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Title Page:

Acute and Chronic CRF_1 Receptor Blockade Inhibits Cocaine-induced Dopamine Release: Correlation with Dopamine Neuron Activity

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Running Title Page:

Running Title: CRF_1 blockade inhibits cocaine-induced DA release.

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Abbreviations

5-HT	5-hydroxy-tryptamine
Acb	Nucleus Accumbens
ACTH	Adrenocorticotropic Hormone
ANOVA	Analysis of Variance
CRF	Corticotropin Releasing Factor
CRF_1	Corticotropin Releasing Factor Type 1 Receptor
CRF_2	Corticotropin Releasing Factor Type 2 Receptor
DA	Dopamine
EPSC	Excitatory Post-synaptic Potential
GABA	γ-aminobutyric acid
HPLC	High Performance Liquid Chromatography
i.c.v.	Intracerebroventricular
NMDA	N-methyl-D-aspartate
PBS	Phosphate Buffered Saline
PPTg	Pedunculopontine Tegmental Nucleus
SEM	Standard Error of the Mean
VTA	Ventral Tegmental Area

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Abstract

Corticotropin releasing factor (CRF) is a neuropeptide associated with the integration of the physiological and behavioral responses to stress. More recently, CRF_1 receptor antagonists have been shown to decrease cocaine self-administration and inhibit stress-induced reinstatement of cocaine-seeking behavior. The exact mechanisms underlying this effect are not clear. Based on the large literature demonstrating an association between dopaminergic neurotransmission and reward-related behavior, the aim of the present study was to examine the effects of acute vs. chronic CRF_1 receptor blockade on mesencephalic dopamine (DA) neuron activity (determined by *in vivo* extracellular recordings) and extracellular DA levels in the nucleus accumbens (Acb) (using *in vivo* microdialysis). In addition, the effect of CRF_1 receptor antagonism on cocaine-induced DA overflow in the Acb was examined and correlated with DA neuron activity in the ventral tegmental area (VTA). Acute (but not chronic) CRF_1 receptor blockade (by CRA-0450) was found to significantly increase DA neuron population activity without affecting burst firing, average firing rate or Acb DA levels. In addition, both acute and chronic CRF_1 receptor antagonism significantly reduced cocaine-stimulated DA overflow in the Acb, and this reduction was correlated with an attenuated cocaine-induced inhibition of DA population activity. Taken as a whole, these data demonstrate that, although DA neuron population activity exhibits tolerance to chronic CRF_1 receptor antagonism (by CRA-0450), tolerance does not develop to the selective inhibition of cocaine-induced DA release (in the Acb) and as such may be of benefit in the treatment of cocaine addiction.

Introduction

Corticotropin releasing factor (CRF) is a peptide neurotransmitter found in high abundance throughout the neuraxis where its actions include control of the secretion of adrenocorticotropic hormone (ACTH) from the pituitary in response to stressful stimuli (Chalmers et al., 1996; Carrasco and Van de Kar, 2003; Rivier et al., 2003). Recent research into the neurobiological actions of CRF has implicated this peptide in a variety of biological actions including anxiety (Takahashi, 2001), depression (Reul and Holsboer, 2002), cardiovascular control (Briscoe et al., 2000), appetite regulation (Hope et al., 2000) and central reward processing (Sarnyai et al., 2001). The CRF system has also been shown to play a role in the actions of drugs of abuse, in particular cocaine (Goeders and Guerin, 2000; Lu et al., 2001; Sarnyai et al., 2001). This was originally suggested following the demonstration that acute cocaine administration induces elevated plasma ACTH and cortisol levels via an action dependent on central CRF receptor activation (Rivier and Lee, 1994), while other reports have shown that cocaine administration significantly alters extrahypothalamic markers of central CRF neurotransmission (Sarnyai et al., 1993; Zhou et al., 1996; Gardi et al., 1997). These findings have led to the suggestion that the central CRF system may play a role in cocaine self-administration (Sarnyai et al., 2001); this is further supported by a recent study reporting that CRF_1 receptor blockade (by CP-154,526) can decrease cocaine self-administration without affecting responding for food (Goeders and Guerin, 2000). In addition to decreasing cocaine self-administration, CRF receptor antagonists reduce stress-induced reinstatement of drug seeking behavior and attenuate cocaine withdrawal-induced anxiety (Erb et al., 1998; Shaham et al., 1998; Basso et al., 1999; Erb and Stewart, 1999).

