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VK4-40, a Novel D_3R Partial Agonist, Attenuates Cocaine Reward and Relapse in Rodents

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Running Title: D_3R Partial Agonist Attenuates Cocaine Reward

Abstract (250 words)

Background and Purpose. Despite widespread abuse of cocaine, there are no approved treatments for cocaine use disorder. Chronic cocaine use is associated with upregulated dopamine D_3 receptor (D_3R) expression in the brain, and therefore, most D_3R-based medication development has focused on D_3R antagonists. However, D_3R antagonists do not attenuate cocaine intake under "easy" self-administration conditions when response requirements are low. Here we evaluated a novel, highly selective and metabolically stable D_3R partial agonist, VK4-40, for its efficacy in reducing cocaine intake and relapse to drug seeking.

Experimental Approach. The impact of VK4-40 on cocaine intake and relapse were evaluated using intravenous self-administration procedures under a fixed-ratio 2 reinforcement schedule and cocaine-primed reinstatement conditions in rats. Optogenetic brain-stimulation reward procedures were used to evaluate the interaction of VK4-40 and cocaine in the mesolimbic dopamine system. Sucrose self-administration and a conditioned place preference paradigm was used to evaluate the abuse potential of VK4-40 alone and other unwanted effects.

Key Results. VK4-40 dose-dependently reduced cocaine self-administration and cocaine-primed reinstatement of drug-seeking behavior. In addition, VK4-40 inhibited cocaine-enhanced brain-stimulation reward caused by optogenetic stimulation of dopamine neurons in the ventral tegmental area. VK4-40 alone decreased brain-stimulation reward, and produced neither conditioned place preference nor place aversion. This new D_3R partial agonist also failed to alter oral sucrose self-administration.

Conclusions and Implications. The novel D_3R partial agonist, VK4-40, attenuates cocaine reward and relapse in rodents, without significant unwanted effects. These findings support further investigation of D_3R partial agonists as putative treatments for cocaine use disorder.

What is already known

- Dopamine D_3 receptor-based medication development has focused on D_3 receptor antagonists.
- However, D_3 receptor antagonists are less effective in attenuation of cocaine self-administration.

What this study adds

- VK4-40 is a novel highly selective and metabolically stable D_3 receptor partial agonist
- VK4-40 inhibits cocaine self-administration and cocaine-primed reinstatement of drug seeking
- VK4-40 also inhibits optical brain-stimulation reward and cocaine-enhanced brain-stimulation reward
- VK4-40 neither has abuse potential by itself nor inhibits food-taking behavior

What is clinically significant

- These new findings suggest that dopamine D_3 receptor partial agonists may be promising for the treatment of cocaine abuse and addiction
- These findings may lead to the discovery of new pharmacotherapies for cocaine use disorders

Keywords: Addiction, Brain-stimulation reward, Cocaine, Dopamine D_3 Receptor, Reinstatement, Self-administration

1. INTRODUCTION

Cocaine abuse remains a major public health problem across the globe. In 2019, 35 million people worldwide were estimated to suffer from a substance use disorder, while only 1 in 7 received treatment (World Drug Report, 2019). While recent research efforts and media attention have focused on opioid abuse and overdoses, global manufacture of cocaine reached a historical high in 2017 (World Drug Report, 2019). Cocaine continues to be consumed by more than 18.1 million people (World Drug Report, 2019), an estimate which will likely rise in upcoming years as availability of the drug increases and costs decrease (Gregory, 2019). In Britain alone, cocaine use has more than doubled in the last 5 years (Young-Powell, 2029).

Despite ongoing and widespread use of cocaine, there are no accepted treatments currently approved for cocaine use disorder. In the brain, cocaine acts by binding to the dopamine transporter (DAT) and inhibiting dopamine reuptake, thereby prolonging dopaminergic activity at its receptors. There are five major dopamine receptor subtypes in the brain, designated D_1-D_5, which are G-protein coupled. Of these, the D_1 and D_5 receptor subtypes are primarily coupled to G_s, whereas the D_2, D_3, and D_4 subtypes are primarily G_i-coupled (Galaj, Ewing & Ranaldi, 2018). Unlike D_1, D_4 and D_5, the D_2 and D_3 receptor subtypes are expressed both pre-synaptic dopamine terminals where activation serves to suppress dopamine release as well as post-synaptic in the brain where their activation inhibits downstream neuronal signaling (Galaj, Ewing & Ranaldi, 2018; McGinnis, Siciliano & Jones, 2016).

The D_3 receptor (D_3R) in particular represents an attractive therapeutic target for cocaine use disorders. Unlike the other receptor subtypes which are expressed widely throughout the brain, D_3R expression is restricted primarily to the mesolimbic dopamine system, amygdala, and select cortical regions affected by cocaine abuse (Galaj, Ewing & Ranaldi, 2018). In addition, D_3R has the highest affinity to endogenous dopamine, suggesting a more important role than other dopamine receptors in synaptic transmission (Levant, 1997; Sokoloff, Le Foll, Perachon, Bordet, Ridray & Schwartz, 2001). Post-mortem studies have shown increased D_3R expression in the nucleus accumbens (NAc), ventromedial caudate/putamen basolateral amygdala and striatum in cocaine overdose cases (Mash & Staley, 1999; Segal, Moraes & Mash, 1997; Staley

& Mash, 1996). Similarly, PET studies using the D_3R-preferring radioligand [^{11}C](+)PHNO revealed upregulated D_3R binding in the substantia nigra, hypothalamus and amygdala in cocaine users, with elevated binding correlating with years of cocaine use (Matuskey et al., 2014; Payer et al., 2014).

Based on reports of upregulated D_3R following cocaine abuse, preclinical studies have focused on development of D_3R-selective ligands as putative treatments for cocaine use disorder, with particular emphasis on D_3R antagonists. In rodent models, D_3R antagonists attenuate conditioned place preferences for cocaine (Ashby, Rice, Heidbreder & Gardner, 2015; Hachimine, Seepersad, Ananthan & Ranaldi, 2014; Song et al., 2013) and reduce cocaine seeking in high-demand/low-reinforcement conditions, such as under progressive ratio schedules or during reinstatement of cocaine seeking (Galaj, Ananthan, Saliba & Ranaldi, 2014; Payer et al., 2014; Song et al., 2014; Song et al., 2012; Thomsen, Barrett, Butler, Negus & Caine, 2017; Xi et al., 2013). However, D_3R antagonists have been less effective in reducing cocaine intake under "easy" self-administration conditions when response requirements are low, such as under fixed-ratio (FR) reinforcement schedules (Payer et al., 2014; Song et al., 2012).

