 50)} = 0.41$, n.s.) or the β-AR antagonist propranolol (m; Prop; 0.3, 0.6 µg/side; dose: $F_{(2, 46)} = 0.40$, n.s.; lever: $F_{(1, 46)} = 56.54$, $p < 0.001$; dose × lever: $F_{(2, 46)} = 0.36$, n.s.) had no effect on responses for either lever. Analysis of lever responding across time confirmed those results (i, j; RX: dose: $F_{(1, 38)} = 1.16$, n.s.; dose × lever: $F_{(1, 38)} = 0.33$, n.s.; dose × time: $F_{(5, 190)} = 0.56$, n.s.; dose × time × lever: $F_{(5, 190)} = 0.47$, n.s.; n, o; propranolol: dose: $F_{(1, 38)} = 0.11$, n.s.; dose × lever: $F_{(1, 38)} = 0.63$, n.s.; dose × time: $F_{(5, 190)} = 0.35$, n.s.; dose × time × lever: $F_{(5, 190)} = 0.35$, n.s.). Subjects in all treatment groups discriminated between the active and inactive levers during sucrose seeking (c, h, m: lever factor: $p > 0.001$, *post hoc* test $p < 0.001$ for active versus inactive lever responding; asterisks not shown). Results of Veh-treated group shown in panel f–o were obtained from a single group ($n = 13$) as intra-VTA micro-infusions of Veh, RX and Prop were performed in a single experiment using one cohort of animals. Intra-VTA canulae placements are shown in Fig. 7. Data are presented as the mean + SEM

(Velasquez-Martinez *et al.* 2012) but decreases GABA release (Velasquez-Martinez *et al.* 2015), potentially leading to increased postsynaptic neuronal activation, whereas postsynaptic α_1-AR activation enhances DA and non-DA neuronal activity (Grenhoff *et al.* 1995; Williams *et al.* 2014; Goertz *et al.* 2015). In contrast, NA signaling via α_2-AR decreases DAergic activity (Inyushin *et al.* 2010). Thus, downstream DA signaling is expected to be decreased by VTA α_1-AR blockade, but increased by intra-VTA α_2-AR antagonist. Accordingly, intra-VTA micro-infusion of phenylephrine—a selective α_1-AR agonist—induces burst activity of DA neurons, whereas intra-VTA administration of prazosin blocks the cocaine-evoked increase in phasic DA release in the NAc (Goertz *et al.* 2015). Our behavioral results, demonstrating opposite effects of α_1-AR agonist and antagonists and α_1-AR and α_2-AR antagonists, are consistent with α_1-AR and α_2-AR regulation of DA signaling in the VTA-NAc circuitry and its role in cocaine seeking. We hypothesize that cocaine CSs during abstinence upregulate NAergic activity that drives burst activity of the VTA DA neurons and subsequent phasic

