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Cocaine Increases Dopaminergic Neuron and Motor Activity via Midbrain α1 Adrenergic Signaling

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Cocaine reinforcement is mediated by increased extracellular dopamine levels in the forebrain. This neurochemical effect was thought to require inhibition of dopamine reuptake, but cocaine is still reinforcing even in the absence of the dopamine transporter. Here, we demonstrate that the rapid elevation in dopamine levels and motor activity elicited by cocaine involves α1 receptor activation within the ventral midbrain. Activation of α1 receptors increases dopaminergic neuron burst firing by decreasing the calcium-activated potassium channel current (SK), as well as elevates dopaminergic neuron pacemaker firing through modulation of both SK and the hyperpolarization-activated cation currents (I_h). Furthermore, we found that cocaine increases both the pacemaker and burst-firing frequency of rat ventral-midbrain dopaminergic neurons through an α1 adrenergic receptor-dependent mechanism within the ventral tegmental area and substantia nigra pars compacta. These results demonstrate the mechanism underlying the critical role of α1 adrenergic receptors in the regulation of dopamine neurotransmission and behavior by cocaine.
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INTRODUCTION

Like other abused substances, many of the behavioral effects of cocaine arise from its ability to increase extracellular dopamine levels in forebrain regions (Hurd *et al*, 1989; Reith *et al*, 1997; Wise, 2004). It is commonly assumed that this elevation of extracellular dopamine arises solely from the inhibition of dopaminergic transporters in terminal regions (Licata *et al*, 1995). However, cocaine also increases both dopamine-release probability in anesthetized animals (Venton *et al*, 2006) and the firing rate of dopaminergic neurons in awake animals (Koulchitsky *et al*, 2012). The latter effect appears paradoxical because of the actions of cocaine on dopaminergic transporters in the midbrain, which increases somatodendritic dopamine levels (Chen and Reith, 1994) that in turn activate autoreceptors to decrease firing in brain slices (Lacey *et al*, 1987) or anesthetized animals (Koulchitsky *et al*, 2012). Therefore, it is likely that other neurotransmitter systems contribute to the enhancement of dopamine neurotransmission by cocaine. Consistent with this notion, mice lacking the dopaminergic transporter still self-administer cocaine (Rocha *et al*, 1998), and exhibit increases in dopamine levels in response to the psychostimulants (Carboni *et al*, 2001). Identifying these additional effects of cocaine on dopamine transmission is essential to understand the full mechanism of cocaine reinforcement, and provide novel therapeutic targets for the treatment of substance-abuse disorders.

In addition to its direct actions on dopamine transmission, cocaine also elevates extracellular norepinephrine levels by inhibiting the norepinephrine transporter (Reith *et al*, 1997) and increasing dopamine levels in dopamine transporter-deficient mice (Carboni *et al*, 2001), thus implicating norepinephrine signaling in psychostimulant-mediated modulation of dopamine function (Weinshenker and Schroeder, 2007). Indeed, a number of dopamine-dependent behaviors elicited by cocaine involve noradrenergic signaling, including conditioned place preference (Jasmin *et al*, 2006), escalated cocaine intake by self-administration (Zhang and Kosten, 2007; Wee *et al*, 2008), and reinstatement of cocaine seeking (Zhang and Kosten, 2005). Noradrenergic nuclei project to the ventral midbrain (Jones *et al*, 1977; Phillipson, 1979; Simon *et al*, 1979) and establish synaptic connections onto dopaminergic neurons (Liprando *et al*, 2004; Mejias-Aponte *et al*, 2009). Lesions of noradrenergic nuclei decrease dopamine release in projection areas (Lategan *et al*, 1992), whereas stimulation of noradrenergic nuclei excites the dopaminergic neurons in a manner that is attenuated by systemic administration of α1 adrenergic receptor antagonists (Collingridge *et al*, 1979; Grenhoff and Svensson, 1993; Grenhoff *et al*, 1995). However, the locus of action and the mechanism by which norepinephrine signaling mediates the effects of cocaine remain unknown. Therefore, we tested the mechanism by which cocaine mediates increased locomotor activity and

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increased dopaminergic neuron activity via α1 adrenergic neurotransmission in the midbrain.

MATERIALS AND METHODS

Animals

All experiments were approved by the University of Washington and University of Texas at San Antonio Institutional Animal Care and Use Committees in accordance with National Institute of Heath guidelines.

In vitro Recordings

Experiments were conducted on Sprague-Dawley rats of either sex with a median 21 (range 14–90) days old. The animals were anesthetized with a lethal dose of ketamine/xylazine and decapitated. The brains were quickly removed and placed into an ice-cold, oxygenated cutting solution containing (in mM): 110 cholineCl, 2.5 K Cl, 1.25 NaH_2PO_4, 4 $MgCl_2$, 2 $CaCl_2$, 10 dextrose, 25 $NaHCO_3$, 1.3 ascorbic acid, 2.4 sodium pyruvate, and 0.05 glutathione. Horizontal slices (240 μm) containing the ventral tegmental area (VTA) and substantia nigra pars compacta were obtained using a vibrating tissue slicer (Microm HM 650 V). The slices were then transferred to an incubation chamber containing warm (35 °C) artificial cerebral spinal fluid (ACSF) for one hour prior to recordings and then stored at room temperature. The slices were transferred to a recording chamber for the experiments where they were submerged in oxygenated ACSF. The ACSF was equilibrated with 95% O_2–5% CO_2, had a of pH 7.2 and contained (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 10 dextrose, 25 $NaHCO_3$, 1.3 ascorbic acid, and 2.4 sodium pyruvate. The slices were superfused with ACSF at a rate of 2 ml/min by a gravity feed system and all experiments were conducted at 34–36 °C.

While the majority of neurons were recorded using perforated-patch and whole-cell recordings, some neurons were recorded using the cell-attached technique. No differences were detected among the recording techniques in terms of spontaneous frequency and this data was pooled. The cells were visualized using gradient contrast illumination through a 40x water-immersion lens on an Olympus BX51 upright microscope. Patch pipettes were pulled from borosilicate glass (o.d. = 1.5 mm, i.d. = 0.84 mm) using a P-97 Flaming/Brown electrode puller (Sutter Instruments). Pipettes were filled with a solution containing (in mM): 138 K-gluconate, 10 Hepes, 0.0001 $CaCl_2$, 0.2 EGTA, 4 NaATP, 0.4 NaGTP, and 2 $MgCl_2$, with an osmolarity of 270–275 mOsm adjusted to a pH of 7.3 with KOH. Perforations were accomplished by adding the antibiotic gramicidin D (dissolved in DMSO, 100 μg/ml of internal solution) to the internal solution. A final tip resistance between 4–8 MΩ was used for both perforated-patch and whole-cell recordings. Iontophoresis of aspartate was used to evoke bursts. A positive holding current (8–10 nA) was applied to sharp electrodes with a typical tip resistance of 30–70 MΩ. 1 M aspartate (pH 8.0) was ejected from the pipette near the soma using an ejection current typically between −30 and −50 nA (duration 100–200 ms). All experiments involving iontophoresis were conducted in the presence of the AMPA receptor antagonist, NBQX. Recordings were made using a Multiclamp 700B amplifier (Molecular Devices). Signals were digitized at 15–30 kHz and saved to a hard drive for analysis using the software program, Axograph X (Axograph Scientific).