In contrast to full antagonists that functionally block their receptor targets, partial agonists exert the unique profile of acting as both antagonists and low efficacy agonists. As such, partial agonists are more effective at maintaining abstinence and mitigating relapse than antagonist therapies for substance use disorders, such as those involving nicotine or opioids (Jordan, Cao, Newman & Xi, 2019; Jordan & Xi, 2018). For example, nicotinic receptor antagonists like mecamylamine and DHβE occupy nicotinic acetylcholine receptors and block nicotine reward, but also produce precipitated withdrawal symptoms (Jordan & Xi, 2018). Likewise, the opioid receptor antagonist naloxone reverses opioid overdose, but induces acute opioid withdrawal and increases relapse risk (Jordan, Cao, Newman & Xi, 2019). Partial agonists such as varenicline ($\alpha_4\beta_2$ nicotinic receptors) and buprenorphine (μ opioid receptors) act as antagonists in the presence of nicotine or opioids, blocking the rewarding effects of these drugs of abuse. However, during periods of abstinence, these compounds elicit partial activation of their receptor targets, thereby mitigating withdrawal symptoms and reducing the risk of relapse (Jordan, Cao, Newman & Xi, 2019; Jordan & Xi, 2018).

Although the D_3 receptor is not a direct target of cocaine, cocaine indirectly activates D_3R via blockade of DAT and elevation of synaptic dopamine levels (Galaj, Ewing & Ranaldi, 2018). Here, we applied the treatment rationale of partial agonism in other substance use disorders to examine the efficacy of a new, highly selective and metabolically stable D_3R partial agonist, VK4-40 (Kumar et al., 2016; Shaik et al., 2019), as a putative treatment for cocaine abuse using preclinical rodent models. In this study, we evaluated the pharmacological effects of VK4-40 on cocaine self-administration under "easy" low-demand (FR2) reinforcement conditions and cocaine-primed reinstatement in rats, as well as cocaine-enhanced optogenetic brain-stimulation reward in transgenic DAT-Cre mice. Finally, we evaluated the effects of VK4-40 on non-drug (sucrose) self-administration and the rewarding vs. aversive properties of VK4-40 alone in a traditional conditioned place preference paradigm.

2. MATERIALS AND METHODS

2.1 Animals

Long-Evans rats (males, 275-325 g; Charles River Laboratories, Raleigh, N.C.) were used for self-administration and reinstatement experiments. Heterozygous male DAT-Cre +/- mice (25-40 g), bred at the National Institute on Drug Abuse Intramural Research Program (NIDA IRP), were used for optogenetics experiments. Evaluation of these behaviors in rodents is well-established to recapitulate aspects of cocaine abuse in humans (Jordan et al. 2019a; Galaj et al. 2018; Song et al. 2014; You et al. 2019). Group sizes were determined based adequate statistical power to detect meaningful statistically significant effects in previous studies. All animals were housed in the NIDA IRP animal vivarium under a reverse 12-hr light-dark cycle (lights on at 7:00 PM) with ad libitum access to food and water. All procedures were approved by the Animal Care and Use Committee (ACUC) and were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals, 8th edition* (National Research Council, 2011).

2.2 Drugs and solutions

VK4-40 was synthesized at the NIDA IRP and dissolved in 25% 2-hydroxypropyl beta-cyclodextrin in distilled H_20 (Jordan et al., 2019a; Jordan et al., 2019b; Kumar et al., 2016; Shaik et al., 2019). Cocaine was obtained from the NIDA Drug Supply Program, dissolved in sterile saline and diluted to concentrations appropriate for i.v. drug self-administration and i.p. injections in rats and mice. All drugs given were in a volume of 1 ml/kg in rats and 0.1 ml/kg in mice by i.p. injections.

2.3 Cocaine self-administration

To determine whether VK4-40 attenuates cocaine intake, rats underwent intravenous catheter surgeries and i.v. self-administration procedures were as described previously (Jordan et al., 2019a). Rats were anesthetized under xylazine/ketamine anesthesia (10/90 mg/kg, i.p.) and implanted with a micro-renathane catheter into the right jugular vein. The catheter was secured to the vein with suture silk and inserted subcutaneously to exit at an incision at the back of the skull, where it was joined to a 22-gauge stainless steel cannula and mounted to the skull with stainless steel screws and dental acrylic. Rats were flushed daily after surgery with a gentamicin-heparin-saline solution (0.1 mg/ml gentamicin, 30 IU heparin) to prevent infection and preserve patency.

Cocaine self-administration training began one week after surgery in operant chambers equipped with response levers, a house light, a cue light mounted above the active lever, and syringe pumps located outside the chamber (Med Associates Inc., Georgia, VT, USA). During the initial training phase, rats were allowed to press an active lever to earn a 1.0 mg/kg infusion of cocaine (4.6 sec in duration, paired with illumination of the cue light and extinguishment of the house light) under a fixed-ratio 1 (FR1) schedule of reinforcement during daily, 3-hr sessions. Responses on the inactive lever were recorded but had no scheduled consequences.

After 2 weeks of training on the 1.0 mg/kg dose, rats transitioned to a lower cocaine dose (0.5 mg/kg/infusion) and a FR2 schedule of reinforcement. Training continued until animals earned at least 20 cocaine infusions per session, exhibited less than 20% variability in responding, and reached an active/inactive lever response ratio of 2:1 or higher for 3 consecutive

days. Once stable self-administration was achieved, animals transitioned to a multiple-dose self-administration program, during which ascending doses of cocaine were available for self-administration in 20-min intervals (0.031, 0.0625, 0.125, 0.25, 0.5 and 1 mg/kg/infusion), with 10-min intervals between each dose (Keck et al., 2013). Cocaine doses were achieved by adjusting the infusion volume and duration. Once stable self-administration was acquired following the criteria above, rats were randomly selected to receive systemic i.p. injections of VK4-40 (0, 3 or 10 mg/kg) 30-min. prior to the test session (doses were chosen based on prior studies; Jordan et al., 2019a; Jordan et al., 2019b; Shaik et al., 2019; You et al., 2019).