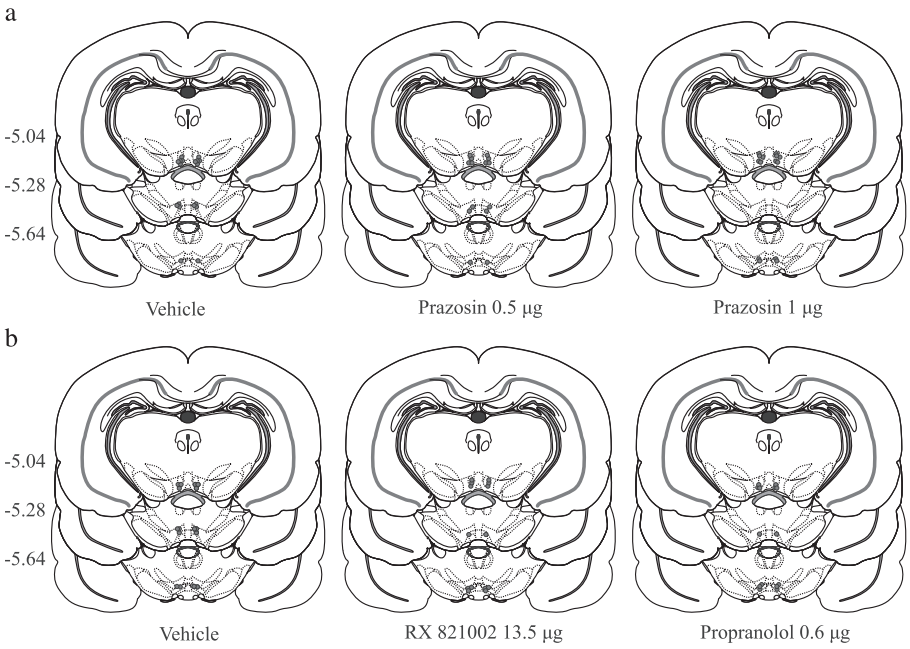

Figure 7 Representative ventral tegmental area (VTA) micro-infusion cannula placements in sucrose seeking under extinction conditions after treatment with (a) prazosin (0.5, 1 μg/side), (b) RX 821002 (13.5 μg/side) and (c) propranolol (0.6 μg/side). Drawings are adapted from (Paxinos & Watson 2007)

DA release at terminals. This may enable behavioral arousal and/or salience detection during early abstinence and lead to cocaine seeking.

Ventral tegmental area AR regulation of arousal and/or salience could impact behavioral responses to other salient cues, such as these related to natural (as opposed to cocaine) rewards. Here, we found that intra-VTA injection of α_1-AR, α_2-AR or β-AR antagonists, in doses effectively attenuating cocaine seeking, had no effects in sucrose seeking under extinction conditions.

Understanding the neural mechanisms involved in drug seeking and craving during abstinence may facilitate methodical development of new therapeutics to treat SUD. Previous clinical study showed that doxazosin—a selective α_1-AR antagonist—reduced cocaine use in cocaine addicts (Shorter *et al.* 2013). Similarly, systemic administration of prazosin attenuates cocaine-induced reinstatement of cocaine seeking (Zhang & Kosten 2005), whereas prazosin treatment only with conjunction with β-AR antagonist decreased cue-induced reinstatement of cocaine seeking in rats (Smith & Aston-Jones 2011). Effectiveness of intra-VTA α_1-AR blockade in the present study is contrasted by lack of prazosin effects after systemic administration demonstrated by Smith & Aston-Jones (2011). Such results discrepancy might be related to different subjective effects of systemic prazosin in comparison to intra-VTA administration. In addition, intra-VTA administration of prazosin has little effects on periphery in contrast with systemic treatment. Lastly, results discrepancy might be related to differences in behavioral paradigm used in present study in which cocaine-seeking test was not preceded by extinction training. Responding under extinction conditions after forced abstinence provides a measure of cue-induced cocaine craving (Lu *et al.* 2004; Wolf 2016); however, alternative interpretation could also be provided. For example, decreased responding after intra-VTA AR antagonist treatment might indicate increased rate of within-session extinction learning rather than attenuated cocaine craving. Importantly, both processes would manifest as decreased cocaine seeking.

In conclusion, our results identified a novel functional role of VTA NAergic signaling in the regulation of cocaine seeking under extinction conditions. Demonstration of the roles of VTA α_1-AR and α_2-AR signaling may help to tailor pharmacotherapy for selected symptoms of SUD such as drug craving during abstinence.

Acknowledgements

The authors declare no conflict of interest. We thank Dr Zuzanna Setkowicz for technical assistance with histological verification. This work was supported by the Polish National Science Center (Research grants UMO-2013/11/D/NZ4/02371 and UMO-2014/13/B/NZ4/00146; both to WBS) and the statutory funds of the Institute of Pharmacology Polish Academy of Sciences.