Although there is a vast literature examining the central processes underlying the actions of CRF in altered behavioral states such as anxiety and stress (for review see: Bale and Vale, 2004), there have been fewer reports on how CRF affects central reward processing. Nonetheless, CRF

and CRF receptors are distributed throughout brain regions associated with the control of emotive processing and hormone regulation, as well as throughout regions centrally involved in the actions of abused drugs such as the ventral mesencephalon, amygdala, bed nucleus of the stria terminalis and prefrontal cortex (Behan et al., 1996). Moreover, immunohistochemical investigations have demonstrated that CRF_1 receptors are localized on a proportion of neurons throughout the ventral tegmental area (VTA) that may synthesize DA and project to forebrain regions such as the nucleus accumbens (Acb) (Sauvage and Steckler, 2001). These findings suggest that CRF may be able to modulate DAergic neurotransmission throughout the rat mesolimbic system. For these reasons, the present study utilized a potent and selective CRF_1 receptor antagonist, CRA-0450 (Chaki et al., 2004), to examine the effects of acute vs. chronic CRF_1 receptor blockade on mesencephalic DA neuron activity (determined by *in vivo* extracellular recordings) and DA overflow in the Acb (using *in vivo* microdialysis). In addition, the effect of CRF_1 receptor antagonism on cocaine-induced DA overflow in the Acb was examined and correlated with DA neuron activity in the VTA.

Methods

All experiments were performed in accordance with the guidelines outlined in the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Survival Surgery

Chronic i.c.v drug/vehicle administration (CRA-0450: 0.3µg/0.5µl/hour – 14 days: vehicle: 0.5µl/hour – 14 days) was performed using Alzet micro-osmotic minipumps (model 2002) in conjunction with Alzet brain infusion kits (Type I). Briefly, male Sprague Dawley rats (250-400g) were anaesthetized with a mixture of ketamine (80mg/kg i.p.) and xylazine (12mg/kg i.p.) and placed in a stereotaxic apparatus. A burr hole was drilled overlying the third ventricle (bregma: AP -0.8mm, ML +1.5mm) and an L-shaped (28 gauge) cannula was lowered into the third ventricle (5mm ventral of skull surface) and fixed in place with dental cement covering the pedestal of the cannula and two small anchor screws. A pocket was made subcutaneously between the scapulae and the attached minipump was implanted, the skin sutured and treated with a topical antibacterial/analgesic ointment (Pramoxine HCl 1% w/w: Neomycin 0.35% w/w). All rats received post-operative analgesia for 2 days in the form of Children's Tylenol Syrup mixed with softened rat chow. Rats were returned to a central animal facility and monitored daily for a period of two weeks before subsequent experimentation.

Extracellular DA neuron recordings

Male Sprague Dawley (250-400g) rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic apparatus. Anesthesia was maintained by supplemental administration of chloral hydrate as required to maintain suppression of limb compression withdrawal reflex and a core body temperature of 37^{o}C was sustained by a thermostatically controlled heating pad. For acute administration of drug/vehicle, a subset of rats were implanted with a 23 gauge injection cannula (AP -0.8, ML +1.5, DV -5.0 mm from bregma) that was fixed in place with dental cement. A burr hole was drilled overlying the contralateral VTA (AP -5.3, ML -0.8 mm from bregma) and dura carefully removed. Extracellular recording microelectrodes (2.0mm OD borosilicate glass capillary tubing, 1μm tip diameter, impedance 5-10 MΩ) were filled with 2M NaCl containing 2% w/v pontamine sky blue. The electrode signal was amplified, filtered and discriminated from noise using a combination amplification and window discrimination unit (WDR-420: Fintronics), and data was acquired, stored and analyzed using custom designed computer software (Neuroscope).

Extracellular microelectrodes were lowered into the VTA (-6.5 to -9.0mm ventral of brain surface) using a hydraulic microdrive and the activity of the population of DA neurons was determined by recording from spontaneously active DA neurons encountered while making 5-9 vertical passes, separated by 200μm, throughout the VTA. Spontaneously active DA neurons were identified using previously established electrophysiological criteria (Grace and Bunney, 1983) and once isolated, their activity was recorded for 2-3 mins. Three parameters of activity were measured: i) population activity (defined as the number of spontaneously active DA neurons recorded per electrode track), ii) basal firing rate, and (iii) the proportion of action potentials occurring in bursts (defined as the occurrence of two spikes with an interspike interval of <80ms, and the termination of the burst defined as the occurrence of an interspike interval of >160ms; (Grace and Bunney, 1984). At the cessation of the experiment, the recording site was

marked via iontophoretic ejection of Pontamine sky blue dye from the tip of the recording electrode (30μA constant current: 20-30 mins).