2.4 Cocaine-primed reinstatement

Next, to determine whether VK4-40 could reduce cocaine seeking, rats were tested for cocaine-primed reinstatement. Following completion of multiple-dose testing, rats were re-trained for cocaine self-administration under classical experimental conditions (0.5 mg/kg/infusion, 3-hr sessions) until stable self-administration was achieved. Then the animals were transitioned to extinction training, during which time lever pressing was recorded but had no scheduled consequences. Extinction training continued during daily, 3-hr sessions until active lever presses were reduced to <15% of FR2 self-administration levels (You et al., 2019), requiring approximately 12-14 days. Once extinction was established, rats were randomly selected to receive systemic i.p. injections of vehicle of VK4-40 (3 or 10 mg/kg) 30-min. prior to a priming dose of cocaine (10 mg/kg, i.p.) and placement in the self-administration chambers for a 3-hr reinstatement test session. During this test, active lever presses produced delivery of the previous cocaine-paired cues (illumination of the cue light, extinguishment of the house light, and running of the syringe pump), but cocaine infusions were not delivered.

2.5 Optogenetic intracranial self-stimulation (oICSS)

To determine whether VK4-40 alter cocaine reward via dopamine-dependent mechanisms, adult male DAT-Cre mice were injected with recombinant adeno-associated virus encoding channelrhodopsin and enhanced yellow fluorescent protein (AAV-EF1α-DIO-ChR2-

EGFP, ~2 × 1012 genomes/ml, University of North Caroline Gene Therapy Center) into the ventral tegmental area (VTA; AP -3.28; ML 0.43; DV -4.41 mm relative to Bregma, 150 nl), as described previously (Jordan et al., 2019a). After 4 weeks' recovery, mice were allowed to respond on an active lever in standard mouse operant chambers (Med Associates Inc.) for a 1-s pulse train of laser stimulation (473 nm, 20 mW, 5 ms duration, 25Hz) in daily, 1-hr sessions. Inactive lever responses were recorded but had no scheduled consequences. After acquiring stable responding for 1-week, mice were trained on a rate-frequency program during which 6 stimulation frequencies (100, 50, 25, 10, 5 and 1 Hz) were available for self-stimulation in descending order for 10-min each. Once stable oICSS responding was established, mice were randomly selected for systemic treatment with cocaine (2 or 10 mg/kg, i.p., 5-min pretreatment time) or a combination of VK4-40 (3 or 10 mg/kg, i.p., 30-min pretreatment time) or 25% 2-hydroxypropyl beta-cyclodextrin vehicle (30-min pretreatment time), prior to cocaine injections (10 mg/kg, i.p.) and oICSS sessions.

2.6 Sucrose self-administration

The procedures for oral sucrose self-administration were identical to those for cocaine self-administration, except that active lever presses under a FR1 schedule led to a delivery of 0.08 ml of 5% sucrose solution into a liquid food tray located on the operant chamber wall. Seven rats were used for sucrose self-administration training and testing. Sucrose deliveries were capped at 100 per session to prevent food satiation and a reduction in motivation for sucrose-taking behavior. After training, the animals were randomly selected for testing with vehicle treatment or one of two doses of VK4-40 (1 or 5 mg/kg, i.p.). The treatment was counterbalanced in each rat and each test was separated by two additional training sessions. The total number of sucrose deliveries during the 3-h self-administration session, the time spent to earn the total sucrose deliveries, and the rate of sucrose deliveries as calculated by total sucrose deliveries/time spent (min) were used to evaluate the effects of VK4-40 on sucrose self-administration.

2.6 Conditioned place preferences (CPP)

To determine whether VK4-40 alone produces rewarding or aversive effects, additional mice were tested in a traditional conditioned place preference paradigm, as described previously (Song et al., 2013). Briefly, on days 1-2, mice were pre-conditioned to the CPP chambers by being allowed to freely explore the center corridor and adjacent compartments for 15-min. Days 3-7 comprised conditioning days. On days 3, 5, and 7, mice were treated with either VK4-40 or cocaine (drug conditioning) and, 15-min later, confined to one of the two compartments for 30-min. On the intervening days (4 and 6), mice were treated with either 25% 2-hydroxypropyl beta-cyclodextrin (VK4-40 vehicle control) or saline (cocaine vehicle control) and, 15-min later, confined to the opposing chamber (counterbalanced across subjects). On day 8-9, mice were placed in the center corridor and allowed to freely explore the adjacent compartments for 15-min. The total time spent in the compartments was recorded pre- and post-conditioning (averaged across the two days in each phase).

2.7 Data analyses

All data are presented as mean ± SEM. One- or two-way analysis of variance (ANOVA), with repeated measures for dose, phase (self-administration, extinction and reinstatement) or laser frequency were used to analyze data in Experiments 1-3 (cocaine self-administration, reinstatement, and oICSS), followed by post-hoc Tukey tests. For Experiment 4, CPP scores were calculated by subtracting the total time spent in the drug-paired compartment post-conditioning from the total time spent in that same compartment in the pre-conditioning phase. CPP data were analyzed using paired t-tests. Levels of probability of $p < 0.05$ or lower were considered the threshold for statistical significance. All tests were performed using SigmaStat 12.5 for Windows (Systat Software, Inc.).

3. RESULTS

3.1 VK4-40 inhibits cocaine self-administration

Figure 1 shows results indicating that VK4-40 pretreatment significantly inhibited cocaine self-administration, shifted cocaine dose-response curves downward, and decreased cocaine intake under a FR2 schedule of reinforcement. Two-way ANOVA of infusions earned (Fig.1A) revealed main effects of cocaine dose ($F_{5, 138} = 8.91$, $p < 0.05$) and treatment ($F_{2,138} = 3.84$, $p < 0.05$). Post-hoc testing revealed that 10 mg/kg VK4-40 treatment significantly reduced the number of cocaine infusions earned compared to vehicle at the 0.0625 mg/kg/infusion dose ($p < 0.05$). A similar reduction neared statistical significance at the 0.125 mg/kg/infusion cocaine dose ($p = 0.06$, 10 mg/kg VK4-40 vs. vehicle treatment).

Two-way ANOVA of active lever responses (Fig. 1B) similarly revealed main effects of cocaine dose ($F_{5, 138} = 7.99$, $p < 0.05$) and treatment ($F_{2, 138} = 6.03$. $p < 0.05$). Post-hoc testing indicated that treatment with 10 mg/kg VK4-40 significantly reduced active lever responding at the 0.0625 and 0.125 mg/kg/infusion cocaine doses ($p < 0.05$). There were no significant group differences in inactive lever responding (Fig. 1C).

