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Research paper

Cocaine self-administration augments kappa opioid receptor system-mediated inhibition of dopamine activity in the mesolimbic dopamine system

Paige M. Estave, Haiguo Sun, Emily G. Peck, Katherine M. Holleran, Rong Chen, Sara R. Jones *

Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27101, USA

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ABSTRACT

Prior studies examining the effects of cocaine on the dynorphin/kappa opioid receptor (Dyn/KOR) system primarily focus on non-contingent cocaine exposure, but the effects of self-administration, which more closely reflects human drug-taking behaviors, are not well studied. In this study we characterized the effects of escalated intravenous cocaine self-administration on the functional state of the Dyn/KOR system and its interaction with mesolimbic dopamine signaling. Rats self-administered cocaine in an extended access, limited intake cocaine procedure, in which animals obtained 40 infusions per day (1.5 mg/kg/inf) for 5 consecutive days to ensure comparable consumption levels. Following single day tests of cue reactivity and progressive ratio responding, quantitative real-time polymerase chain reaction was used to measure levels of *Oprk* and *Pdyn* transcripts in the ventral tegmental area and nucleus accumbens. Additionally, after self-administration, ex vivo fast-scan cyclic voltammetry in the NAc was used to examine the ability of the KOR agonist U50,488 to inhibit dopamine release. We found that KOR-induced inhibition of dopamine release was enhanced in animals that self-administered cocaine compared to controls, suggesting upregulated Dyn/KOR activity after cocaine self-administration. Furthermore, expression levels of *Pdyn* in the nucleus accumbens and ventral tegmental area, and *Oprk* in the nucleus accumbens, were elevated in cocaine animals compared to controls. Additionally, *Pdyn* expression in the nucleus accumbens was negatively correlated with progressive ratio breakpoints, a measure of motivation to self-administer cocaine. Overall, these data suggest that cocaine self-administration elevates KOR/Dyn system activity in the mesolimbic dopamine pathway.

Introduction

The dynorphin/kappa opioid receptor (Dyn/KOR) system plays an important role in regulation of reward, stress and pain (for review see Estave et al., 2020 and Paton et al., 2020). $G_{i/o}$-coupled KORs are found ubiquitously throughout the central nervous system, with a notable density in the mesolimbic dopamine (DA) pathway projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) (Mansour et al., 1988; Slowe et al., 1999). Behavioral studies have shown an upregulation of the Dyn/KOR system after chronic intake of drugs including opioids, alcohol, and psychostimulants (for review, see Wee and Koob, 2010; Koob, 2021). For example, repeated cocaine exposure increases KOR binding in limbic brain regions of humans, nonhuman primates, and rodents (Daunais et al., 1993, 1995; Hurd and Herkenham, 1993; Unterwald et al., 1994; Spangler et al., 1996; Fagergren et al., 2003; Frankel et al., 2008). However, most of these preclinical studies utilize non-contingent cocaine exposure, which (1) does not reflect human drug-taking behaviors (Panlilio and Goldberg, 2007); (2) induces a stress state that can impact results (Ploense et al., 2018); and (3) causes differential alterations in gene expression (Stefański et al., 2007; Fumagalli et al., 2013), protein expression and/or function (Caffino et al., 2014), and DA activity (Hemby et al., 1997; Stuber et al.,

Abbreviations: Dyn/KOR, dynorphin/kappa opioid receptor; DA, dopamine; qPCR, quantitative real-time polymerase chain reaction; NAc, nucleus accumbens; VTA, ventral tegmental area; FSCV, ex vivo fast-scan cyclic voltammetry.

* Correspondence to: Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC 27157, USA.

E-mail address: srjones@wakehealth.edu (S.R. Jones).

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2005). Therefore, this study examined the effects of cocaine self-administration on Dyn/KOR activity in the mesolimbic DA system.