Dopaminergic neurons were identified by having a slow spontaneous-firing rate (<10 Hz), a long action potential duration (>1.5 ms), and a large hyperpolarization-activated cation current (I_h) current in response to hyperpolarizing pulses. The presence of a large mGluR1-mediated hyperpolarization to aspartate iontophoresis (Morikawa *et al*, 2003) was also used to properly identify dopaminergic neurons (Marino *et al*, 2001). Drugs were applied to the slice by superfusion at the indicated concentration, NBQX (25 μM), phenylephrine (PE; 10 μM), cyclopiazonic acid (20 μM), apamin (100 nM), prazosin (100 nM), and ZD 7288 (30 μM). All drugs were obtained from Tocris Biosciences or Sigma-Aldrich.

Action potentials were detected using an amplitude threshold, and spike frequency was calculated as the reciprocal of the inter-spike interval. A burst was defined as a series of action potentials that occurred in a 1 s time-window after the onset of iontophoresis. All effects are given in terms of mean ± SE of the mean. Significance was tested using a two-tailed Student's *t*-test and a *p*-value of <0.05, unless otherwise stated.

In vivo Recordings

Experiments were conducted on adult Sprague-Dawley rats weighing between 250 and 350 g at the time of recording. Rats were anesthetized with urethane (1.8 g/kg) through intraperitoneal injections and installed into a stereotaxic frame. The wounds and points of contact were treated with 2% lidocaine ointment. Body temperature was maintained at 37 °C by a heating pad. After scalp removal, small holes above substantia nigra pars compacta and VTA (AP: −5.0; ML: 1.0/−1.0; DV: −6.5 to −8.0) were drilled for insertion of a recording electrode. Attached to the recording electrode was another glass micropipette (~200–300 μm from the recording tip) that was used to pressure eject (5–30 p.s.i.) the α1 adrenergic receptor agonist, PE. Single-unit extracellular recordings of a midbrain dopaminergic neuron were established. Dopaminergic neurons were identified by the following criteria: a slow firing rate (<10 spikes/sec) and a duration greater than 1.1 ms from the onset of the spike to the negative trough. At the end of some experiments, recording sites were marked with injection of pontamine sky blue. A dose–response curve (0, 10, 30, 100 mM) was performed to determine the extent of excitation induced by PE application upon the spontaneous activity of dopaminergic neurons. Spontaneous activity was defined as all tonic activity with an interspike interval duration greater than 100 ms. A burst was defined as a collection of action potentials with the first interspike interval having a duration of less than 80 ms and the terminating action potential immediately following an interspike interval duration greater than 160 ms (Grace and Bunney, 1983). Statistical analysis was performed using the software program GraphPad Prism (Graphpad Software). All effects are given in terms of mean ± SE of the mean. Significance was tested using a one-tailed Student's *t*-test and a *p*-value of <0.05.

Fast-Scan Cyclic Voltammetry

Twelve male Sprague-Dawley rats (~300 g) underwent voltammetry electrode implantation surgeries. Rats were anesthetized with isofluorane and holes were drilled in the skull for anchor screws, a Ag/AgCl reference electrode, recording electrodes, and guide cannula. The reference electrode was implanted, carbon fiber electrodes were lowered bilaterally into the nucleus accumbens shell (relative to bregma: AP 1.5 mm; ML ± 0.6 mm; DV −7.5 mm), a bilateral guide cannula was placed above the VTA (AP −5.6 mm; ML ± 0.5 mm, DV −7.0 mm), and all components were secured with cranioplastic cement. Animals were allowed to recover for at least three weeks before intravenous jugular catheter surgeries, followed by a minimum of 1 week of recovery before experimental manipulations commenced. The location of electrodes and guide cannula were later verified histologically (Supplementary Figure S1).

Rats were exposed to a single habituation session prior to experimental manipulations. These sessions consisted of attaching the rat to the voltammetry recording station (see below) where they received a 3 mg/kg intravenous cocaine infusion (~0.275 ml). Rats that would receive systemic injections of prazosin would also be given an intraperitoneal injection of saline 15 min prior to the cocaine infusion. Prazosin or appropriate controls were administered in a counterbalanced design and a minimum of 1 day separated experimental sessions. For systemic intraperitoneal injections, prazosin was dissolved in 10% methanol/90% water at a stock concentration of 1.5 mg/ml. Systemic injections of prazosin were given 15 min prior to cocaine infusions at doses of 0, 0.3, 1, and 3 mg/kg. Intra-VTA injectors extended 1 mm past the end of the guide cannula. Intra-VTA injections were administered 15 min prior to the 3 mg/kg intravenous cocaine infusion at a volume of 0.5 μl and were visually monitored to ensure successful injection of either vehicle (water) or 200 ng prazosin.

During voltammetry recording sessions, the chronically-implanted carbon-fiber microelectrodes were connected to a head-mounted voltammetric amplifier for dopamine detection by fast-scan cyclic voltammetry as described in detail elsewhere (Clark *et al*, 2010; Willuhn *et al*, 2010). In brief, the potential applied to the carbon fiber was ramped from −0.4 V (*vs* Ag/AgCl) to +1.3 V and back at a rate of 400 V/s during a voltammetric scan and was held at −0.4 V between scans, which repeated at a frequency of 10 Hz throughout the session. Voltammetric data analysis was carried out using software written in LabVIEW and low-pass filtered at 2000 Hz. Dopamine was isolated from the voltammetric signal using chemometric analysis (Heien *et al*, 2005) using a standard training set of stimulated dopamine release detected by chronically implanted electrodes as performed previously (Wanat *et al*, 2010). Dopamine concentration was estimated on the basis of average post-implantation sensitivity of electrodes (Stuber *et al*, 2008; Clark *et al*, 2010). Data were smoothed using a 0.5 s moving average. The locomotor activity score was calculated as the sum of the number of laps around the operant box plus the number of stereotypic behaviors observed when the rat was stationary. Peak dopamine release and activity counts were calculated for the 100 s following the cocaine infusion. Statistical analyses were carried out in Prism (GraphPad, CA) and used repeated measures one-way analyses of variance (ANOVA) followed by *post-hoc* Dunnet's test for systemic injections and paired *t*-tests for intra-VTA injections. Data are presented as mean ± SEM.

RESULTS

Effect of α1 Adrenergic Receptor Antagonism on Cocaine-Induced Increase in Dopamine Release and Locomotor Activity *In Vivo*

We first examined how systemic administration of the specific α1 adrenergic receptor antagonist, prazosin, affects cocaine-evoked increases in dopamine release and locomotor activity in rats. Using fast-scan cyclic voltammetry, we found that an intravenous infusion of 3 mg/kg cocaine elicited a robust increase in dopamine release in the nucleus accumbens shell that was dose-dependently attenuated by intraperitoneal injections of prazosin (peak cocaine-evoked dopamine release: vehicle = 249.8 ± 47.6 nM, 0.3 mg/kg prazosin = 191.8 ± 35.3 nM, 1.0 mg/kg prazosin 141.1 ± 23.3 nM, 3 mg/kg prazosin = 118.4 ± 20.1 nM; one-way repeated measures ANOVA: $F_{3,30} = 7.3$, $p < 0.001$ with *post-hoc* Dunnett's test relative to vehicle: $p < 0.01$ for 1 mg/kg and $p < 0.001$ for 3 mg/kg prazosin, $n = 11$, Figure 1a, b). The effect of prazosin on dopamine release was paralleled by a corresponding decrease in the locomotor-stimulating effects of cocaine (average cocaine-evoked activity counts: vehicle = 39.7 ± 7.0, 0.3 mg/kg prazosin = 33.8 ± 7.0, 1.0 mg/kg prazosin 23.3 ± 1.8, 3 mg/kg prazosin = 21.6 ± 3.5; one-way repeated measures ANOVA: $F_{3,21} = 4.1$, $p < 0.05$ with *post-hoc* Dunnett's test relative to vehicle: $p < 0.05$ for 1 mg/kg and 3 mg/kg prazosin, $n = 8$, not shown). We next assessed whether the systemic effects of prazosin could be mimicked by local infusions of prazosin into the VTA, which sends a dense dopamine projection to the nucleus accumbens shell. Indeed, the systemic effect of prazosin was recapitulated by intra-VTA injections of prazosin, which significantly reduced cocaine-evoked dopamine release in the nucleus accumbens shell (peak cocaine-evoked dopamine release: vehicle = 176.3 ± 21.4 nM, 200 ng prazosin = 64.2 ± 18.2 nM, paired *t*-test $p < 0.01$, $n = 5$, Figure 1c), as well as cocaine-elicited motor activity (average cocaine-evoked activity counts: vehicle = 45.7 ± 5.5, 200 ng prazosin = 31.5 ± 7.6, paired *t*-test $p < 0.05$, $n = 3$; see online video). Therefore, cocaine-mediated activation of α1 adrenergic receptors within the ventral-midbrain results in elevated dopamine levels and increased locomotor activity.