Microdialysis

Concentric microdialysis probes with 2mm exposed membrane (CMA/12: 20kDa permeability, 0.5mm outer diameter) were implanted in the contralateral Acb (AP +1.6, ML -1.2, DV -7.5 mm from bregma) and perfused at a rate of 2μl/min with artificial cerebrospinal fluid (composition in mM: NaCl 124, KCl 2.2, KH_2PO_4 1.3, $MgSO_4$ 1.3, $NaHCO_3$ 20 & $CaCl_2$ 2.0). After 60-90 min equilibration, samples were collected at 20 min intervals into an equal volume (40μl) of HPLC mobile phase (modified MD-TM 70-1332: pH = 4.69, 50mg/L sodium octyl sulfate) to minimize transmitter degradation. Samples were immediately injected into an HPLC system via an autosampler (ESA model 540) and separated on an analytical column (ESA: 150 x 3.2 mm, MD-150/RP-C18) perfused with HPLC mobile phase (0.6ml/min). DA was detected by oxidation using an ESA Coulcoum II detector equipped with a guard cell (+375mV) and a dual electrode analytical cell (ESA 5014; E1 = -175mV, E2 = +185mV). Chromatographic data were acquired and analyzed using an ESA 501 data system. The HPLC system was calibrated at the start of each experiment using external DA standards. For acute experiments, three basal samples were collected before the administration of CRA-0450 (5ug/5ul i.c.v) or vehicle (5ul i.c.v) and drug effects were measured for 40 mins after which cocaine was injected (10mg/kg i.p.) and data recorded for a further 140mins. For chronic experiments, 4 baseline samples were collected before administration of cocaine (10mg/kg i.p.) and subsequent analysis for 140mins.

Histology

At the cessation of the experiment, rats were killed by an overdose of anesthetic, decapitated and their brains removed, fixed for at least 48 hours (8%w/v paraformaldehyde in phosphate buffered saline: PBS), and cryoprotected (25%w/v sucrose in PBS) until saturated. Brains were then sectioned (60μm thick coronal sections), mounted onto gelatin-chrom alum coated slides and stained with cresyl violet for histochemical verification of electrode dye markers, probe and cannula sites with reference to a stereotaxic atlas (Paxinos and Watson, 1986).

Analysis

Electrophysiological analysis of DA neuron activity was performed using custom-designed computer software (Neuroscope) and microdialysis samples were analyzed against DA standards of known concentration using the ESA501 software package (ESA). All data are represented as the mean ± the standard error of the mean (SEM). Differences in electrophysiological recording parameters or DA content between CRA-0450/vehicle and cocaine/saline treated rats were examined using a two-way analysis of variance (ANOVA) followed by a Dunnett's post-hoc test. All statistics were calculated using the SigmaStat software program (Jandel).

Drug administration

CRA-0450 was administered directly into the lateral ventricle for both acute and chronic studies. Central administration was preferred over a peripheral route for a number of reasons: 1) to curtail the known peripheral effects of CRF_1 receptor blockade and to minimize effects on peripheral ACTH and cortisol secretion, and 2) to maintain a continuous, steady-state level of drug in the brain which is beneficial in determining its potential for inducing tolerance.

The doses utilized for i.c.v. administration were based on brain levels of CRA-0450 reported after oral administration of an effective dose of compound (Chaki et al., 2004). These levels were in the order of 2500ng/g or ~4-5µg/brain. Moreover, chronic infusions of 0.3µg/hour were employed to result in a daily dose of ~7µg/day, slightly higher than that administered acutely to compensate for degradation/clearance of the drug.

Materials

CRA-0450 (1-[8-(2,4-dichlorophenyl)-2-methylquinolin-4-yl]-1,2,3,6-tetrahydropyridine-4-carboxamide benzenesulfonate: (Chaki et al., 2004) was a gift from Taisho Pharmaceuticals (Japan) and was dissolved by sonication in 2% v/v Tween 80 in dH_20. Cocaine hydrochloride was dissolved in saline and purchased from Sigma (USA), whilst all other chemicals and reagents were of either analytical or laboratory grade and purchased from various suppliers.

Results

Effect of CRA-0450 administration on DA neuron activity

These data were collected from 430 neurons recorded in 51 rats. Rats that received either acute or chronic vehicle infusions (n=14 rats; 125 neurons) exhibited an average of 1.06 ± 0.09 spontaneously active DA neurons per electrode track (cells/track), with an average firing rate of 4.25 ± 0.18 Hz and 26.7 ± 2.4% of action potentials occurring in bursts (Figs 1-3), consistent with previous findings (Floresco et al., 2003). Acute i.c.v infusion of the CRF_1 receptor antagonist, CRA-0450 (n=6 rats; 73 neurons) resulted in a significant increase in DA neuron population activity (Acute Vehicle: 1.04 ± 0.28 cells/track; Acute CRA: 1.86 ± 0.21 cells/track; $p<0.05$) (Fig 1a). Interestingly, acute CRA-0450 administration alone had no significant effect on either average firing rate (Acute Vehicle: 4.31 ± 0.28 Hz; Acute CRA: 4.06 ± 0.20 Hz) or the percent of action potentials fired in bursts (Acute Vehicle: 25.0 ± 3.4%; Acute CRA: 20.0 ± 2.8%) (Fig 2a, 3a). In contrast, chronic CRA-0450 administration (n=7 rats; 59 neurons) did not significantly influence any of the basal electrophysiological properties measured (Fig 1b, 2b & 3b).