Figure 1D shows the cumulative cocaine intake (i.e., cocaine infusions shown in Fig. 1A X unit cocaine dose, mg/kg) in rats during cocaine self-administration in the presence or absence of VK4-40. Two-way ANOVA of cocaine intake (Fig. 1D) also revealed main effects of cocaine dose ($F_{5, 138} = 24.4$, $p < 0.05$) and VK4-40 treatment ($F_{2, 138} = 3.5$. $p < 0.05$). However, post-hoc testing did not reveal statistically significant differences between vehicle and VK4-40 treatment groups.

3.2 VK4-40 inhibits cocaine-primed reinstatement

Figure 2A show the general experimental procedures. Figure 2B shows the effects of VK4-40 pretreatment on cocaine-primed reinstatement of cocaine-seeking behavior. Two-way ANOVA of active lever responding (Fig. 2B) revealed a main effect of phase ($F_{2,66} = 9.4$, $p < 0.05$). Post-hoc testing indicated that overall, lever responding was significantly lower during the extinction phase compared to self-administration or reinstatement levels ($p < 0.05$). Vehicle-treated rats significantly reinstated active lever responding following the cocaine priming dose ($p < 0.05$ compared to extinction response levels), which was blocked by pretreatment with 3 or 10

mg/kg VK4-40. There were no significant differences in inactive lever responding between any of the groups (Fig. 2C).

3.3 Optogenetic intracranial self-stimulation (oICSS)

Figure 3 shows the general experimental procedures (panels 3A, 3B, and 3C) and the effects of cocaine and/or VK4-40 oICSS maintained by stimulation of VTA DA neurons (Fig. 3D, 3E, 3F). Systemic administration of cocaine dose-dependently shifted the stimulation frequency-lever response curves upward and leftward, indicating an enhancement of brain-stimulation reward. Two-way ANOVA of active lever responses following vehicle or cocaine treatment (Fig. 3D) revealed main effects of frequency ($F_{5, 50} = 21.8$, $p < 0.05$), cocaine dose ($F_{2, 50} = 4.9$, $p < 0.05$), and a frequency X dose interaction ($F_{10, 50} = 4.3$, $p < 0.05$). Post-hoc testing indicated that 10 mg/kg cocaine increased oICSS responding at the 10 Hz and 25 Hz frequencies compared to vehicle treatment ($p < 0.05$).

We next examined the impact of VK4-40 pretreatment on cocaine-enhanced oICSS responding (Fig. 3E). Two-way ANOVA of active lever responses revealed main effects of frequency ($F_{5, 75} = 13.7$, $p < 0.05$) and treatment ($F_{3, 75} = 3.6$, $p < 0.05$), and a frequency X treatment interaction ($F_{15, 75} = 2.7$, $p < 0.05$). Post-hoc testing indicated that 10 mg/kg cocaine increased oICSS responding at the 25 Hz and 10 Hz frequencies ($p < 0.05$), which was dose-dependently blocked by VK4-40 pre-treatment.

We also sought to determine the effects of VK4-40 alone on oICSS responding in a separate cohort of mice (Fig. 3F). Two-way ANOVA revealed main effects of VK4-40 treatment ($F_{2,10} = 58.56$, $p < 0.05$), frequency ($F_{5,25} = 53.17$, $p < 0.05$) and a frequency X treatment interaction ($F_{10,50} = 9.06$, $p < 0.05$). Post-hoc testing indicated that VK4-40 pretreatment reduced oICSS responding compared to vehicle at the 10, 25, 50, and 100 Hz frequency ($p < 0.05$).

3.4 VK4-40 does not reduce sucrose self-administration

To determine whether VK4-40 also alters non-drug reinforcement, we observed the effects of VK4-40 treatment on oral sucrose self-administration. Figure 4A shows that all rats took the maximally allowed 100 sucrose deliveries within the initial 30–60 min of the 3-h session in the presence or absence of the VK4-40 treatment. We also compared the time needed for the maximal 100 deliveries (Fig. 4B). There was no difference between the vehicle and VK4-40 treatment groups in the time needed for the maximal 100 deliveries.

3.5 VK4-40 does not produces conditioned place preferences (CPP)

To determine whether VK4-40 itself has abuse potential, we observed the CPP response to cocaine or VK4-40. A paired t-test on cocaine data (Fig. 4C) revealed that post-conditioning time spent in the drug paired chamber was significantly greater than time spent in the drug-paired chamber pre-conditioning ($t_2 = -2.7$, $p < 0.05$). By contrast, a paired t-test on VK4-40 data (Fig. 4D) revealed no significant differences between the time spent in the drug-paired chamber post-conditioning compared to pre-conditioning ($t_8 = 0.9$, $p = 0.4$).

4. DISCUSSION

There are currently no approved treatments for cocaine use disorder. In prior preclinical studies, D_3R antagonists have shown efficacy in reducing cocaine reward and cocaine seeking under progressive ratio reinforcement schedules and reinstatement models (Galaj, Ananthan, Saliba & Ranaldi, 2014; Payer et al., 2014; Song et al., 2014; Song et al., 2012; Thomsen, Barrett, Butler, Negus & Caine, 2017; Xi et al., 2013). However, D_3R antagonists are comparably ineffective at reducing cocaine intake under conditions involving “easy” response requirements and/or a high reinforcing value of cocaine, such as FR1 or FR2 schedules. In the current study, we found that pretreatment with a partial D_3R agonist, VK4-40, significantly reduced cocaine intake under an easy, low response-requirement schedule (FR2) and mitigated reinstatement of cocaine seeking. Consistent with these findings, VK4-40 also dose-dependently reduced cocaine-enhanced optical brain-stimulation reward driven by activation of VTA DA neurons, implicating a dopamine-dependent mechanism in VK4-40 attenuation of cocaine reward. Importantly, VK4-40 alone neither altered oral sucrose self-administration nor

produced rewarding or aversive effects when administered systemically, suggesting that this D_3R partial agonist may not exert unwanted side-effects in translational studies.

These findings with VK4-40 are consistent to a previous report that CJB090, another D_3R partial agonist, reduced cocaine self-administration in rhesus monkeys (Martelle, Claytor, Ross, Reboussin, Newman & Nader, 2007) and methamphetamine self-administration under both FR and progressive ratio schedules of reinforcement in rats (Orio, Wee, Newman, Pulvirenti & Koob, 2010). Similarly, cariprazine (RGH-188), a D_3R-preferring D_3R/D_2R partial, also reduced cocaine intake under a FR1 schedule and suppressed cue-induced reinstatement of cocaine seeking in rats (Roman, Gyertyan, Saghy, Kiss & Szombathelyi, 2013). In contrast, BP 897, the prototypic D_3R partial agonist, failed to alter cocaine or amphetamine self-administration (Aujla, Sokoloff & Beninger, 2002; Pilla et al., 1999), although this compound was able to reduce conditioned locomotor activity to cocaine and amphetamine (Aujla, Sokoloff & Beninger, 2002; Le Foll, Frances, Diaz, Schwartz & Sokoloff, 2002) and suppress reinstatement responding to cocaine-associated cues (Cervo, Carnovali, Stark & Mennini, 2003; Gal & Gyertyan, 2006). The D_3R partial agonist, RGH-237, also failed to alter cocaine intake under a FR1 schedule, but inhibited acquisition of cocaine conditioned place preferences and attenuated cue-induced reinstatement of cocaine seeking, (Gyertyan et al., 2007).