Dyn/KOR hyperactivity is involved in behavioral dysregulation after cocaine self-administration. For example, pharmacological KOR blockade prevents stress-induced reinstatement of cocaine seeking (Beardsley et al., 2005), decreases cocaine breakpoints on a progressive ratio schedule of reinforcement (Wee et al., 2009), and attenuates anxiety- and depressive-like behaviors after chronic cocaine self-administration in rats (Valenza et al., 2016). These behavioral outcomes may be due to alterations in either endogenous dynorphin tone or responsiveness of KORs. PET imaging of the striatum in active cocaine users showed that higher baseline KOR binding availability correlated with greater cocaine choice (Martinez et al., 2019). Indeed, after cocaine overdose, human postmortem studies showed an upregulation of both mRNA encoding prodynorphin (the precursor peptide for dynorphin) and autoradiographic KOR binding in the striatum (Hurd and Herkenham, 1993; Staley et al., 1997; Mash and Staley, 1999).

When KORs are activated by dynorphin or exogenous agonists, DA release is suppressed through multiple mechanisms ultimately leading to subsequent terminal hyperpolarization (for review, see Margolis and Karkhanis, 2019). This inhibition of DA release after chronic cocaine self-administration may contribute to a hypofunctioning DA system (for review, see Trifilieff and Martinez, 2013), and dopamine hypofunction is involved in the reward deficits and negative affective states seen during drug withdrawal, which may drive further cocaine use and promote relapse (for review see Koob et al., 2014 and Koob, 2021). While there is evidence that the Dyn/KOR system is upregulated after chronic, repeated cocaine exposure (for review see, Shippenberg et al., 2007; Chavkin and Koob, 2016), the mechanisms through which the Dyn/KOR system contributes to cocaine-induced DA hypofunction are not well understood.

In this study, we examine how the Dyn/KOR system in both the VTA and NAc adapts after a high-dose, binge-like cocaine self-administration procedure that produces an escalation of intake and neurobiological changes associated with cocaine use disorder (Ferris et al., 2011, 2012; Calipari et al., 2013, 2014). Further, to better understand the functional state of the Dyn/KOR system following cocaine self-administration, we used fast-scan cyclic voltammetry (FSCV) in brain slices to measure the ability of KOR agonism to inhibit DA release in the NAc. In another subset of animals, we measured gene expression levels of *Oprk* and *Pdyn* encoding KOR and prodynorphin, respectively, after cocaine self-administration and correlated these mRNA levels with behavioral measures taken in the same animals. Overall, these studies provide a more comprehensive view of Dyn/KOR and DA system interactions and dysregulation after contingent cocaine exposure.

Methods

Animals

Male Sprague-Dawley rats (n = 21, 9–12 per group; 325–400 g; Envigo, Indianapolis, IN) were maintained on a 12:12 h light/dark cycle (lights on at 1500) with food and water ad libitum. The Institutional Animal Care and Use Committee at Wake Forest School of Medicine approved the experimental protocol, and all animals were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities.

Drugs

For FSCV, Cocaine HCl and U50,488 were acquired from National Institute on Drug Abuse Drug Supply Program (Bethesda, MD) and dissolved in deionized water. For behavior, Cocaine HCl was dissolved in sterile saline.

Catheter implantation

Rats were anesthetized with ketamine (100 mg/kg, i.p) and xylazine (8 mg/kg, i.p.) and given ketoprofen (5 mg/kg, s.c.) for analgesia before being implanted with a chronic indwelling jugular catheter as described previously (Liu et al., 2005). Following surgery, animals were individually housed in 30 × 30 × 30 cm custom-made operant conditioning chambers, which served as both a housing and experimental chamber as described previously (Siciliano et al., 2019). All self-administration sessions occurred in the active/dark cycle (0900–1500). The first cocaine self-administration session began 3 days after surgery.

Cocaine self-administration: extended access, limited intake procedure

Without prior operant training, rats were given access to cocaine (1.5 mg/kg/infusion over approximately 4 s depending on animal weight) on a fixed-ratio one (FR1) schedule of reinforcement. Each response resulted in an infusion of cocaine, retraction of the lever and illumination of a stimulus light for the duration of the infusion. Sessions were terminated when 40 infusions were reached or 6 h elapsed, whichever occurred first. Acquisition was considered met when the animals administered 40 infusions of cocaine within a 6 h session. Animals were required to complete at least 4 additional days of 40 infusions per day, for a total of 5 consecutive days. A subset of animals were sacrificed the following morning (~0900) for FSCV experiments (approximately 18 h after the last self-administration session) or quantitative real-time PCR (described below). All control animals in this study were implanted with a port on the animal's dorsum, allowing animals to be tethered in cages similar to cocaine counterparts to control for stress exposures including anesthesia, surgery, tethering and housing conditions (hearing pump sounds, animal activity).