Acute cocaine administration (10mg/kg i.p.) resulted in a significant decrease in DA neuron population activity (Saline: 1.04 ± 0.28 cells/track; Cocaine: 0.43 ± 0.06 cells/track; $p<0.05$) and burst firing (Saline: 25. 0 ± 3.4%; Cocaine: 13.0 ± 3.5%; $p<0.05$) to a similar degree in both acute (n=6; 19 neurons) and chronic (n=6; 23 neurons) vehicle treated rats (Fig 1). In addition, a statistically significant decrease in firing rate was observed in acute vehicle and CRA-0450 treated rats (Fig 2a); however the small magnitude of this effect suggests that it is unlikely to be physiologically relevant and was not observed in chronically treated animals (Fig 2b). On

the other hand, both acute (n=6; 72 neurons) and chronic (n=6; 59 neurons) CRF_1 receptor blockade completely inhibited the effect of cocaine on population activity (Fig 1), without significantly affecting burst firing (Fig 3), suggesting a dissociation between the regulation of these two parameters.

DA microdialysis

As demonstrated in Fig 4, the systemic administration of cocaine (10mg/kg i.p.) induced a robust increase (>150%; $p<0.05$) in Acb DA release in both acute and chronic vehicle treated rats, consistent with previous reports (Lu et al., 2003). In addition, neither acute (5µg/5µl i.c.v.) nor chronic (0.3µg/hour/14days i.c.v.) CRA-0450 administration had any significant effect on basal extracellular DA levels determined by microdialysis (Fig 4). However, both acute and chronic CRF_1 receptor blockade significantly attenuated (by >50%; $p<0.05$) the cocaine-induced increase in Acb DA release (Fig 4).

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Discussion

In the present study, a combined neurochemical/neurophysiological analysis was used to assess the effects of acute vs. chronic CRF_1 receptor blockade on the activity of the midbrain DA system. As such, acute (but not chronic) CRA-0450 administration induced a selective increase in DA neuron population activity, suggestive of a tonic inhibitory role for CRF on the activity of VTA DA neurons. In addition, we report that both acute and chronic CRF_1 receptor blockade potently inhibit cocaine-induced DA overflow in the Acb, which is correlated with a selective attenuation of the effects of cocaine on DA neuron population activity. As such, these data add further weight to the suggestion that the CRF system plays a role in central reward processing.

Effect of CRF_1 receptor antagonism on the midbrain DA system.

The present study demonstrates that the CRF_1 antagonist CRA-0450 selectively increases DA neuron population activity in the VTA, without significantly altering average burst firing or firing rate. This dissociation between the regulation of DA neuron activity states has been investigated previously and demonstrated to be associated with distinct afferent inputs to the VTA (Floresco et al., 2003). As such it has been reported that spontaneous activity in DA neurons is associated with spontaneous membrane depolarizations and subsequent modulation via GABAergic transmission to/within the VTA (Grace, 1987). Therefore, given the results of the present study, it is likely that CRF exerts a tonic modulatory control on population activity either via a direct effect on the DA neuron, or via modulation of GABAergic transmission to/within the ventral mesencephalon. Given the relatively few reports on the effects of extrahypothalamic CRF throughout the CNS, the exact neurochemical processes associated with the CRF_1 receptor

antagonist-induced increase in DA population activity are unclear. However, it has been demonstrated previously that the VTA possesses a high degree of CRF receptor immunoreactivity suggested to be localized to DAergic neurons (Sauvage and Steckler, 2001). In addition, the effects of CRF receptor activation on DA neuron activity have been recently investigated utilizing *in vitro* intracellular recordings (Ungless et al., 2003). These studies reported the presence of a CRF_2 receptor-mediated potentiation of NMDA EPSC's, whereas modulation of CRF_1 receptor activity was without effect. One possible reason for the apparent disparity with the current results is that a large proportion of the afferent inputs to the VTA are severed during slice preparation for *in vitro* recordings. Moreover, DA neurons recorded from slice preparations display significantly different firing patterns and membrane characteristics when compared to those observed *in vivo*. Thus, DA neurons recorded *in vitro* display a significantly higher degree of spontaneous activity and an extremely regular firing rate (Grace, 1987; Grace and Onn, 1989). As such the modulation of population activity by CRA-0450 observed in the present study may not be observed in the slice preparation due to the high degree of spontaneous activity present in that preparation secondary to severing of afferent processes. In addition, the effect of CRA-0450 on population activity observed in the present study may result from an altered modulation of GABAergic afferents to the VTA, which again would not be observed in the *in vitro* preparation.