Authors Contribution

WBS designed and performed experiments, analyzed results, performed histological verifications and wrote the manuscript. KS performed parts of behavioral experiments and histological verifications and wrote parts of the results section of the manuscript. WBS, KS, KP and GD performed animals' surgeries. KK and GD performed parts of behavioral experiments.

References

Addy NA, Nunes EJ, Wickham RJ (2015) Muscarinic, but not nicotinic, acetylcholine receptor blockade in the ventral tegmental area attenuates cue-induced sucrose-seeking. Behav Brain Res 291:372–376.

Allchin RE, Batten TF, McWilliam PN, Vaughan PF (1994) Electrical stimulation of the vagus increases extracellular glutamate recovered from the nucleus tractus solitarii of the cat by in vivo microdialysis. Exp Physiol 79:265–268.

Azami NS, Piri M, Oryan S, Jahanshahi M, Babapour V, Zarrindast MR (2010) Involvement of dorsal hippocampal alpha-adrenergic receptors in the effect of scopolamine on memory retrieval in inhibitory avoidance task. Neurobiol Learn Mem 93:455–462.

Bromberg-Martin ES, Matsumoto M, Hikosaka O (2010) Dopamine in motivational control: rewarding, aversive, and alerting. Neuron 68:815–834.

Do-Monte FHM, Allensworth M, Carobrez AP (2010) Impairment of contextual conditioned fear extinction after microinjection of alpha-1-adrenergic blocker prazosin into the medial prefrontal cortex. Behav Brain Res 211:89–95.

Ecke LE, Elmer GI, Suto N (2012) Cocaine self-administration is not dependent upon mesocortical alpha 1 noradrenergic signaling. Neuroreport 23:325–330.

Fox H, Sinha R (2014) The role of guanfacine as a therapeutic agent to address stress-related pathophysiology in cocaine-dependent individuals. Adv Pharmacol 69:217–265.

Geisler S, Zahm DS (2005) Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. J Comp Neurol 490:270–294.

Goertz RB, Wanat MJ, Gomez JA, Brown ZJ, Phillips PEM, Paladini CA (2015) Cocaine increases dopaminergic neuron and motor activity via midbrain alpha 1 adrenergic signaling. Neuropsychopharmacology 40:1151–1162.

Grenhoff J, North RA, Johnson SW (1995) Alpha(1)-adrenergic effects on dopamine neurons recorded intracellularly in the rat midbrain slice. Eur J Neurosci 7:1707–1713.

Inyushin MU, Arencibia-Albite F, Vazquez-Torres R, Velez-Hernandez ME, Jimenez-Rivera CA (2010) Alpha-2 noradrenergic receptor activation inhibits the hyperpolarization-activated cation current (Ih) in neurons of the ventral tegmental area. Neuroscience 167:287–297.

Jasmin L, Narasaiah M, Tien D (2006) Noradrenaline is necessary for the hedonic properties of addictive drugs. Vascul Pharmacol 45:243–250.

Jobes ML, Ghitza UE, Epstein DH, Phillips KA, Heishman SJ, Preston KL (2011) Clonidine blocks stress-induced craving in cocaine users. Psychopharmacology (Berl) 218:83–88.

King SO, Williams CL (2009) Novelty-induced arousal enhances memory for cued classical fear conditioning: interactions between peripheral adrenergic and brainstem glutamatergic systems. Learn Mem 16:625–634.

Lu L, Grimm JW, Hope BT, Shaham Y (2004) Incubation of cocaine craving after withdrawal: a review of preclinical data. Neuropharmacology 47:214–226.

Masana M, Bortolozzi A, Artigas F (2011) Selective enhancement of mesocortical dopaminergic transmission by noradrenergic drugs: therapeutic opportunities in schizophrenia. Int J Neuropsychopharmacol 14:53–68.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 21:8655–8663.

Mejías-Aponte CA (2016) Specificity and impact of adrenergic projections to the midbrain dopamine system. Brain Res 1641:258–273.