The mechanisms through which the novel partial D_3R agonist VK4-40 appears to be superior to D_3R antagonists in reducing cocaine self-administration are unclear. One possibility is that antagonists fully block the D_3R, thereby inactivating both post-synaptic D_3R inhibitory signaling as well as the pre-synaptic D_3R autoreceptor "brake" on dopamine release (Jordan, Cao, Newman & Xi, 2019; Jordan & Xi, 2018). When there is little cost to earning cocaine (e.g., under a FR2 schedule), subjects may therefore seek equal or greater amounts of the drug to compensate for the reduction in cocaine reward after D_3R antagonist treatment. This is supported by the finding that genetic deletion of presynaptic D_3Rs causes an increase in basal levels of extracellular dopamine (Song, Zhang, Li, Bi, Gardner & Xi, 2012; Zhan et al., 2018), which may subsequently activate other dopamine receptors to counteract the loss of D_3R function. By contrast, a D_3R partial agonist elicits partial activation of pre- and post-synaptic receptors under basal conditions and thereby decreases dopamine release. This effect of D_3R

partial agonism may functionally attenuate cocaine-induced increases in dopamine (Orio, Wee, Newman, Pulvirenti & Koob, 2010), and therefore, reduce cocaine reward and cocaine intake even under low-cost self-administration conditions.

It is also unknown why VK4-40 is more effective than other D_3R partial agonists such as BP897 and RGH237 in its ability to attenuate cocaine self-administration under a FR2 schedule of reinforcement. Notably, BP 897 is only ~70-fold selective for $D_3R > D_2R$ (Garcia-Ladona & Cox, 2003) and CJB090 approximately 60-fold selective for $D_3R > D_2R$ (Achat-Mendes, Platt, Newman & Spealman, 2009; Hachimine, Seepersad, Ananthan & Ranaldi, 2014), whereas VK4-40 is over 200-fold selective for $D_3R > D_2R$ (Kumar et al., 2016). Although RGH-237 exhibits ~150-fold selectivity for $D_3R > D_2R$, this compound has significantly poorer brain penetrability and poor bioavailability, as assessed by plasma drug levels that near baseline by 5 hours post-administration (Gyertyan et al., 2007). By contrast, VK4-40 exhibits excellent brain penetration ability, with brain levels remaining high for 8 hours or more after oral administration in the rat (Jordan et al., 2019a; Kumar et al., 2016).

In addition to poor selectivity and metabolic profiles, translation of D_3R-selective ligands to the clinic has been barred by other issues such as cardiotoxicity, poor absorption, distribution, and excretion, and increases in blood pressure when combined with cocaine (Appel, Li, Holmes & Acri, 2015; Keck, Burzynski, Shi & Newman, 2014). Because the D_3R is highly expressed in the kidneys and can participate in the pathogenesis of hypertension (Jordan et al., 2019b), we recently examined the cardiovascular impact of structurally different D_3R ligands, both individually and in combination with cocaine or oxycodone (Jordan et al., 2019b). Briefly, we found that older-generation antagonists (e.g., SB-277,011A) increased blood pressure both alone and in combination with cocaine, as observed previously (Appel, Li, Holmes & Acri, 2015; Jordan et al., 2019b). In contrast, *R*-VK4-40, an enantiomer of VK4-40, and the structurally similar sister compound *R*-VK4-116, either failed to increase or dose-dependently attenuated blood pressure responses to cocaine and oxycodone (Jordan et al., 2019b). While the effects of partial D_3R agonism on blood pressure are unknown, it is unlikely that VK4-40 increases blood pressure in the presence of cocaine when its enantiomer failed to do so (Jordan et al., 2019b). Together, these findings suggest that peripheral side effects of D_3R-selective ligands can be

circumvented with molecular modifications, supporting the further development of D_3R-selective treatments for cocaine abuse.

There are several limitations to this study that warrant further investigation. First, the current study was conducted solely in male rats. While no sex differences in behavioral or dopaminergic responses to D_3R/D_2R ligands have been reported previously (Chang, Swerdlow, Breier, Thangaraj & Weber, 2010; Jordan et al., 2019b; McGinnis, Siciliano & Jones, 2016), investigations of VK compounds in female rats are currently underway. Second, the long-term effects of chronic administration of D_3R partial agonists (i.e., the probable conditions under which these compounds would be prescribed to human subjects), remain unknown. Finally, the impact of VK4-40 on non-drug reinforcement needs further study. Whereas BP 897 and RGH-237 did not impact sucrose seeking or sucrose intake (Gal & Gyertyan, 2006; Gyertyan et al., 2007), CJB090 reduced food intake (Martelle, Claytor, Ross, Reboussin, Newman & Nader, 2007). Our data indicate that VK4-40 (1 and 5 mg/kg) did not alter sucrose self-administration, but further work will be necessary to confirm the effects of higher doses and chronic treatment on both sucrose and food consumption as well as body weight maintenance over time.

In conclusion, the highly selective and metabolically stable D_3R partial agonist, VK4-40, attenuates cocaine reward and cocaine seeking in rodents, without exerting rewarding or aversive effects when administered alone. These results support the further development for VK4-40 and structurally related D_3R partial agonists as treatments for cocaine and other psychostimulant use disorders.

AUTHOR CONTRIBUTIONS

C.J.J., Z-X.X., A.H.N. designed the experiments. C.J.J., Y.H., G-H.B., and Z-B.Y. performed the experiments. J.C. synthesized the VK4-40 compound. C.J.J. and Z-X.X. analyzed the data and prepared the figures. C.J.J., Z-X.X., and A.H.N. wrote the manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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Figure Legends

Figure 1. Impact of VK4-40 on cocaine self-administration under the FR2 reinforcement schedule in rats. (A) Cocaine self-administration dose-response curves in the presence or absence of VK4-40 treatment, indicating that VK4-40 dose-dependently reduced the number of cocaine infusions earned. (B) Active lever responses for various doses of cocaine in the presence or absence of VK4-40 treatment, indicating that VK4-40 dose-dependently reduced cocaine seeking. (C) Inactive lever responses during access to various doses of cocaine reveal no non-specific impact of VK4-40 pretreatment on inactive lever responding. (D) Calculated cocaine intake (mg/kg) during cocaine self-administration in the presence or absence of VK4-40 treatment. ** $p < 0.01$, * $p < 0.05$, compared to vehicle control group.