Cue reactivity task

Eighteen hours after the last cocaine self-administration session, animals were tested for cue reactivity. In this task, lever presses resulted in illumination of a cue light, pump noise and retraction of a lever on a FR1 schedule; however, saline was delivered in place of cocaine. Sessions lasted for 6 h, and the total number of lever presses was recorded.

Cocaine self-administration on a progressive ratio schedule of reinforcement

One day after the cue reactivity task, animals were allowed to self-administer cocaine on a progressive ratio schedule of reinforcement (PR, 0.19 mg/kg/infusion of cocaine) for one day. A single day of testing was used since breakpoints have been shown to be stable over time after animals self-administer high doses of cocaine, as in the extended access, limited intake procedure used here (Morgan et al., 2006; Liu et al., 2007). During progressive ratio schedule of reinforcement, response requirements systematically increased following each earned reinforcer in the following ratio sequence: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, etc. as described previously (Richardson and Roberts, 1996). Breakpoints were defined as the number of reinforcers earned prior to one hour elapsing without completion of the next response ratio requirement (Richardson and Roberts, 1996).

Ex vivo fast scan cyclic voltammetry (FSCV)

FSCV was used to examine changes in DA dynamics in rat NAc core following completion of extended access, limited intake procedure. Approximately 18 h after the last self-administration session (0900–1000), animals were rapidly decapitated, brains were removed and immediately transferred to ice-cold artificial cerebrospinal fluid. A vibrating tissue slicer (Leica VT1200S, Leica Biosystems, Wetzler, Germany) was used to prepare 400 μM thick coronal brain slices containing

the NAc core (see Mauterer et al., 2018 for details). Brain slices were transferred to recording wells containing 32°C oxygenated ASCF flowing at 1 mL/min. A carbon fiber microelectrode (150–200 μm length, 7 μm diameter) and a bipolar stimulating electrode were positioned into the NAc core. A single electrical pulse (750 μA, 4 msec, monophasic) was applied every 5 min to evoke DA release. The extracellular DA concentration was recorded by applying a triangular waveform (−0.4 to +1.2 to −0.4 V vs. Ag/AgCl, at a rate of 400 V/s) to the carbon fiber microelectrode. Once DA release was stable, a concentration-response curve of U50,488 (0.01, 0.03, 0.10, 0.30, 1.0, 3.0 μM) was obtained by adding the KOR agonist cumulatively to the slice superfusion buffer at approximately 45 min intervals, or after the effect of the drug on DA release was stable.

Quantitative Real-time PCR (qPCR)

Animals were anesthetized with isoflurane and rapidly decapitated approximately 18 h after the PR cocaine self-administration session. The NAc and VTA were immediately collected using a rat brain block and stored at − 80 °C until tissue processing. One side of NAc or VTA tissue was homogenized in TRIzol, and the total RNA was extracted using an RNeasy isolation kit (Qiagen, Valencia, CA, USA). The purity and quantity were determined using a NanoDrop 2000 Spectrometer (Thermo Fisher Scientific, Wilmington, DE, USA). The total RNA (1 μg) was reverse transcribed to a single-stranded cDNA using a cDNA Reverse Transcription Kit (Invitrogen). The sequences of oligonucleotide primers for amplification of the opioid genes were described previously (Sun et al., 2020): *Oprk* forward primer: 5′-TCCCTGGTCATGTTTGTCATC-3′; *Oprk* reverse primer: 5′-TGGAAGGGCATAGTGGTAGTA-3′), *Pdyn* forward primer: 5′-CTTGGAGAATGAGGTTGCTTTG-3′; *Pdyn* reverse primer: 5′-GAGACGCTGGTAAGGAGTTG-3′), and housekeeping gene *Gapdh* forward primer: 5′-TGATGCTGGTGCTGAGTATGTCGT-3′; *Gapdh* reverse primer: 5′-TTCTCGTGGTTCACACCCATCACA-3′. The primers were synthesized by Integrated DNA technologies (Coralville, IA, USA).