Effect of α1 Adrenergic Receptor Activation on Dopaminergic Neuron Firing Activity *In Vivo*

Given the effect of local midbrain α1 adrenergic receptor antagonism on cocaine-evoked locomotor activity and dopamine release, we next assessed whether local activation of α1 adrenergic receptors by itself could affect the firing activity of dopaminergic neurons *in vivo*. Single unit recordings were conducted on dopaminergic neurons from anesthetized rats (Figure 2a). Pressure ejection of the α1 agonist, PE(30 mM) resulted in increasing the background (pacemaker) firing frequency from a mean firing rate of

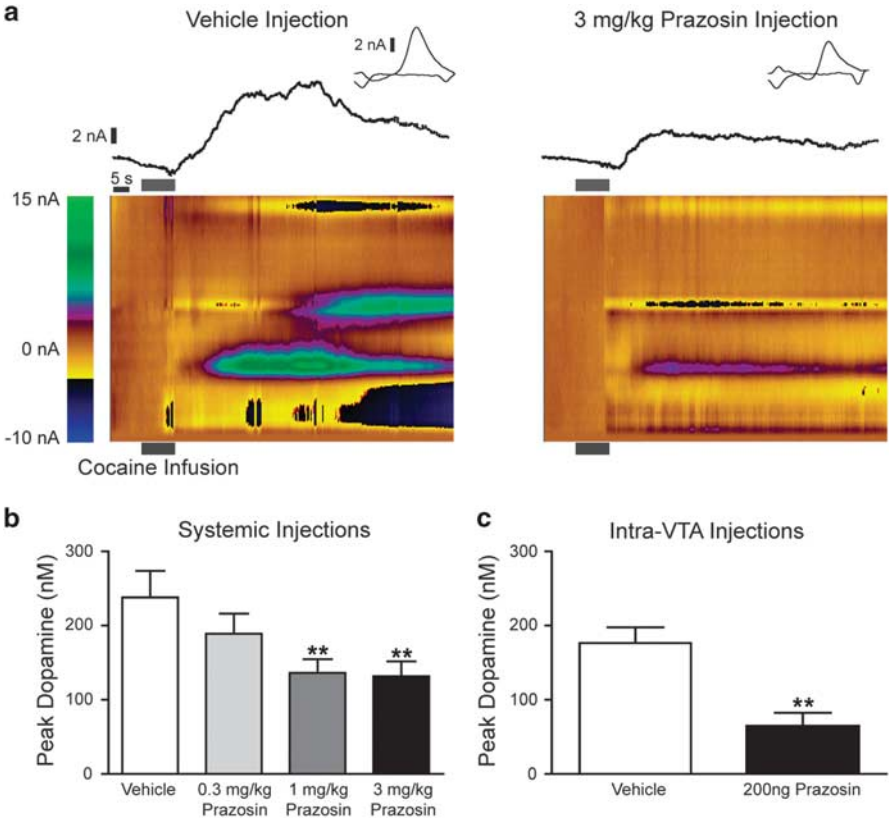

Figure 1 Antagonizing α1 adrenergic-receptor activity attenuates cocaine-evoked dopamine release and locomotor activity. (a) Representative voltammetry recordings using a chronically-implanted electrode in the nucleus accumbens shell from a single rat on different days. Systemic administration of prazosin reduces dopamine efflux elicited by intravenous infusion of 3 mg/kg cocaine. (Top) Current at the peak oxidation potential of dopamine is plotted as a function of time with the inset showing the cyclic voltammogram, identifying the current as dopamine. (Bottom) Two-dimensional pseudocolor plots of cyclic voltammograms over time. (b) Systemic injections of prazosin dose-dependently attenuate cocaine-evoked dopamine release in the nucleus accumbens shell. (c) Similarly, intra-VTA injections of prazosin attenuate cocaine-evoked dopamine release in the nucleus accumbens shell. $^{*}p<0.05$ and $^{**}p<0.01$.

2.64 Hz ± 0.24 to 3.75 Hz ± 0.54 (paired *t*-test, $p<0.01$; $n=10$, Figure 2b, top left). Dopaminergic neurons *in vivo* also fire action potentials in bursts (Grace and Bunney, 1984; Lodge and Grace, 2007). Activation of α1 adrenergic receptors significantly increased burst activity in dopaminergic neurons. The mean intra-burst frequency increased from 24.37 ± 3.4 to 27.85 ± 3.1 Hz (paired *t*-test, $p<0.01$; $n=7$, Figure 2b, top right). Also, the percent of spikes that occurred in the burst compared with spontaneous firing increased from 27.3 to 58% (paired *t*-test, $p<0.05$; $n=7$, Figure 2b, bottom left) and the rate of incidence of bursts was increased from 0.19 ± 0.08 to 0.48 ± 0.12 bursts/second (paired *t*-test, $p<0.05$; $n=7$, Figure 2b, bottom right). The mean burst length in terms of both time and spike number was not significantly changed (paired *t*-test, $p>0.05$; $n=7$) during α1 adrenergic receptor activation. Therefore, activating α1 adrenergic receptors increases pacemaker and burst firing of dopaminergic neurons *in vivo*.

Mechanism of Adrenergic Receptor Excitation of Dopaminergic Neurons

To determine the mechanism underlying the α1 adrenergic receptor-mediated excitation of dopaminergic neurons, we examined how PE (10 μM) affected both the pacemaker and burst-firing activity of dopaminergic neurons *in vitro*. PE (10–20 min wash) increased the mean spontaneous-firing frequency by 19.6% from 2.96 ± 0.20 Hz up to 3.54 ± 0.15 Hz (paired *t*-test, $p<0.0001$, $n=20$, Figure 3a). The regularity of firing, as assessed by the mean coefficient of variation of the inter-spike interval (CV), was unaltered by PE application (baseline 0.156 ± 0.09, PE 0.10 ± 0.07, paired *t*-test, $p>0.05$; $n=20$). Notably, the peak afterhyperpolarization potential (AHP) was reduced by 11.73% from −58.15 ± 1.74 mV during whole-cell recordings in control conditions to −51.33 ± 1.56 mV in the presence of PE (paired *t*-test, $p<0.001$; $n=8$, Figure 3b). Dopaminergic neuron bursts are mediated by NMDA receptor activation (Chergui *et al*, 1993; Overton and Clark, 1997; Morikawa *et al*, 2003; Deister *et al*, 2009). Bursts of action potentials were generated every 30 s by iontophoretic application of the glutamate analog, aspartate, onto the recorded neuron in the presence of 25 μM NBQX, an AMPA receptor antagonist. Under these conditions 10 μM PE was also sufficient to increase the mean intra-burst firing frequency from 12.48 ± 0.79 to 14.22 ± 1.08 Hz (paired *t*-test, $p<0.01$, $n=12$, Figure 3c, d), as well as the maximum intra-burst firing frequency from 15.49 ± 1.15 Hz to 17.55 ± 1.52 Hz ($p<0.01$, $n=12$). The increase in the intra-burst firing frequency was not accompanied by a significant increase in the mean