Although acute CRF receptor blockade significantly increased DA neuron population activity, DA levels in the Acb were not significantly affected by this treatment. Given the previous literature demonstrating the robustness of the correlation between population activity and DA release (determined by *in vivo* microdialysis) (Moore et al., 1998; Floresco et al., 2003), it is likely that the increased population activity observed in the present study may be associated with either a compensatory response to a transient CRA-0450 mediated decrease in Acb DA

release or with an increased activity restricted to a subpopulation of neurons not projecting to the Acb such as those projecting to cortical regions (i.e. prefrontal cortex). Although this could be examined using antidromic activation, stimulation of postsynaptic targets itself is likely to change the baseline activity states of the DA neurons.

Effect of CRF_1 receptor antagonism on cocaine-induced DA release

Since there is increasing literature suggesting a role for CRF in the central actions of abused drugs, including cocaine (Sarnyai et al., 2001), we examined the effect of CRF_1 receptor blockade on the DAergic responses to systemic cocaine administration. It is well known that cocaine significantly increases DA release in terminal regions throughout the rat brain (Kuhar et al., 1991). Moreover, this supraphysiological increase in terminal DA levels induces compensatory changes in mesencephalic DA neurons, such as a decrease in population activity and burst firing, in the direction of normalizing DA levels. Consistent with this, the present study demonstrates that vehicle-treated rats display a robust increase in Acb DA release after systemic cocaine administration and this is correlated with a significant decrease in both population activity and average burst firing. Moreover, both acute and chronic CRF_1 receptor antagonism potently inhibited the effect of cocaine on DA overflow in the Acb, and this was correlated with a reversal of the cocaine-induced decrease in DA neuron population activity. Interestingly CRF_1 receptor blockade did not attenuate the effect of cocaine on burst firing, consistent with the dissociation between the control of population activity and burst firing reported previously (Floresco et al., 2003). Since it has been demonstrated that cocaine not only blocks DA transporters, but also 5-HT and noradrenaline transporters (Ritz et al., 1990; Kuhar et al., 1991), it is possible that the persistent effects of cocaine on DA neuron burst firing are associated with

a lack of effect of CRF on non-DAergic monoamine release, particularly 5-HT and noradrenaline. Indeed, previous studies have demonstrated a potent inhibitory effect of serotonin on the activity of PPTg neurons (Leonard and Llinás, 1994), which have been shown to regulate burst firing in VTA DA neurons (Floresco et al., 2003). This suggests that an increase in 5-HT throughout this region, such as that purportedly induced by cocaine, may lead to a decreased burst firing in the VTA secondary to PPTg inhibition. In addition, it has been demonstrated that systemic 5-HT transport blockade (by fluoxetine) decreases DA neuron activity in the VTA (Prisco and Esposito, 1995). As such it is plausible that the persistent effect of cocaine on burst firing observed in the present study may be associated with an increase in non-DAergic monoamine transmission not affected by CRF_1 receptor blockade.

There is significant evidence demonstrating that the principal effects of cocaine on DAergic transmission result from a pharmacological blockade of the DA transporters (Ritz et al., 1987). The demonstration that acute CRA-0450 administration can inhibit cocaine-induced DA release likely suggests an interaction with the DA transporter. However, this is clearly not a direct pharmacological action since it has been demonstrated that CRA-0450 displays little affinity for any of the monoamine transporters throughout the rat brain (Chaki et al., 2004). In addition, previous studies have demonstrated an inhibition of cocaine-induced behavioral measures and DA release with the acute administration of structurally distinct CRF receptor antagonists (Lu et al., 2003), demonstrating that this effect is attributable to CRF_1 receptor blockade and not a non-specific action of the drug. Remarkably, acute CRF_1 receptor inhibition did not significantly affect basal DA release, consistent with previous observations (Lu et al., 2003), suggesting that CRA-0450 administration leads to a CRF_1 receptor-specific decreased efficacy of cocaine without influencing 'normal' transporter function. Unfortunately given the relatively small literature regarding CRF/DA interactions, the exact mechanisms underlying the

effect of CRF_1 receptor blockade on cocaine-induced DA release and associated changes in neurophysiology are yet to be elucidated.

An important consideration is the recent report that CRA-0450 may also inhibit σ_1 receptors throughout the CNS (Chaki et al., 2004), however it is unlikely that this is associated with the results obtained in the present study for a number of reasons: 1) Previous studies have demonstrated σ_1 receptor inhibition to have no significant effect on DA neuron activity (Ceci et al., 1988) and 2) the inhibitory effects of CRA-0450 on cocaine-induced DA release and subsequent changes in population activity reported in the current study are consistent with previous reports demonstrating a reduction by CRF_1 receptor antagonists of cocaine-induced behavioral changes and DA release (Lu et al., 2003). Therefore, it is likely that the neurochemical/neurophysiological changes observed in the present study are associated with CRF_1 receptor blockade and not with any purported activity at the σ_1 receptor.