To determine the mRNA levels of *Oprk* and *Pdyn*, qPCR was conducted with Fast SYBR Green Master Mix (Invitrogen) in a 96-well format using an ABI 7500 Fast real-time PCR System (Applied Biosystems, Forester City, CA, USA). The PCR reaction mixture contained primers (100 nM) and transcribed cDNA (10 ng) in a total volume of 20 μl. Samples were denatured first at 95 °C for 10 min followed by 36 cycles of PCR (15 s at 95 °C, 20 s at 60 °C and 15 s at 72 °C). Control and cocaine self-administration samples were run concurrently on the same 96-well plate, and each sample was run in triplicate. The mRNA levels of *Oprk* and *Pdyn* were normalized to the housekeeping gene *Gapdh*, which is not altered by cocaine self-administration (Graham and Self, 2010). The relative fold change was calculated using the ΔΔCt method (Livak and Schmittgen, 2001). Data are presented as relative to controls.

Data analysis

Demon Voltammetry and Analysis software was used to evaluate evoked DA release and reuptake kinetics. Recording electrodes were calibrated by recording current responses to a known concentration of DA (3 μM), which was used to convert current (nA) to DA concentration (μM). Michaelis–Menten based modeling was then used to determine the amount of stimulated DA release and the maximal rate of DA uptake (V_{max}). See (Yorgason et al., 2011) for further details.

All analyses were conducted using GraphPad Prism 8 (Graph Pad Software, La Jolla, CA) and R software (Vienna, Austria). Data are presented as mean ± standard error, and significance level is set at $p < 0.05$. When appropriate, Mauchly's Test of Sphericity was used to determine if data violated the assumption of sphericity, and Greenhouse–Geisser correction was used if data violated this assumption. For cocaine self-administration, a mixed effects model with repeated measures was used to determine if there was a significant reduction in the inter-infusion interval and, similarly, the rate of infusions per hour across session days (n = 12). Since a single data point was missing, these data were analyzed using a mixed effects model instead of an ANOVA. This missing value was attributed to a random occurrence (software malfunction) and not a function of animal responding. Posthoc Dunnett's multiple comparisons tests was used to evaluate whether there were any differences in these parameters between sessions on days 2–5 and day 1. For FSCV, a Student's t-test was conducted to compare baseline DA release and the uptake rate between cocaine-exposed (n = 21 slices) and naïve rats (n = 36 slices). Concentration-response curves were then subjected to a two-way ANOVA with repeated measures, with concentration as the within-subject factor and experimental group as the between-subjects factor. DA release was the dependent variable. Two-way ANOVAs were followed by targeted pairwise comparisons between groups using a Sidak post hoc test. A Student's t-test was used to determine if there were a difference in maximal effect of U50,488 between cocaine and control animals. Due to unequal variance between groups, a Welch's T-test was used to determine if there were a difference in potency. For mRNA, a Student's t-test was conducted to determine differences between cocaine-exposed and control rats (n = 6–7 per group). Lastly, Pearson's correlation coefficient was used to measure the strength of correlations between mRNA levels and cue reactivity responding or progressive ratio breakpoint.

Results

Escalation of the rate of cocaine intake

We have shown previously that 5 days of 40 cocaine infusions (1.5 mg/kg/inf) on an FR1 schedule in a 6-hr session results in an escalated rate of intake across cocaine self-administration sessions (Ferris et al., 2011, 2012; Calipari et al., 2013, 2014). This escalation can be expressed as a decrease in the average inter-infusion interval between the 40 infusions, an increased rate of infusions per hour, or a decrease in the length of time to complete 40 infusions. A mixed effects model with repeated measures revealed a significant main effect of cocaine session on the inter-infusion interval ($F_{(4, 43)} = 10.16$, $p < 0.0001$, Fig. 1 C) and the rate of infusions per hour ($F_{(4, 43)} = 8.147$, $p < 0.0001$, Fig. 1D). Posthoc Dunnett's multiple comparisons tests revealed a significant decrease in the inter-infusion intervals on days 2–5 when compared to day 1 (day 2, $p = 0.0086$; day 3, $p = 0.0003$; Day 4, $p < 0.0001$; Day 5, $p < 0.0001$; Fig. 1 C). There was also a significant increase in the rate of infusions on days 3–5 when compared to day 1 (day 3, $p = 0.0044$; day 4, $p = 0.0003$; day 4, $p < 0.0001$, Fig. 1D).