Figure 2. Impact of VK4-40 on cocaine-primed reinstatement of drug-seeking behavior following extinction in rats previously trained to self-administer cocaine. (A) General experimental procedures; (B) Active lever responding during the last session (day) of cocaine self-administration, the last session (day) of extinction, and the reinstatement test. (C) Inactive lever responding during the last session (day) of cocaine self-administration, last session (day) of extinction, and reinstatement test. A priming dose of cocaine (10 mg/kg, i.p.) significantly reinstated active lever responding compared to extinction levels, which was blocked by pretreatment with 3 or 10 mg/kg VK4-40. * $p < 0.05$, vehicle treatment group. ^ $p < 0.05$ compared to the last session of extinction.

Figure 3. Impact of VK4-40 and/or cocaine on optogenetic intracranial self-stimulation (oICSS) of VTA dopamine neurons by DAT-Cre mice. (A) Schematic of experimental model, illustrating AAV-DIO-ChR2-eGFP was microinjected into the VTA and an optical fiber was implanted into the VTA in DAT-Cre mice. (B) Representative images, showing that AAV-DIO-ChR2-eGFP is selectively expressed in VTA dopamine neurons in DAT-Cre mice. (C) Schematic diagram showing the expression of ChR2 in VTA DA neurons that can be activated by 473 nm laser activation. (D) Systemic administration of cocaine dose-dependently increased active lever responding for VTA dopamine neuron activation. (E) Pretreatment with VK4-40 blocked cocaine-induced increases in active lever responding for dopamine stimulation. (F) Systemic

administration of VK4-40 alone inhibited oICSS. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to vehicle treatment group.

Figure 4. Impact of VK4-40 on oral sucrose self-administration and conditioned place preferences to VK4-40 or cocaine. (A) VK-40 did not alter oral sucrose self-administration and all animals reached the maximally allowed 100 sucrose deliveries. (B) VK4-40 also failed to alter the time spent to reach the maximal 100 sucrose deliveries. (C) Cocaine produced significant conditioned place preferences to the cocaine-paired chamber. (D) VK4-40 produced neither conditioned place preferences nor place aversions to the VK4-40-paired side of the apparatus.