Mejías-Aponte CA, Drouin C, Aston-Jones G (2009) Adrenergic and noradrenergic innervation of the midbrain ventral tegmental area and retrorubral field: prominent inputs from medullary homeostatic centers. J Neurosci 29:3613–3626.

Mitrano DA, Schroeder JP, Smith Y, Cortright JJ, Bubula N, Vezina P, Weinshenker D (2012) Alpha-1 adrenergic receptors are localized on presynaptic elements in the nucleus accumbens and regulate mesolimbic dopamine transmission. Neuropsychopharmacology 37:2161–2172.

Newton TF, De la Garza R, Brown G, Kosten TR, Mahoney JJ, Haile CN (2012) Noradrenergic alpha(1) receptor antagonist treatment attenuates positive subjective effects of cocaine in humans: a randomized trial. PLoS One 7:e30854.

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates: hard cover edition, 6th edn. San Diego: Elsevier.

Phillips PEM, Stuber GD, Helen MLAV, Wightman RM, Carelli RM (2003) Subsecond dopamine release promotes cocaine seeking (vol 422, pg 614, 2003). Nature 423:461–461.

Robertson SD, Plummer NW, de Marchena J, Jensen P (2013) Developmental origins of central norepinephrine neuron diversity. Nat Neurosci 8:1016–1023.

Rommelfanger KS, Mitrano DA, Smith Y, Weinshenker D (2009) Light and electron microscopic localization of alpha-1 adrenergic receptor immunoreactivity in the rat striatum and ventral midbrain. Neuroscience 158:1530–1540.

Schroeder JP, Epps SA, Grice TW, Weinshenker D (2013) The selective dopamine beta-hydroxylase inhibitor nepicastat attenuates multiple aspects of cocaine-seeking behavior. Neuropsychopharmacology 38:1032–1038.

Selken J, Nichols DE (2007) Alpha 1-adrenergic receptors mediate the locomotor response to systemic administration of (+/−)-3,4-methylenedioxymethamphetamine (MDMA) in rats. Pharmacol Biochem Be 86:622–630.

Shaham Y, Hope BT (2005) The role of neuroadaptations in relapse to drug seeking. Nat Neurosci 8:1437–1439.

Shorter D, Lindsay JA, Kosten TR (2013) The alpha-1 adrenergic antagonist doxazosin for treatment of cocaine dependence: A pilot study. Drug Alcohol Depen 131:66–70.

Sinha R, Kimmerling A, Doebrick C, Kosten TR (2007) Effects of lofexidine on stress-induced and cue-induced opioid craving and opioid abstinence rates: preliminary findings. Psychopharmacology (Berl) 190:569–574.

Smith RJ, Aston-Jones G (2008) Noradrenergic transmission in the extended amygdala: role in increased drug-seeking and relapse during protracted drug abstinence. Brain Struct Funct 213:243–261.

Smith RJ, Aston-Jones G (2011) Alpha(2) adrenergic and imidazoline receptor agonists prevent cue-induced cocaine seeking. Biol Psychiatry 70:712–719.

Solecki W, Wickham RJ, Behrens S, Wang J, Zwerling B, Mason GF, Addy NA (2013) Differential role of ventral tegmental area acetylcholine and N-methyl-D-aspartate receptors in cocaine-seeking. Neuropharmacology 75:9–18.

Stamatakis AM, Stuber GD (2012) Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance. Nat Neurosci 15:1105.

Stefanik MT, Kalivas PW (2013) Optogenetic dissection of basolateral amygdala projections during cue-induced reinstatement of cocaine seeking. Front Behav Neurosci 7:213.

Stefanik MT, Kupchik YM, Brown RM, Kalivas PW (2013a) Optogenetic evidence that pallidal projections, not nigral projections, from the nucleus accumbens core are necessary for reinstating cocaine seeking. J Neurosci 33:13654–13662.

Stefanik MT, Moussawi K, Kupchik YM, Smith KC, Miller RL, Huff ML, Deisseroth K, Kalivas PW, LaLumiere RT (2013b) Optogenetic inhibition of cocaine seeking in rats. Addict Biol 18:50–53.