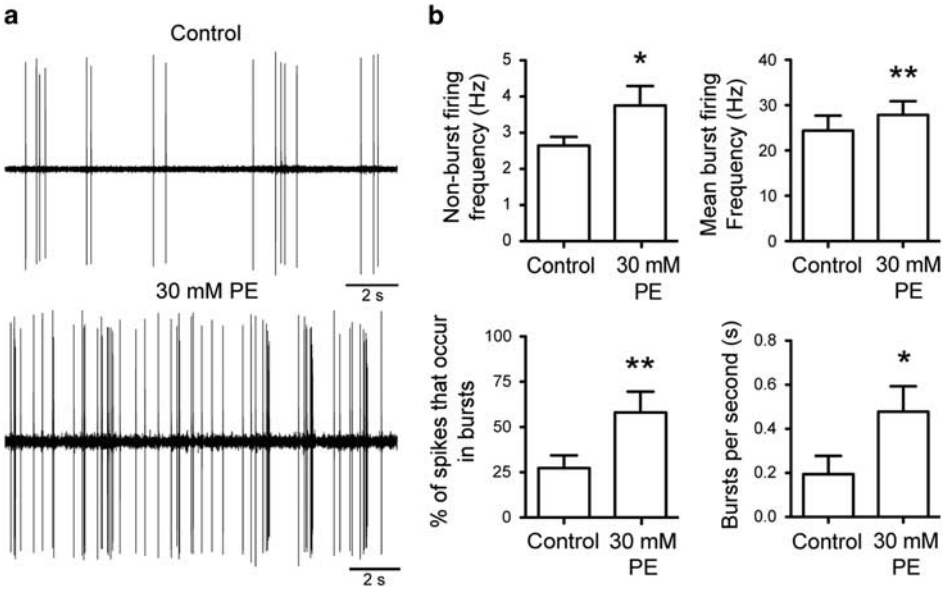

Figure 2 Activation of α1 adrenergic receptors *in vivo* increases both spontaneous firing frequency and inter-burst firing frequency. (a) Single-unit extracellular recordings of DA neurons were established before (top) and after (bottom) local α1 adrenergic receptor activation by 30 mM PE. (b) Summarized data showing that local pressure ejection of PE increases spontaneous single-spike activity (upper left; $n = 10$). Activation of α1 adrenergic receptors also increased burst activity ($n = 7$) with increases in the mean intra-burst firing frequency (upper right), percent of spikes that occur in bursts (lower left), and the number of bursts per second (bottom right). *$p < 0.05$; **$p < 0.01$.

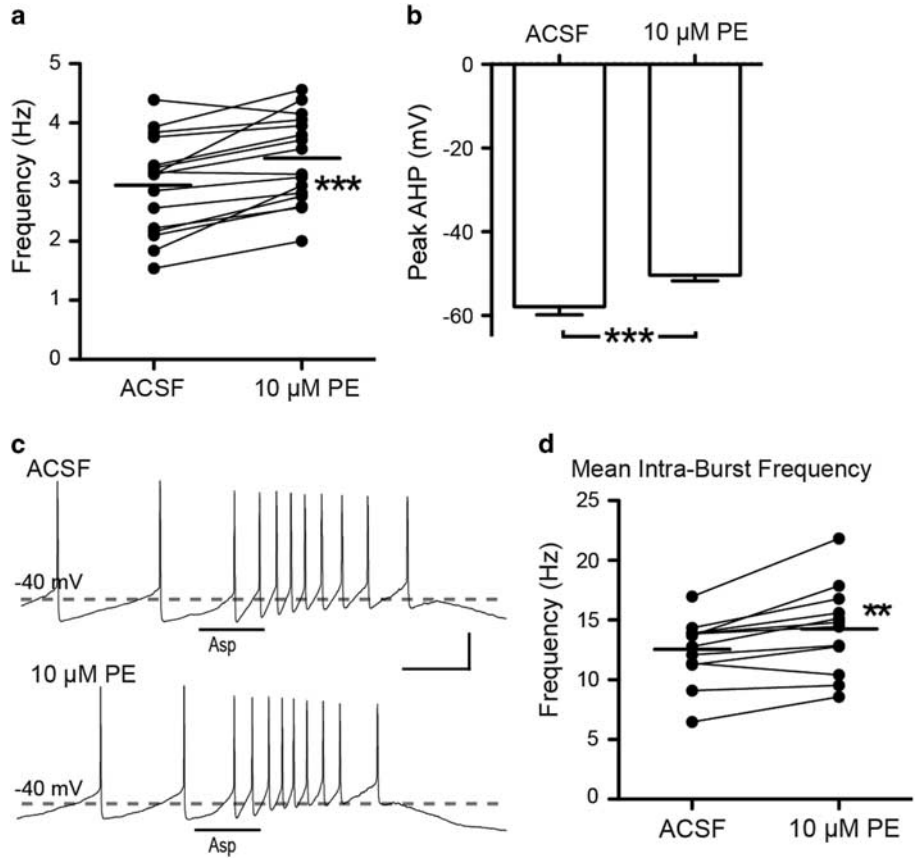

Figure 3 Activation of α1 adrenergic receptors *in vitro* (bath application) increases both spontaneous and intra-burst firing frequency, and decreases the peak amplitude of the single-spike afterhyperpolarization. (a) Summarized data showing the PE-mediated increase in firing frequency (19.6% increase in frequency; $n = 20$). (b) Summarized data displaying the reduction in the peak afterhyperpolarization (11.7% decrease in peak AHP amplitude; $n = 8$) observed with PE. (c. Example traces zoomed in on the burst (aspartate iontophoresis (black bar, Asp)) illustrating the increase in intra-burst firing frequency. (d). Summarized data showing the PE-mediated increase in the mean intra-burst firing frequency (14% increase; $n = 12$). Scale bars are 20 mV (ordinate), 0.2 s (abscissa). Horizontal bars in population data indicate means of the samples. **$p < 0.01$; ***$p < 0.001$.

number of action potentials composing the burst (paired *t*-test, $p>0.05$).

As activating α1 adrenergic receptors increased dopaminergic neuron firing that was accompanied by a reduction in the AHP, we performed voltage-clamp experiments to examine whether currents involved in the generation of the AHP were modulated by α1 adrenergic receptor activation. We first focused on the I_h, which can regulate the size of the AHP and the firing rate of dopaminergic neurons (Neuhoff *et al*, 2002). The I_h was evoked by a hyperpolarizing voltage step from −60 mV to −120 mV for 1 s (Figure 4a). In control conditions the hyperpolarizing pulse evoked a −895.1 ± 95.85 pA inward current that was increased by application of PE to −1003 ± 121.4 pA (paired *t*-test; $p<0.05$; $n=9$; Figure 4b). The effect of PE also persisted while blocking action potentials with TTX (TTX = −537.2 ± 60.77 pA, TTX + PE = −569.0 ± 61.08 pA; paired *t*-test; $p<0.01$; $n=14$; not shown), indicating that the effect of PE is postsynaptic. In separate experiments, the α1 adrenergic receptor antagonist, prazosin (100 nM), reversed the increase in I_h by PE (control = −826.1 ± 105.4 pA, PE = −877.9 ± 115.5 pA, PE + prazosin = −777.5 ± 104.7 pA; one-way repeated measures ANOVA: $F_{2,12}=13.69$, $p<0.05$ with *post-hoc* Tukey's test relative to control: $p<0.05$ for PE, $p>0.05$ for PE + prazosin; relative to PE: $p<0.05$ for PE + prazosin, $n=7$; not shown), demonstrating the specificity of PE. Furthermore, when neurons were voltage clamped at −60 mV, 10 μM PE application evoked a mean inward current of −31.77 ± 5.13 pA ($n=7$). Blocking I_h current with 30 μM ZD 7288 (Neuhoff *et al*, 2002) significantly inhibited the inward current evoked by PE exposure (−7.70 ± 2.53 pA, $p<0.05$, unpaired *t*-test, $n=7$; Supplementary Figure S2). Together, these experiments demonstrate that α1 adrenergic receptor stimulation in dopaminergic neurons elicits an inward current arising from activation of an I_h current.