Figure 1

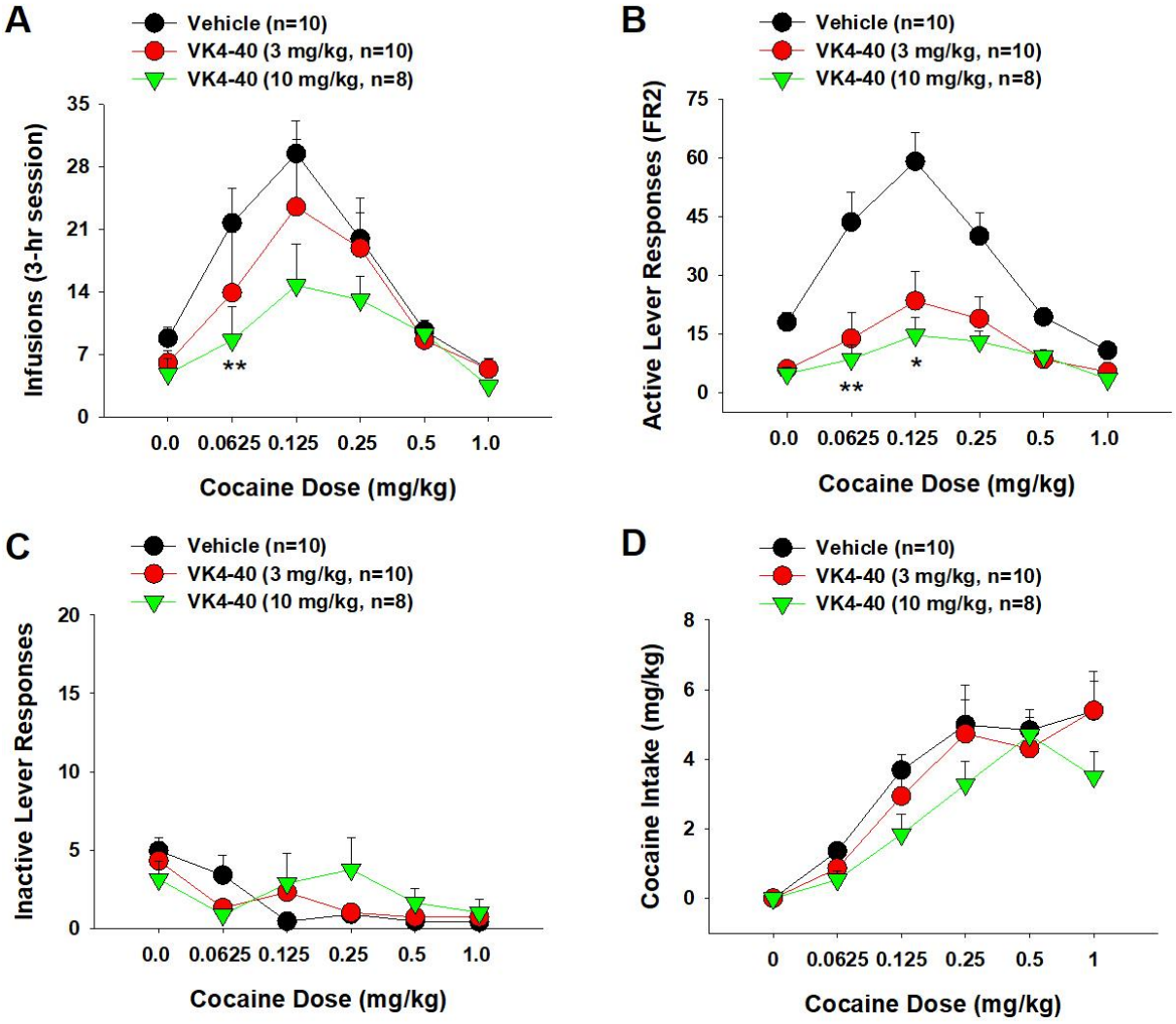

A
Vehicle (n=10)
VK4-40 (3 mg/kg, n=10)
VK4-40 (10 mg/kg, n=8)
Infusions (3-hr session)
35
28
21
14
7
0
**
0.0 0.0625 0.125 0.25 0.5 1.0
Cocaine Dose (mg/kg)
B
Vehicle (n=10)
VK4-40 (3 mg/kg, n=10)
VK4-40 (10 mg/kg, n=8)
Active Lever Responses (FR2)
75
60
45
30
15
0
**
*
0.0 0.0625 0.125 0.25 0.5 1.0
Cocaine Dose (mg/kg)
C
Vehicle (n=10)
VK4-40 (3 mg/kg, n=10)
VK4-40 (10 mg/kg, n=8)
Inactive Lever Responses
20
15
10
5
0
0.0 0.0625 0.125 0.25 0.5 1.0
Cocaine Dose (mg/kg)
D
Vehicle (n=10)
VK4-40 (3 mg/kg, n=10)
VK4-40 (10 mg/kg, n=8)
Cocaine Intake (mg/kg)
8
6
4
2
0
0 0.0625 0.125 0.25 0.5 1
Cocaine Dose (mg/kg)

Figure 2

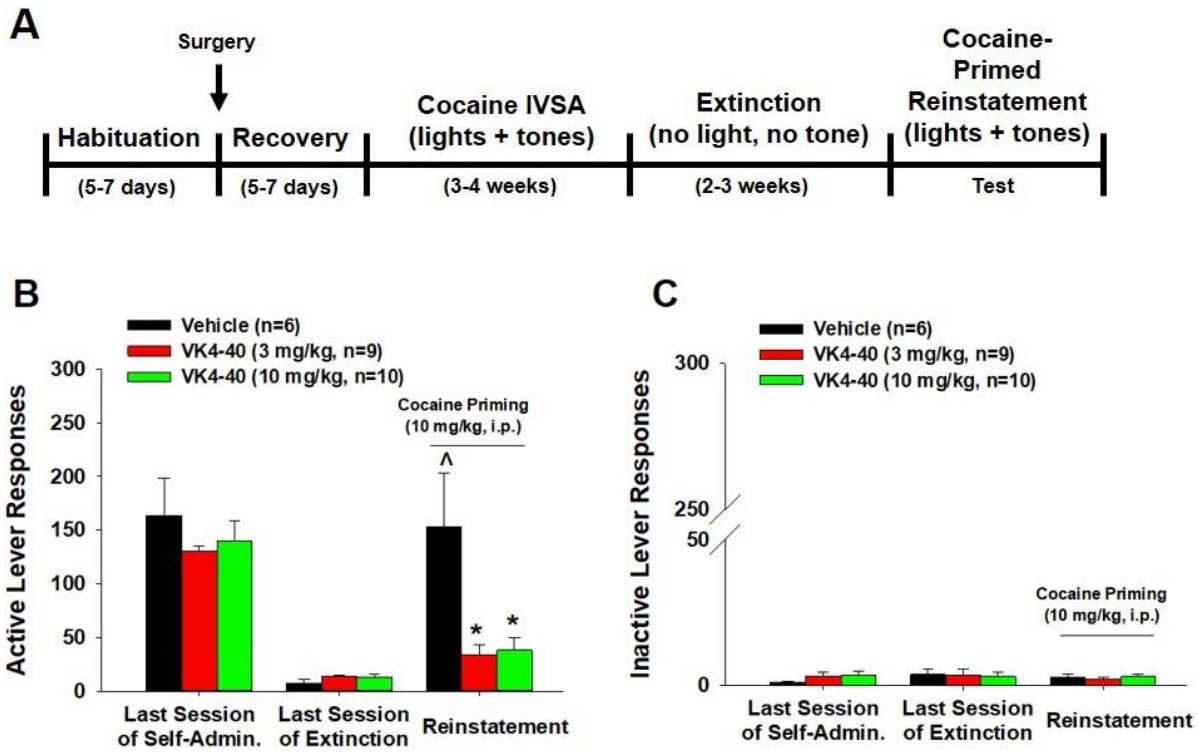

A
Surgery
Habituation
Recovery
Cocaine IVSA
(lights + tones)
Extinction
(no light, no tone)
Cocaine-
Primed
Reinstatement
(lights + tones)
(5-7 days)
(5-7 days)
(3-4 weeks)
(2-3 weeks)
Test
B
Vehicle (n=6)
VK4-40 (3 mg/kg, n=9)
VK4-40 (10 mg/kg, n=10)
Active Lever Responses
300
250
200
150
100
50
0
Cocaine Priming
(10 mg/kg, i.p.)
^
*
*
Last Session
of Self-Admin.
Last Session
of Extinction
Reinstatement
C
Vehicle (n=6)
VK4-40 (3 mg/kg, n=9)
VK4-40 (10 mg/kg, n=10)
Inactive Lever Responses
300
250
50
0
Cocaine Priming
(10 mg/kg, i.p.)
Last Session
of Self-Admin.
Last Session
of Extinction
Reinstatement