Increased kappa opioid receptor activity after IV cocaine self-administration

Pre-drug baseline DA release and uptake parameters were analyzed to evaluate differences in DA kinetics after 5 days of 40 injections (FR1, 1.5 mg/kg/inf per day, 6 hr sessions) cocaine self-administration as shown in Fig. 1. There were no significant differences in baseline stimulated DA release ($t_{(55)} = 1.657$, $p = 0.1033$; Fig. 2B) or the rate of maximal uptake ($t_{(55)} = 0.2092$, $p = 0.8351$; Fig. 2 C). A two-way ANOVA with repeated measures revealed a main effect of cocaine self-administration on inhibition of DA release in response to KOR agonist U50,488, indicating that cocaine self-administration increases responsivity of the KOR system in the NAc core (seen as an increase in inhibition of DA release; $F_{(1,14)} = 10.51$, $p = 0.0059$; Fig. 2D). Sidak's multiple comparisons test revealed a greater inhibition of DA release by U50,488 treatment at 0.01 μM ($p = 0.013$), 0.03 μM ($p = 0.0143$) and 0.1 μM ($p = 0.0251$) in cocaine self-administration animals. This increase in inhibition of DA release by KOR activation appears to be due to a shift in the maximal effect ($t_{(14)} = 2.684$, $p = 0.0178$; Fig. 2E), and possibly a shift in IC50, or dose that induces 50% inhibition, though not significant ($t_{(10.21)} = 2.142$, $p = 0.0573$; Fig. 2F).