Conclusions

Taken as a whole, these data provide the first extensive neurochemical/neurophysiological analysis of the actions of acute and chronic CRF_1 receptor blockade throughout the VTA. More specifically, these data show that acute CRA-0450 administration significantly and selectively increases DA neuron population activity throughout the VTA, suggestive of a tonic CRF-mediated inhibition of DA neuron activity. Furthermore, we report that both acute and chronic CRF_1 receptor blockade significantly attenuates cocaine-induced DA overflow in the Acb, which is correlated with a reversal of the cocaine-induced inhibition of DA neuron population activity. As such these data add further weight to the

suggestion that the CRF system plays a role in central reward processing and moreover suggest that CRF_1 receptor antagonists may provide a new therapeutic approach for the treatment of cocaine addiction.

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Footnotes:

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Legends for Figures:

Figure 1

Effects of acute (A) and chronic (B) CRF_1 receptor blockade (by CRA-0450) on basal (white bars) and cocaine-modulated (black bars) DA neuron population activity. * represents statistically significant difference from control (vehicle alone) ($p<0.05$ 2-way ANOVA: Dunnett's post-hoc) and † represents significant difference between vehicle and CRA treated rats receiving cocaine ($p<0.05$ 2-way ANOVA: Dunnett's post-hoc: n=6-7 rats/group).

Figure 2

Effects of acute (A) and chronic (B) CRF_1 receptor blockade (by CRA-0450) on basal (white bars) and cocaine-modulated (black bars) DA neuron firing rate. * represents statistically significant difference from control (vehicle alone) ($p<0.05$ 2-way ANOVA: Dunnett's post-hoc: n=6-7 rats/group).

Figure 3

Effects of acute (A) and chronic (B) CRF_1 receptor blockade (by CRA-0450) on basal (white bars) and cocaine-modulated (black bars) DA neuron bursting activity. Percentages represent the proportion of spikes that are discharged in a bursting pattern, * represents statistically significant difference from control (vehicle alone) ($p<0.05$ 2-way ANOVA: Dunnett's post-hoc: n=6-7 rats/group).

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Figure 4

Effects of acute (A) and chronic (B) CRF_1 receptor blockade (by CRA-0450: open circles) on cocaine-induced DA release in the Acb compared to vehicle (closed squares). Acute and chronic vehicle data were not significantly different and were pooled. A single arrow denotes acute vehicle/CRA-0450 administration, and a double arrow signifies the time of cocaine administration. † represents significant difference between vehicle and CRA treated rats receiving cocaine ($p<0.05$ 2-way ANOVA: n=6-10 rats/group).