Figure 3

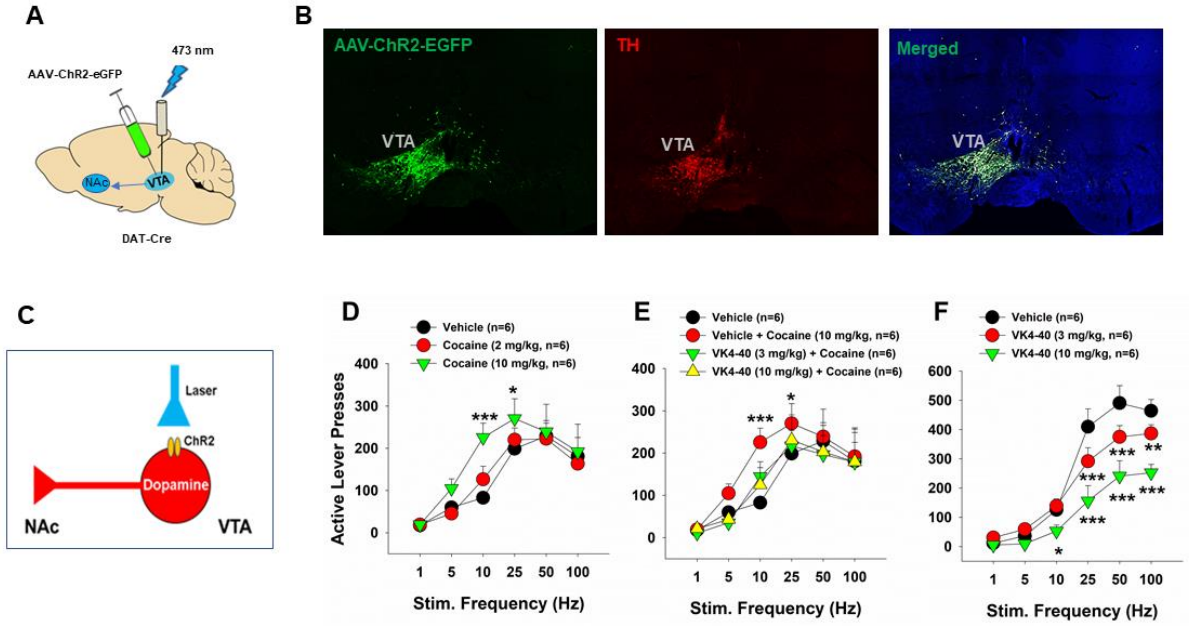
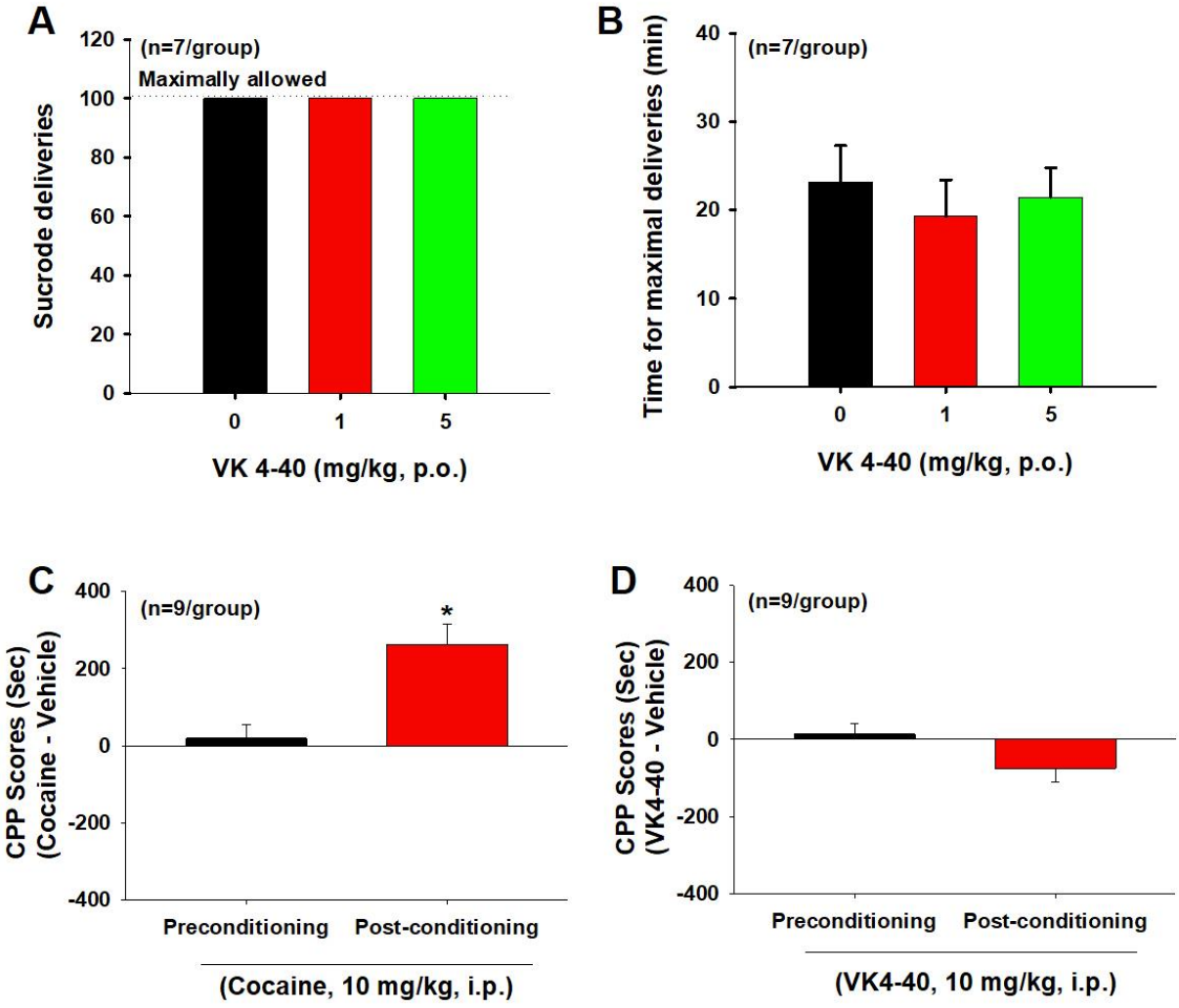

A
473 nm
AAV-ChR2-eGFP
NAc
VTA
DAT-Cre
B
AAV-ChR2-EGFP
VTA
TH
VTA
Merged
VTA
C
Laser
ChR2
Dopamine
NAc
VTA
D
Vehicle (n=6)
Cocaine (2 mg/kg, n=6)
Cocaine (10 mg/kg, n=6)
Active Lever Presses
Stim. Frequency (Hz)
E
Vehicle (n=6)
Vehicle + Cocaine (10 mg/kg, n=6)
VK4-40 (3 mg/kg) + Cocaine (n=6)
VK4-40 (10 mg/kg) + Cocaine (n=6)
Stim. Frequency (Hz)
F
Vehicle (n=6)
VK4-40 (3 mg/kg, n=6)
VK4-40 (10 mg/kg, n=6)
Stim. Frequency (Hz)

Figure 4


A
(n=7/group)
Maximally allowed
Sucrode deliveries
120
100
80
60
40
20
0
0
1
5
VK 4-40 (mg/kg, p.o.)
B
(n=7/group)
Time for maximal deliveries (min)
40
30
20
10
0
0
1
5
VK 4-40 (mg/kg, p.o.)
C
(n=9/group)
*
CPP Scores (Sec)
(Cocaine - Vehicle)
400
200
0
-200
-400
Preconditioning
Post-conditioning
(Cocaine, 10 mg/kg, i.p.)
D
(n=9/group)
CPP Scores (Sec)
(VK4-40 - Vehicle)
400
200
0
-200
-400
Preconditioning
Post-conditioning
(VK4-40, 10 mg/kg, i.p.)

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