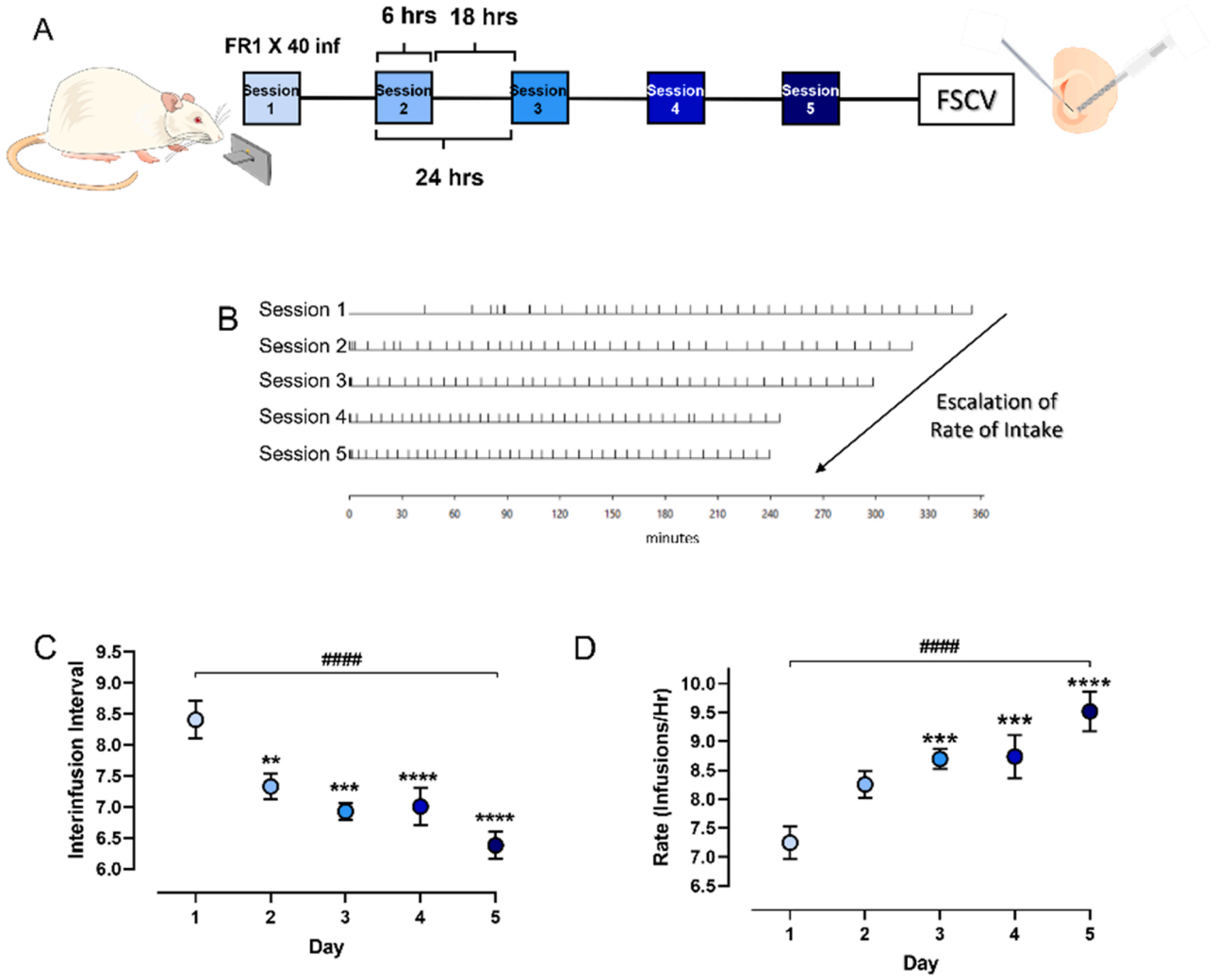

Fig. 1. Escalation of cocaine intake on an extended access, limited intake procedure. A) Schematic of experimental timeline. B) Event record across 5 sessions of a representative animal self-administering cocaine (1.5 mg/kg/inf, 40 infusions). Each vertical tick mark represents a contingent infusion of cocaine. Escalation of cocaine intake can be seen across the five days, with the animals self-administering 40 infusions in shorter session lengths each day. C) Significant decrease in the interinfusion interval (time between each infusion) across 5 days. D) Significant escalation of rate of intake (infusions/hour) across 5 days. ($n = 12$; $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). Main effect: #### $p < 0.0001$ Data shown as mean ± SEM. FR1, fixed-ratio one; inf, infusion; FSCV, fast-scan cyclic voltammetry.

Increased expression levels of Oprk and Pdyn in the mesolimbic DA system after cocaine self-administration

After animals completed 5 days of 40 cocaine infusions (FR1, 1.5 mg/kg/inf, 6 hr sessions), followed by 1 day of cue reactivity (and 1 day of progressive ratio (Fig. 3A, B, C), expression levels of mRNA encoding the kappa opioid receptor (*Oprk)* and the dynorphin precursor, prodynorphin (*Pdyn),* were determined by qPCR analyses (Fig. 3). Cocaine self-administration significantly increased *Oprk* mRNA in the NAc ($t_{(11)}= 4.998$, $p = 0.0004$; Fig. 3D), with no significant change in the VTA ($t_{(12)}= 1.851$, $p = 0.089$; Fig. 3E) compared to controls. Cocaine self-administration significantly increased *Pdyn* mRNA levels in the NAc ($t_{(12)}= 4.561$, $p = 0.0007$; Fig. 3F) and VTA ($t_{(11)}= 2.432$, $p = 0.0333$; Fig. 3G).