Velasquez-Martinez MC, Vazquez-Torres R, Jimenez-Rivera CA (2012) Activation of alpha1-adrenoceptors enhances glutamate release onto ventral tegmental area dopamine cells. Neuroscience 216:18–30.

Velasquez-Martinez MC, Vazquez-Torres R, Rojas LV, Sanabria P, Jimenez-Rivera CA (2015) Alpha-1 adrenoreceptors modulate GABA release onto ventral tegmental area dopamine neurons. Neuropharmacology 88:110–121.

Volkow ND, Baler RD (2014) Addiction science: uncovering neurobiological complexity. Neuropharmacology 76:235–249.

Wee S, Mandyam CD, Lekic DM, Koob GF (2008) Alpha(1)-noradrenergic system role in increased motivation for cocaine intake in rats with prolonged access. Eur Neuropsychopharmacol 18:303–311.

Williams MA, Li C, Kash TL, Matthews RT, Winder DG (2014) Excitatory drive onto dopaminergic neurons in the rostral linear nucleus is enhanced by norepinephrine in an alpha(1) adrenergic receptor-dependent manner. Neuropharmacology 86:116–124.

Wolf ME (2016) Synaptic mechanisms underlying persistent cocaine craving. Nat Rev Neurosci 17:351–365.

Zhang XY, Kosten TA (2005) Prazosin, an alpha-1 adrenergic antagonist, reduces cocaine-induced reinstatement of drug-seeking. Biol Psychiatry 57:1202–1204.

Zhang XY, Kosten TA (2007) Previous exposure to cocaine enhances cocaine self-administration in an alpha 1-adrenergic receptor dependent manner. Neuropsychopharmacology 32:638–645.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1. Cocaine intake during cocaine self-administration training across drug treatments. Prior to VTA drug administration on withdrawal day 3, there were no pre-existing differences in the number of cocaine infusions administered during cocaine self-administration training between rats in the 'to be Veh' and (A) 'to be Praz' groups (dose: $F_{(2,\ 22)} = 0.03$, n.s., time: $F_{(10,\ 220)} = 55.32$, $P < 0.001$; dose × time: $F_{(20,\ 220)} = 0.74$, n.s.) as well as the (B) 'to be Teraz' (dose: $F_{(1,\ 14)} = 1.24$, n.s., time: $F_{(8,\ 112)} = 11.67$, $P < 0.001$; dose × time: $F_{(8,\ 112)} = 0.04$, n.s.), (C) 'to be Phenyl' (dose: $F_{(1,\ 13)} = 0.02$, n.s., time: $F_{(10,\ 130)} = 20.61$, $P < 0.001$; dose × time: $F_{(20,\ 130)} = 1.02$, n.s.), (D) 'to be RX' (dose: $F_{(2,\ 32)} = 0.61$, n.s., time: $F_{(8,\ 256)} = 6.94$, $P < 0.01$; dose × time: $F_{(16,\ 256)} = 0.71$, n.s.), (E) 'to be RX + Teraz' group (dose: $F_{(1,\ 14)} = 0.19$, n.s., time: $F_{(10,\ 140)} = 23.17$, $P < 0.001$; dose × time: $F_{(10,\ 140)} = 0.82$, n.s.) and (F) 'to be Prop' subjects (dose: $F_{(2,\ 23)} = 0.23$, n.s., time: $F_{(8,\ 184)} = 18.17$, $P < 0.001$; dose × time: $F_{(16,\ 184)} = 0.94$, n.s). Data are presented as the mean + SEM.