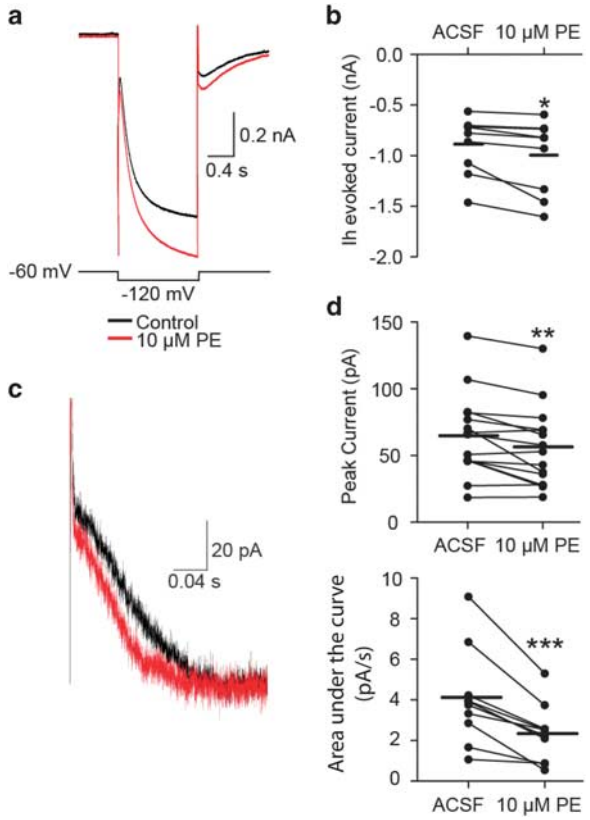

Figure 4 Activation of α1 adrenergic receptors *in vitro* (bath application) increases the hyperpolarization-activated cation current (I_h) and decreases the calcium-activated potassium current (SK). (a) Representative traces during control (black) and after bath application of PE (red). (b) Summary data showing that PE increases I_h (12.1% increase; $n=9$; Left). (c) Representative trace of an SK current during control (black) and after bath application of PE (red). (d) Summary data showing that PE decreases the peak apamin-sensitive component of the SK current (14% decrease; $n=15$; Middle), and also the area under the curve (42.7% decrease; $n=10$; Right). *$p<0.05$; **$p<0.01$; ***$p<0.001$.

The AHP in dopaminergic neurons is also composed of hyperpolarizing currents such as calcium activated K^+ (SK) currents (Shepard and Bunney, 1991; Harris *et al*, 1992; Nedergaard, 2004), which have been shown to be modulated by activation of noradrenergic receptors in other types of neurons (Sherman and Koch, 1986; McCormick and Williamson, 1989; Wang and McCormick, 1993). Therefore, we investigated how activation of α1 adrenergic receptors affects SK currents in dopaminergic neurons. Dopaminergic neurons were held at −60 mV and an action potential was evoked by applying a 2 ms (or 10 ms when in TTX) depolarizing pulse (40–70 mV from holding), producing an outward current (Figure 4c) that was sensitive to the application of the specific SK antagonist, apamin (100 nM) (Sah, 1996). Activation of α1 adrenergic receptors resulted in a 14% reduction in the peak SK current (from 64.80 ± 7.95 pA to 55.71 ± 7.78 pA; paired *t*-test, $p<0.01$; $n=15$; Figure 4d top) and a 43% reduction in the area under the curve (from 4.07 ± 0.74 pA/s to 2.33 ± 0.45 pA/s; paired *t*-test, $p<0.001$; $n=10$; Figure 4d bottom). The effect of PE on SK also persisted while blocking action potentials with TTX (TTX = 1.873 ± 0.652 pA/s, TTX + PE = 1.468 ± 0.586 pA/s; paired *t*-test; $p<0.05$; $n=8$; not shown), indicating a postsynaptic effect. Therefore, activation of α1 adrenergic receptors on dopaminergic neurons elicits an increase in I_h current and a decrease in SK current.

Given that activation of α1 adrenergic receptors modulates I_h and SK currents, we next assessed whether these currents were required for the α1 agonist, PE, to increase the spontaneous firing rate of dopaminergic neurons. Blocking the I_h current with 30 μM ZD 7288 did not attenuate the ability of PE to increase the pacemaker firing frequency of dopaminergic neurons (Figure 5a). Specifically, 10 μM PE increased the mean spontaneous firing frequency by 25.1% from 2.43 ± 0.57 to 3.04 ± 0.41 Hz in the presence of ZD 7288 (paired *t*-test, $p<0.05$, $n=6$, Figure 5a, b). Activation of α1 adrenergic receptors also increased the firing frequency by 24.1% when SK currents were blocked with 100 nM apamin (apamin: 2.97 ± 0.42 Hz; apamin + PE: 3.69 ± 0.48 Hz, paired *t*-test, $p<0.01$, $n=7$, Figure 5c, d). Therefore, the magnitude of the α1 adrenergic-mediated increase in firing was not significantly altered by either the I_h or SK antagonist ($p>0.05$). However, PE was ineffective at modulating the spontaneous firing of dopaminergic neurons in the presence of both ZD 7288 and apamin (ZD 7288 + apamin: 3.53 ± 0.64 Hz; ZD 7288 + apamin + PE: 3.52 ± 0.50 Hz, paired *t*-test, $p>0.05$; $n=7$, Figure 5e, f), illustrating that both the I_h and SK currents are required for the increase in dopaminergic neuron pacemaker activity by α1 adrenergic receptor activation.