Figure 1

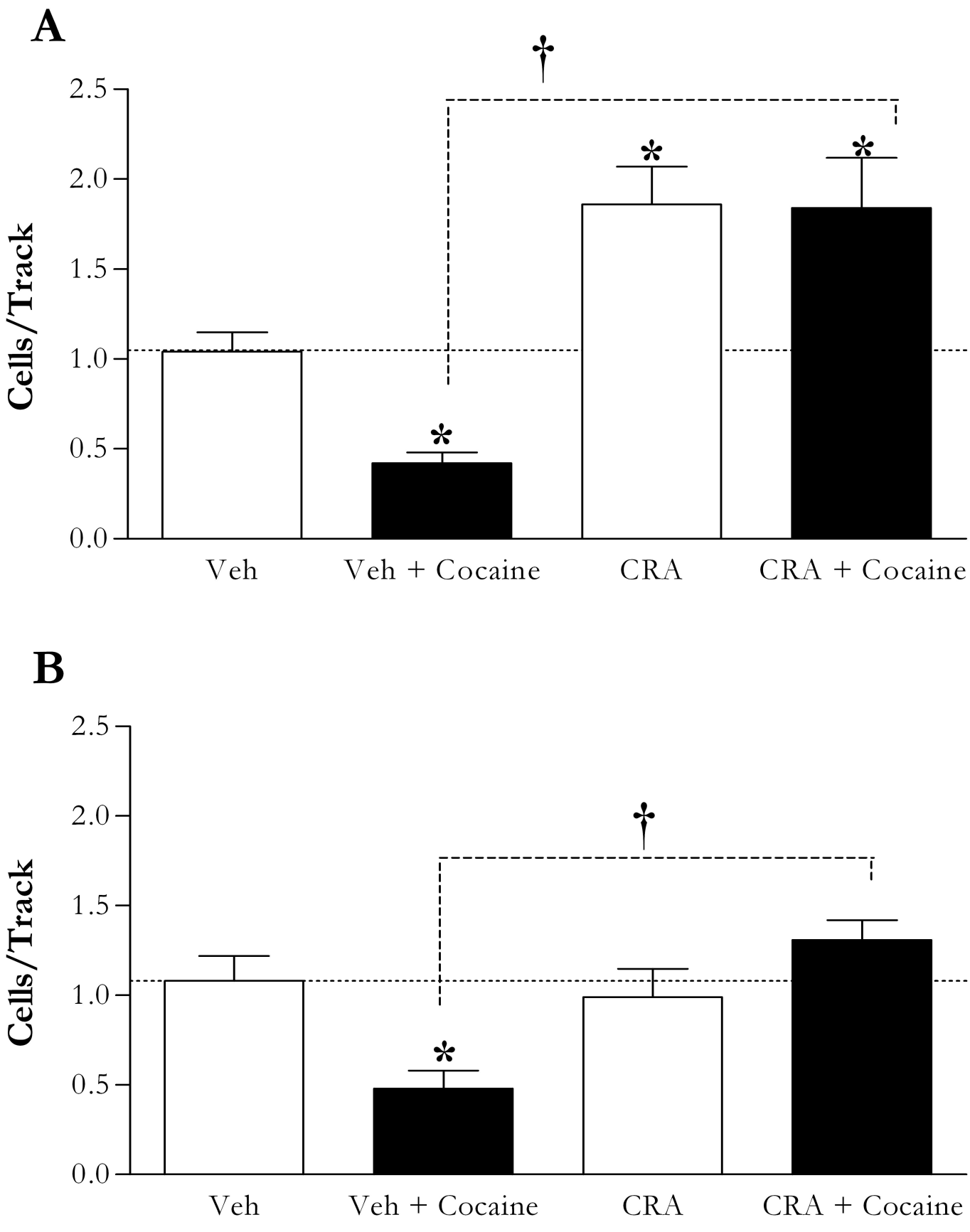

Figure 2