Figure S2. Blockade of noradrenergic signaling in the VTA does not modulate locomotor activity over time. Intra-VTA micro-infusion of prazosin (A; 0.5–1 μg/side; dose: $F_{(2,\ 14)} = 0.57$, n.s.; time: $F_{(5,\ 70)} = 59.31$, $P < 0.001$, *post hoc* test $P < 0.001$ for 5 min versus 15, 20, 15, or 30 min; dose × time interaction: $F_{(10,\ 70)} = 0.82$, n.s.), terazosin (B; 1 μg/side; dose: $F_{(1,17)} = 0.58$, n.s.; time: $F_{(5,\ 85)} = 116.9$, $P < 0.001$, *post hoc* test $P < 0.001$ for 5 min versus 10, 15, 20, 25 or 30 min; dose × time interaction: $F_{(5,\ 85)} = 0.28$, n.s.), phenylephrine (C; 1 μg/side; dose: $F_{(1,15)} = 0.09$, n.s.; time: $F_{(5,\ 75)} = 43.8$, $P < 0.001$, *post hoc* test $P < 0.05$ for 5 min versus 10, 15, 20, 25 or 30 min; dose × time interaction: $F_{(5,\ 75)} = 1.55$, n.s.), RX 821002 (C; 1.35–13.5 μg/side; dose: $F_{(2,\ 34)} = 2.31$, n.s.; time: $F_{(5,\ 170)} = 63.77$, $P < 0.001$, *post hoc* test $P < 0.001$ for 5 min versus 10, 15, 20, 25 or 30 min; dose × time interaction: $F_{(10,\ 170)} = 1.18$, n.s.), RX 821001 + terazosine (E; RX 13.5 μg/side +Teraz 1 μg/side; dose: $F_{(1,13)} = 0.15$, n.s.; time: $F_{(5,\ 65)} = 18.4$, $P < 0.001$, *post hoc* test $P < 0.001$ for 5 min versus 15, 20, 25 or 30 min; dose × time interaction: $F_{(5,\ 65)} = 0.88$, n.s.), or propranolol (F; 0.3–0.6 μg/side; dose: $F_{(2,\ 25)} = 0.76$, n.s.; time: $F_{(5,\ 125)} = 78.59$, $P < 0.001$, *post hoc* test $P < 0.001$ for 5 min versus 10, 15, 20, 25 or 30 min; dose × time interaction: $F_{(10,\ 125)} = 1.40$, n.s.), in comparison to PBS (Veh) did not influence distance traveled over time. Data are presented as the mean + SEM.

Figure S3. Blockade of noradrenergic signaling in the VTA does not influence velocity. Intra-VTA micro-infusion of prazosin (A; 0.5–1 μg/side; $F_{(2,\ 14)} = 0.56$, n.s.), terazosin (B; 1 μg/side; $t = 0.79$, df = 17, n.s.), phenylephrine (C; 1 μg/side; $t = 0.54$, df = 16, n.s.), RX 821002 (C; 1.35–13.5 μg/side; $F_{(2,\ 34)} = 0.30$, n.s.), RX 821001 + terazosine (E; RX 13.5 μg/side +Teraz 1 μg/side; $t = 0.01$, df = 16, n.s.), or propranolol (F; 0.3–0.6 μg/side; $F_{(2,\ 25)} = 0.75$, n.s.), in comparison to PBS (Veh) had no effect on rats' average speed. Data are presented as the mean + SEM of velocity.

Table S1. Behavioral testing. VTA drug micro-infusions were performed on withdrawal day 3 (WD3) in rats previously trained for cocaine or sucrose self-administration. In addition, VTA drug micro-infusions were performed on WD5–6 in cocaine-abstinent rats prior to the open field testing. SA: self-administration.

Table S2. Number of subjects with VTA cannula misplacements. PBS (Veh), prazosin (Praz), terazosin (Teraz), phenylephrine (Phenyl), RX 821002 (RX), propranolol (Prop) n.a.—not applicable. The total number of subjects in each treatment group is indicated in parentheses.

Table S3. A table listing the factors and levels of ANOVA according to the drug micro-infusions and behavioral tests.

Table S4. A table listing the factors and levels as well as statistical details of ANOVA according to the drug micro-infusions and behavioral tests.