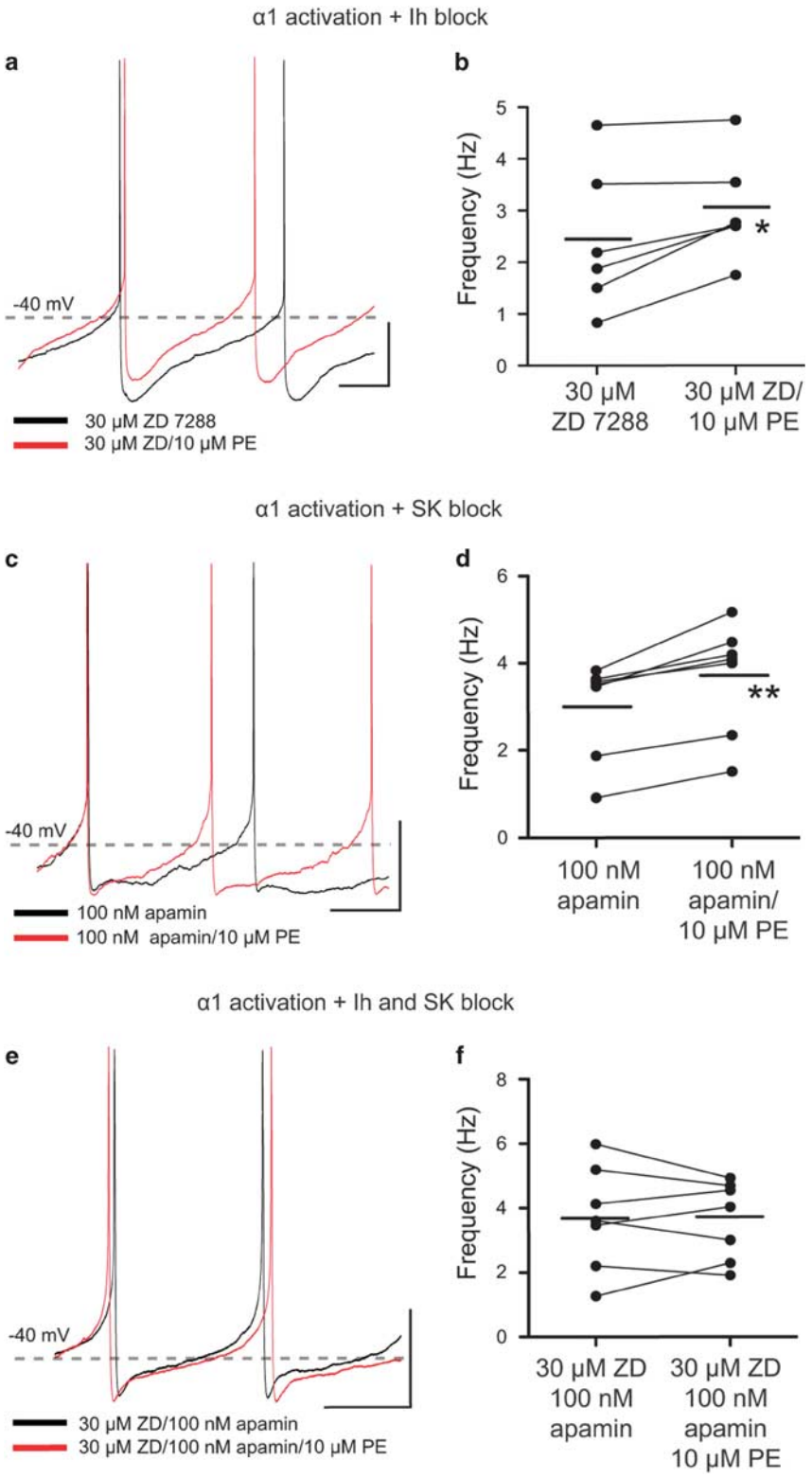

Figure 5 Activation of α1 adrenergic receptors *in vitro* (bath application) increases the spontaneous firing frequency of DA neurons through modulation of both I_h and SK currents. (a) Example traces of action potentials recorded during antagonism of I_h (30 μM ZD 7288; black) and after bath application of 10 μM PE and 30 μM ZD 7288 (red). (b) Summarized data showing that the PE-mediated increase in firing frequency (25.1% increase; $n = 6$) is still evident after blocking I_h with 30 μM ZD 7288. (c) Example of action potentials obtained following blockade of SK channels with 100 nM apamin (black) and after application of 10 μM PE and 100 nM apamin (red). (d) Summarized data showing that blocking SK does not eliminate the PE-mediated increase in spontaneous firing frequency (24.1% increase; $n = 7$). (e) Overlay of action potentials obtained after both 30 μM ZD 7288 and 100 nM apamin (black) and after application of 10 μM PE, 30 uM ZD 7288, and 100 nM apamin (red). (f) Summarized data showing that blocking both I_h and SK eliminates the PE-mediated increase in spontaneous firing frequency ($n = 7$). Scale bars are 20 mV (ordinate), 0.2 s (abscissa). Horizontal bars in population data indicate the means of the samples. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

We next ascertained whether the increase in dopamine burst firing by the α1 adrenergic receptor involves I_h and/or SK currents. Blocking the I_h current did not alter the effect of PE on either the mean (ZD 7288: 17.41 ± 1.55 Hz, ZD 7288 + PE: 20.79 ± 1.51 Hz, $p<0.01$; paired *t*-test, $n=10$, Figure 6a, b) or the maximum (ZD 7288: 21.57 ± 1.97 Hz, ZD 7288 + PE: 25.72 ± 1.88 Hz, paired *t*-test, $p<0.05$, $n=10$) intra-burst frequency, demonstrating that the α1 adrenergic receptor-mediated increase in I_h is not singularly responsible for the increase in burst firing frequency. In contrast, blocking SK currents with apamin prevented the α1 adrenergic receptor-mediated increase in both the mean intra-burst firing rate (apamin: 19.98 ± 1.90 Hz, apamin + PE: 19.91 ± 1.71 Hz, paired *t*-test, $p>0.05$; $n=13$, Figure 6c, d), and the maximum intra-burst firing frequency (apamin: 24.39 ± 2.25 Hz, apamin + PE: 23.95 ± 2.04 Hz, paired *t*-test, $p>0.05$; $n=13$). Activation of α1 adrenergic receptor reduces internal calcium stores in dopaminergic neurons (Paladini *et al*, 2001) that in turn can modulate SK currents (Berridge, 1998; Cui *et al*, 2007). Elimination of the internal calcium stores with 20 μM cyclopiazonic acid (Morikawa *et al*, 2000) did not affect how activation of α1 adrenergic receptors increased both spontaneous and burst firing frequency (22.4% increase in frequency; $n=9$; Supplementary Figure S3). These findings collectively demonstrate the SK current is solely responsible for the increase in burst firing elicited by α1 adrenergic receptor activation, and that this effect is independent of calcium-induced calcium release from internal stores.

Effect of α1 Adrenergic Receptor Antagonism on Cocaine-Induced Increase in Dopaminergic Neuron Firing Activity *In Vitro*

After characterizing the mechanism by which α1 adrenergic receptor activation affects the firing of dopaminergic neurons, we finally examined whether cocaine similarly increased pacemaker and burst firing of dopaminergic neurons of the substantia nigra pars compacta and VTA in an α1 adrenergic receptor dependent manner. Whole-cell and perforated-patch recordings were performed in the presence of the dopamine D2 receptor antagonist, eticlopride (10 μM) (Lacey *et al*, 1990; Shi *et al*, 2000). Cocaine (10 μM) increased the spontaneous pacemaker firing frequency of dopaminergic neurons by 15.7%, from 1.97 ± 0.189 Hz to 2.28 ± 0.22 Hz (paired *t*-test, $p<0.01$; $n=14$; Figure 7a). Blocking α1 adrenergic receptors with prazosin (100 nM) eliminated the cocaine-mediated excitation