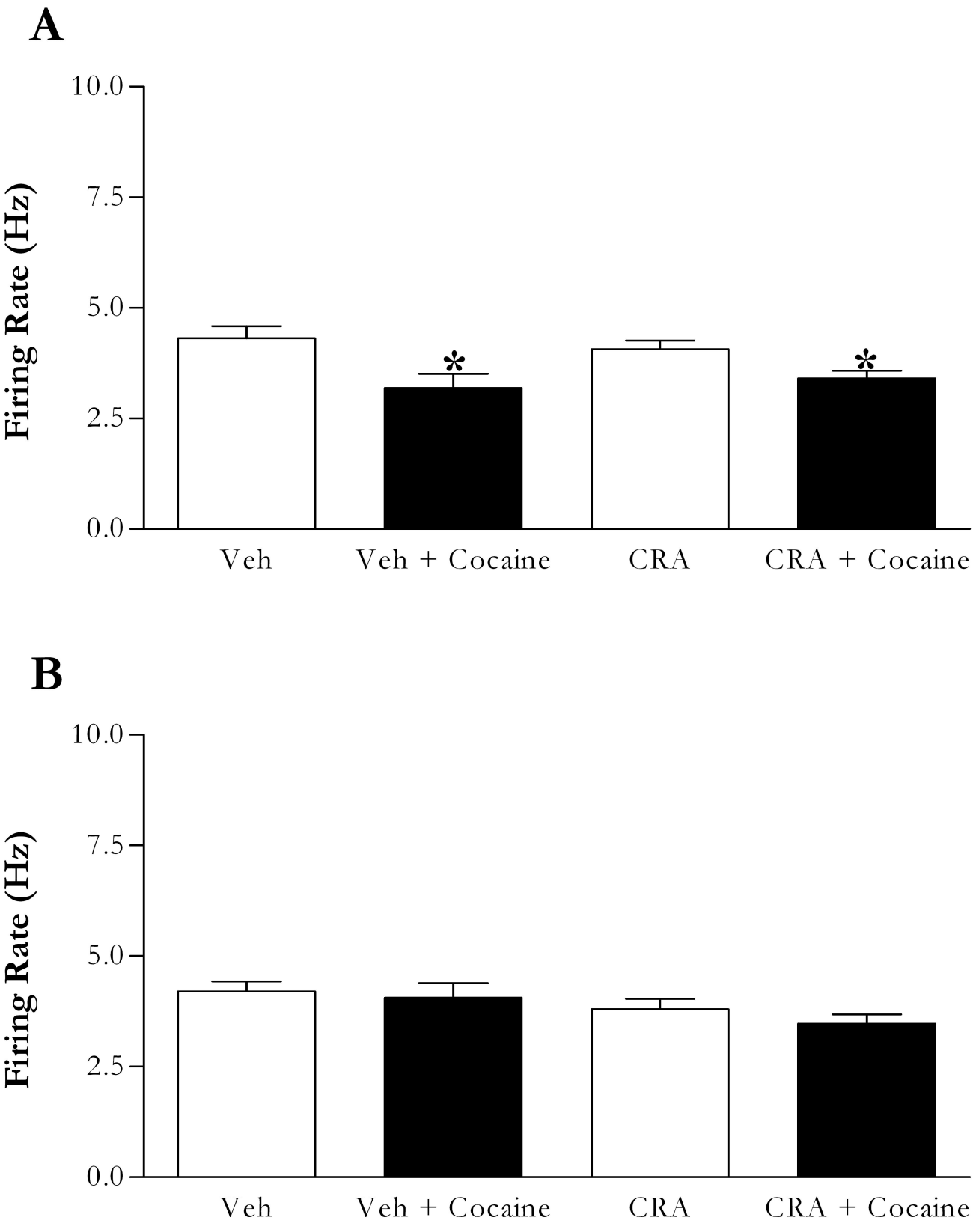

Figure 3

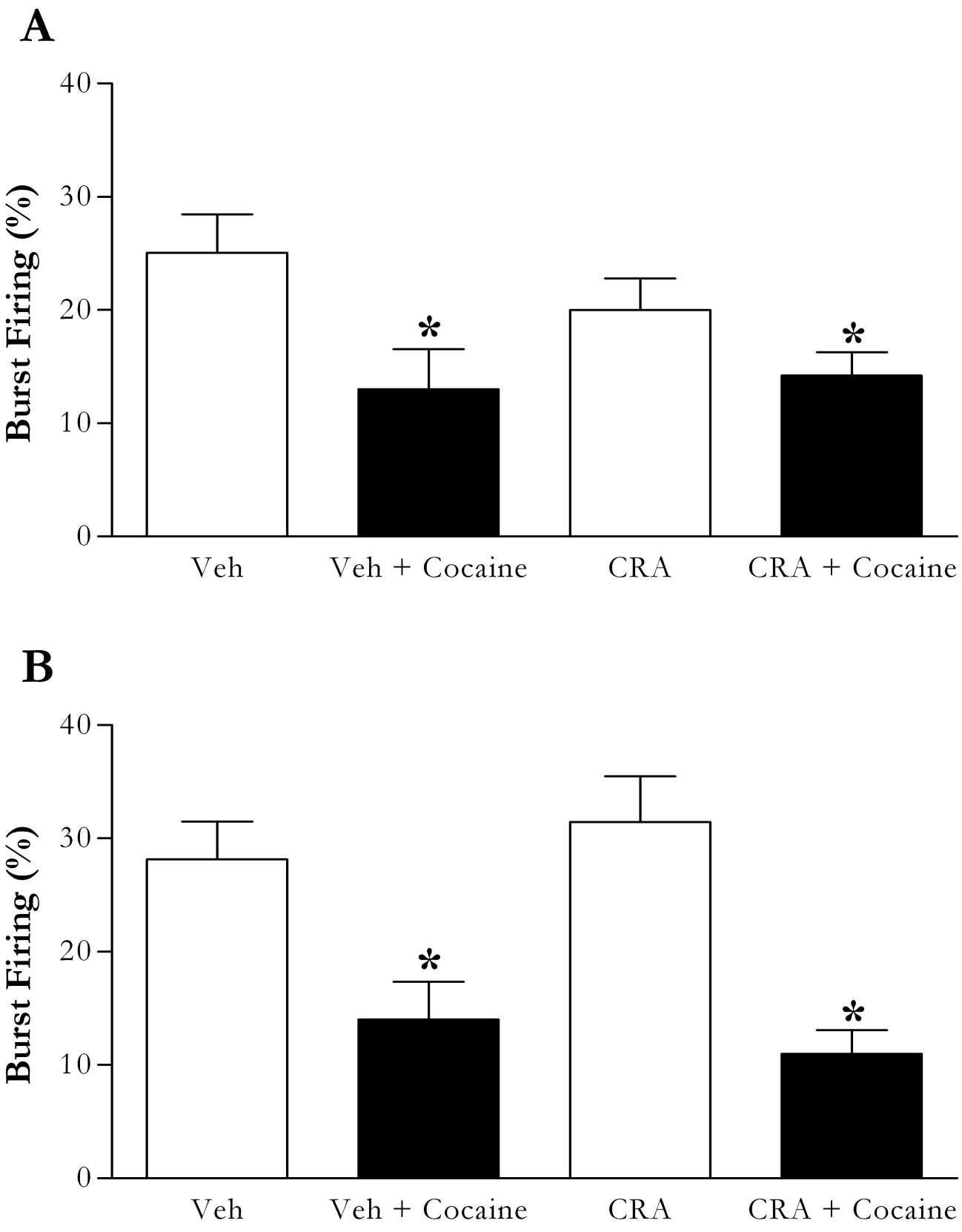

Figure 4