Pdyn mRNA levels are negatively correlated with cocaine breakpoints on PR

We examined whether there were correlations between *Oprk* or *Pdyn* mRNA levels and drug seeking behaviors using Pearson's correlation coefficient analysis. Drug seeking behaviors included cue reactivity (measured by lever presses during a session when saline was substituted for cocaine; cues included cue light, pump noise and lever retraction after press on a FR1 schedule, to measure seeking in the absence of drug) on Day 1, and cocaine breakpoints (measured by number of reinforcers earned during a session with increasing response requirements for each injection of 0.19 mg/kg/inf cocaine, to measure motivation to self-administer cocaine) on Day 2 following 5 days of self-administration (see Fig. 3) in the same animals as Fig. 3. The mRNA levels of *Pdyn,* but not *Oprk,* in the NAc were negatively associated with breakpoints on a PR schedule of reinforcement ($r = -0.85$, $r^2 = 0.73$, $F_{(1,5)}= 13.54$, $p = 0.0143$), suggesting that animals with lower cocaine breakpoints at the 0.19 mg/kg/inf dose tested here are predicted to have higher levels of *Pdyn* mRNA in the NAc (Fig. 4B). Breakpoints were not significantly correlated with *Oprk* or *Pdyn* mRNA levels in the VTA (Fig. 4 C,G). The total number of lever presses during the cue reactivity session was not correlated with *Oprk* or *Pdyn* mRNA levels in the VTA or the NAc (Fig. 4D,E,H,I).

Discussion

This study explored the state of the Dyn/KOR system in the NAc and VTA of rats after high-dose, binge-like cocaine self-administration by examining KOR activity in the NAc using FSCV, and by measuring *Oprk*

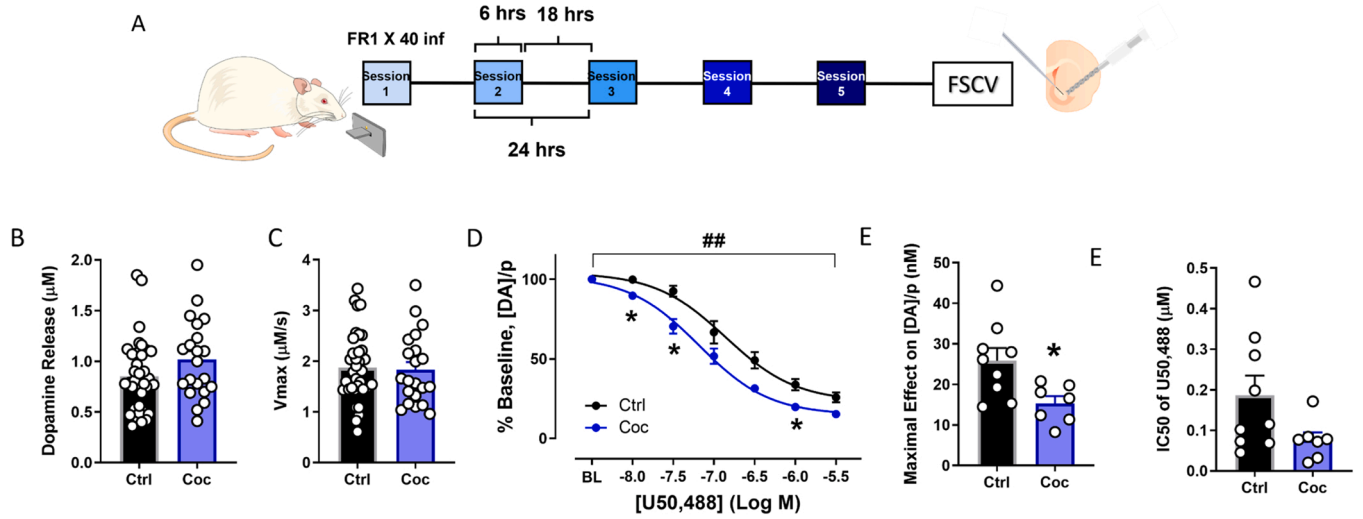

Fig. 2. Increased kappa opioid receptor system function after cocaine self-administration. A) Schematic of experimental timeline. B) Average evoked dopamine release (μM) in the control group (n = 36, black) versus the cocaine group (n = 21, blue). C) Average maximal rate of dopamine uptake (Vmax; Ctrl, n = 36; Coc, n = 21); D) Group data showing that the cocaine group had greater response to KOR agonist U50,488 (Ctrl, n = 9; Coc, n = 7). E) Increased efficacy of U50,488 in the cocaine group, defined by maximal effect on dopamine per pulse ([DA]/p). F) No significant change in potency of U50,488 between groups, defined by IC50. *p < 0.05. Main effect: ## p < 0.01. Data shown as mean ± SEM. FR1, fixed-ratio one; inf, infusion; FSCV, fast-scan cyclic voltammetry; DA, dopamine. Coc, cocaine; Ctrl, control.