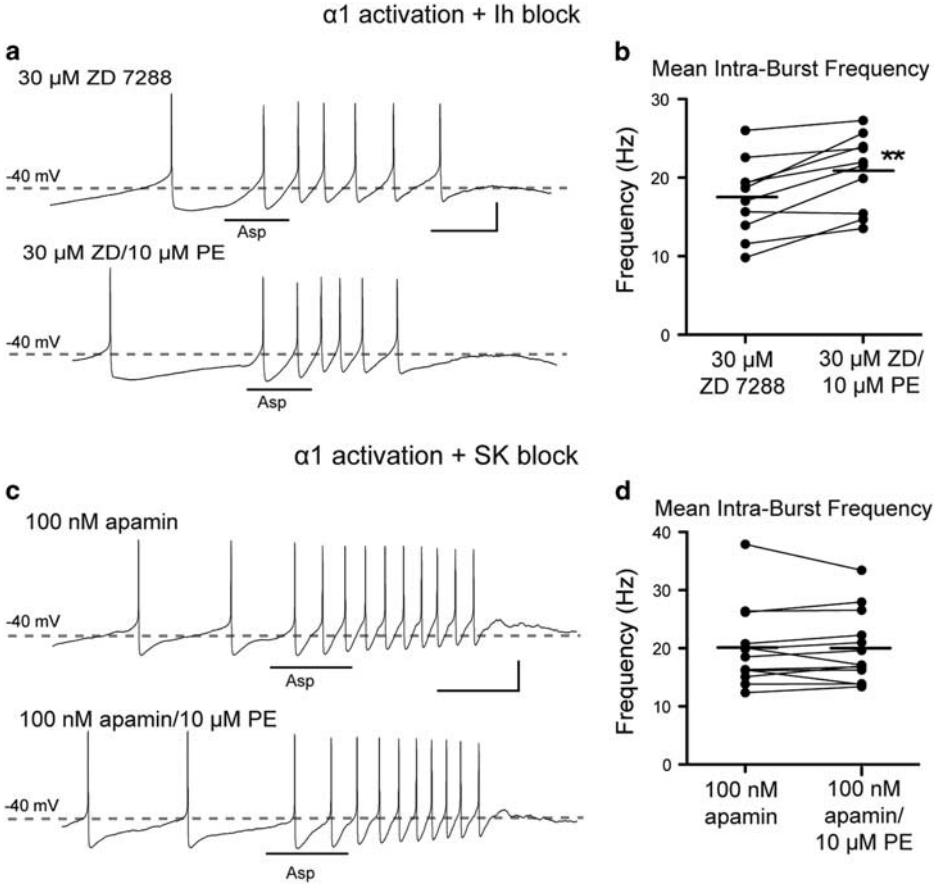

Figure 6 Activation of α1 adrenergic receptors *in vitro* (bath application) increases intra-burst firing frequency by modulating SK currents. (a) Example traces of a burst (aspartate iontophoresis (black bar, Asp)), illustrating that the PE-mediated increase in the intra-burst firing frequency is still evident after blocking I_h with 30 μM ZD 7288. (b) Summarized data showing that the increase in burst firing frequency is not blocked by ZD 7288 (19.4% increase; $n=10$). (c) Burst traces showing that the increase in the intra-burst firing frequency owing to PE is blocked in the presence of SK channel antagonists. (d) Summarized data ($n=13$) showing that blocking SK channels eliminates the increase in mean intra-burst frequency. Scale bars are 20 mV (ordinate), 0.2 s (abscissa). Horizontal bars in population data indicate means of the samples. $^{**}p<0.01$.

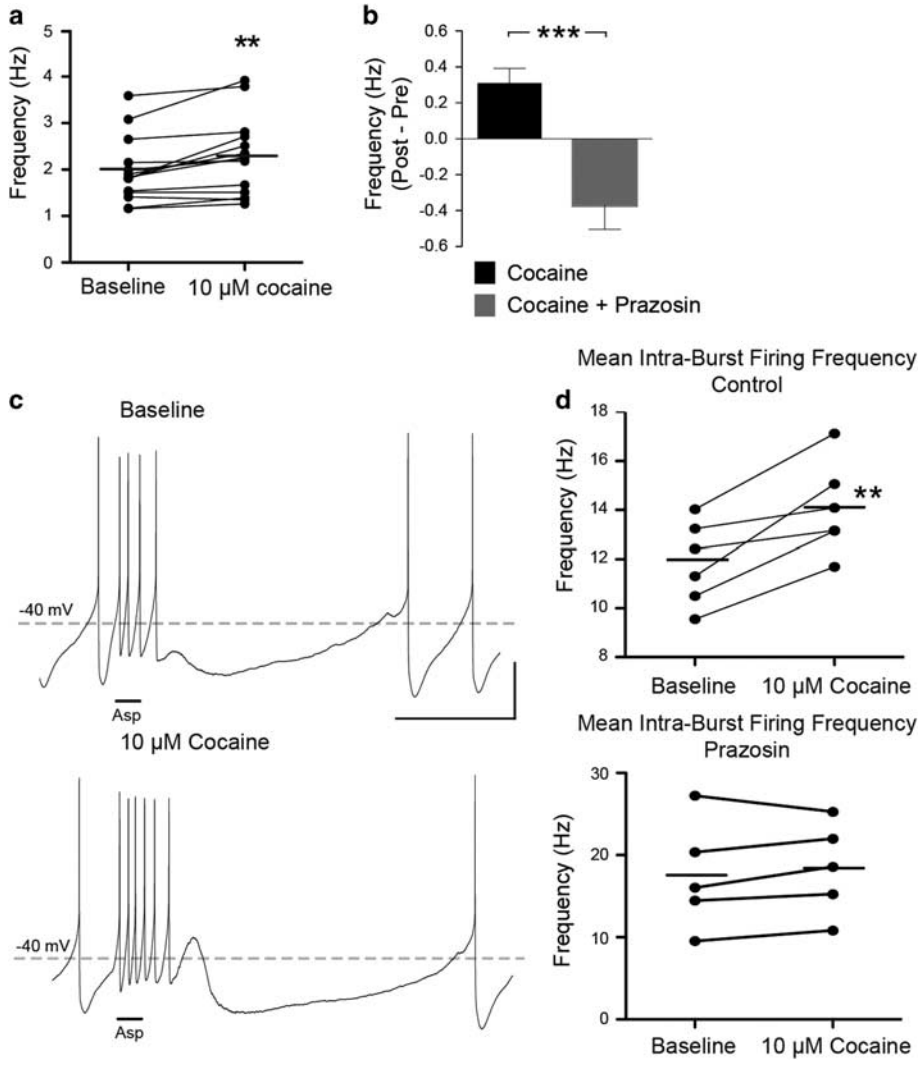

Figure 7 *In vitro* cocaine administration (bath application) activates α1 adrenergic receptors, leading to an increase in spontaneous and burst firing frequency. (a) Summarized data showing that cocaine elicits an excitation in spontaneous activity (15.7% increase in frequency; $n = 14$). (b) Summarized data showing that the α1 adrenergic receptor antagonist, prazosin (100 nM; $n = 15$), blocks the increase in spontaneous activity observed during cocaine administration. (c) Example traces of a burst evoked with aspartate iontophoresis (black bar, Asp), illustrating that cocaine increases the intra-burst firing frequency. (d) Summarized data showing that application of 10 μM cocaine increases mean (18.7% increase; $n = 6$) intra-burst firing frequency. This increase in excitability is eliminated with prazosin ($n = 5$). Scale bars are 20 mV (ordinate), 0.2 s (tonic) and 1.0 s (burst; abscissa). Horizontal bars in population data indicate the means of the samples. $^{**}p < 0.01$; $^{***}p < 0.001$.

(prazosin: 2.60 ± 0.25 Hz, prazosin + cocaine: 2.23 ± 0.23 Hz, unpaired *t*-test, $p < 0.0001$, $n = 14$, Figure 7b), demonstrating that cocaine increases the pacemaker firing frequency of dopaminergic neurons in an α1 adrenergic receptor-dependent mechanism. As psychostimulants induce an increase in dopamine concentration even in animals lacking the dopamine transporter (Carboni *et al*, 2001) we determined whether specifically blocking the norepinephrine transporter mimicked the effect of cocaine. The specific norepinephrine transporter blocker, nisoxetine (100 nM), also increased the pacemaker firing frequency (control = 1.93 ± 0.18 Hz, nisoxetine = 2.23 ± 0.24 Hz; paired *t*-test, $p < 0.05$, $n = 11$; not shown).

Using aspartate iontophoresis to induce bursts, bath application of cocaine increased the mean intra-burst firing frequency of dopaminergic neurons from 11.84 ± 0.70 Hz to 14.05 ± 0.77 Hz (paired *t*-test, $p < 0.01$, $n = 6$, Figure 7c, d) and the maximum intra-burst firing frequency from 14.16 ± 0.64 Hz to 18.40 ± 1.3 Hz (paired *t*-test, $p < 0.01$; $n = 6$). Furthermore, blocking α1 adrenergic receptors with prazosin eliminated the cocaine-induced increase in both mean intra-burst firing frequency (prazosin: 17.50 ± 2.98 Hz, prazosin + cocaine: 18.37 ± 2.53 Hz, paired *t*-test, $p > 0.05$; $n = 5$, Figure 7d) and the maximum intra-burst firing frequency (prazosin: 20.55 ± 3.39 Hz, prazosin + cocaine: 22.38 ± 3.00 Hz, paired *t*-test, $p > 0.05$; $n = 5$). These findings demonstrate that cocaine excites dopaminergic neuron firing activity by activating α1 adrenergic receptors. The increase in firing activity elicited by cocaine was not significantly different from the increase in firing activity evoked by PE ($p > 0.05$). Blocking the norepinephrine transporter with nisoxetine (100 nM) mimicked the effect of cocaine and also increased iontophoresis-induced bursts (burst mean

frequency control = 14.36 ± 2.22 Hz, nisoxetine = 20.61 ± 3.36 Hz; paired *t*-test, $p < 0.05$; $n = 8$; burst maximum frequency control = 19.09 ± 2.85 Hz, nisoxetine = 25.75 ± 3.50 Hz; paired *t*-test, $p < 0.05$; $n = 8$; not shown). Thus, both cocaine and α1 adrenergic receptor agonists were similarly sufficient to increase the pacemaker and burst firing frequency of dopaminergic neurons. Collectively, our *in vitro* and our *in vivo* experimental results provide a mechanism by which cocaine increases dopamine neurotransmission via activation of α1 adrenergic receptor within the midbrain.

DISCUSSION

The cocaine-evoked increase in dopamine levels and corresponding elevation in locomotor activity was dependent upon α1 adrenergic receptor signaling within the ventral midbrain. In this study we demonstrate a novel action of cocaine to increase both pacemaker and burst firing of dopaminergic neurons by activating α1 adrenergic receptors within the ventral midbrain. α1 adrenergic-dependent activation of dopaminergic neurons was observed *in vivo* and *in vitro*, with the elevation in pacemaker firing frequency mediated by a decrease in SK current, and an increase in I_h current, whereas the effect on intra-burst firing frequency was due solely to a decrease in SK current. Therefore, in addition to its established role in blocking dopamine reuptake, cocaine increases dopamine levels and increases locomotor activity through activation of α1 adrenergic receptors within the ventral midbrain.

Evidence from genetic and pharmacological manipulations of the noradrenergic system demonstrate the involvement of adrenergic signaling in mediating the behavioral effects of psychostimulants (Weinshenker and Schroeder, 2007). Inhibiting norepinephrine synthesis prevents the development of conditioned place preference for cocaine (Jasmin *et al*, 2006) and reduces cocaine-seeking behavior (Schroeder *et al*, 2010). Blocking α1 adrenergic receptor signaling decreases psychostimulant-induced locomotor activity (Darracq *et al*, 1998; Drouin *et al*, 2002), reduces cocaine intake after previous drug administration (Zhang and Kosten, 2007; Wee *et al*, 2008), and attenuates the reinstatement of cocaine seeking behaviors (Zhang and Kosten, 2005). Several of these changes in behavior can be mediated by the effect of norepinephrine throughout the brain (eg Mitrano *et al*, 2012, 2014). However, many drug-dependent behaviors require activation of the mesolimbic dopamine system (Kalivas, 1995; Kalivas and McFarland, 2003; Zweifel *et al*, 2008), which indicates that the effect of antagonizing α1 adrenergic signaling on cocaine-elicited behaviors could also be mediated by modulation of the dopamine system. Indeed, α1 adrenergic receptor-knockout mice exhibit lower extracellular dopamine levels and do not exhibit the characteristic psychostimulant-induced rise in dopamine concentration (Auclair *et al*, 2002). The modulation of dopamine neurotransmission by α1 adrenergic receptors occurs on α1-expressing neurons in prefrontal cortex that project to dopaminergic neurons in the midbrain (Darracq *et al*, 1998; Ventura *et al*, 2003; Pascucci *et al*, 2007; Ventura *et al*, 2007). Our findings demonstrate that α1 adrenergic receptor activation within the ventral midbrain is also sufficient to excite dopaminergic neural activity. While α1 adrenergic receptor activation does occur on presynaptic terminals within VTA (eg Velásquez-Martínez *et al*, 2014), our results show that postsynaptic receptor activation on dopaminergic neurons also contributes to the rapid elevation of dopamine levels and motor activity elicited by acute cocaine administration. Furthermore, these results provide a mechanism for how α1 adrenergic receptor antagonism attenuates cocaine-dependent behaviors that engage the mesolimbic dopamine system.

Experiments performed *in vitro,* and in anesthetized rodents *in vivo* demonstrate that acute cocaine administration decreases dopaminergic neuron activity (Branch *et al*, 2013; Einhorn *et al*, 1988; Lacey *et al*, 1990; Koulchitsky *et al*, 2012). This effect has been attributed to increased catecholamine levels activating the D2 autoreceptors, thereby hyperpolarizing dopaminergic neurons and inhibiting their rate of firing (Lacey *et al*, 1987). Indeed, when D2 autoreceptors are desensitized by chronic cocaine administration, cocaine no longer has the degree of inhibition on dopaminergic neuron activity and is even capable of evoking an increase in the number of spontaneously active dopaminergic neurons along with an increase in firing rates when examined in anesthetized animals (Henry *et al*, 1989). Furthermore, preventing D2 autoreceptor activation can unmask excitatory pharmacological effects in both anesthetized preparations (Shi *et al*, 2000) and in awake, behaving animals (Brown and Kiyatkin, 2008). In this regard, we examined the effect of cocaine *in vitro* when blocking D2 autoreceptors and found an increase in the frequency of spontaneous and burst firing in dopaminergic neurons. This increase in firing is consistent with a study demonstrating that cocaine increases dopaminergic neuron firing activity in awake rodents (Koulchitsky *et al*, 2012). Anesthesia silences the noradrenergic system (Mason and Angel, 1983; Kushikata *et al*, 2011), which could account for the discrepancy between recordings in anesthetized and awake rats, where dopaminergic neuronal activity is either decreased or increased, respectively, by cocaine. These studies suggest a complex interplay in dopaminergic neurons between the excitatory actions of α1 adrenergic signaling and the inhibitory actions of D2 autoreceptors.

Cocaine increases dopamine levels by inhibiting reuptake through the dopaminergic transporter. However, this mechanism is insufficient to account for all of the actions of cocaine on the dopaminergic system. Cocaine also modulates dopamine transmission via activation of α1 adrenergic receptors within the ventral midbrain. Antagonizing α1 adrenergic receptor signaling has yielded promising results in both preclinical (Zhang and Kosten, 2005; Forget *et al*, 2010; Verplaetse *et al*, 2012), and clinical settings (Simpson *et al*, 2009) for substance use disorders. The current results offer a neurobiological mechanism of this therapeutic benefit, and provide additional targets by identifying the ionic basis of this action.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)