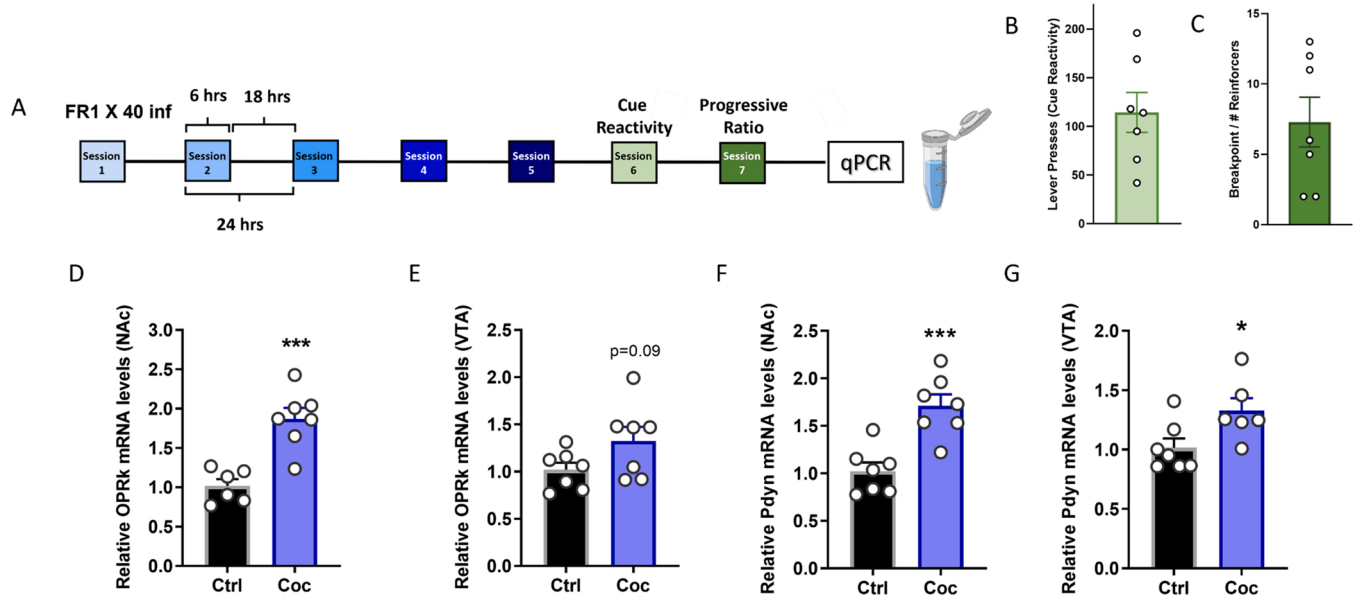

Fig. 3. Effect of contingent cocaine self-administration on mRNA levels of the kappa opioid receptor and prodynorphin genes in the NAc and VTA. A) Schematic of experimental timeline. B) Total lever presses accumulated during cue reactivity session (n = 7) and C) breakpoint during progressive ratio schedule of reinforcement (n = 7). D) Cocaine self-administration (n = 7, blue) significantly increased *Oprk* in the Nac compared to controls (n = 6, black). C) Cocaine self-administration (n = 7, blue) resulted in a modest increase in the VTA compared to controls (n = 7, black). D) Cocaine self-administration (blue, n = 7) significantly increased *Pdyn* in the NAc compared to controls (n = 7, black). E) Cocaine self-administration (n = 6, blue) increased *Pdyn* in the VTA compared to controls, though not significantly (n = 7, black). *p < 0.05, * **p < 0.001. Data was normalized to *Gapdh* gene and shown as mean ± SEM. FR1, fixed-ratio one; inf, infusion; qPCR, quantitative real-time PCR; Coc, cocaine; Ctrl, control; NAc, nucleus accumbens; VTA, ventral tegmental area.

and *Pdyn* mRNA levels in the NAc and VTA. This is the first report to employ FSCV in brain slices to show alterations in the Dyn/KOR system after cocaine self-administration. Additionally, our unique extended access, limited intake self-administration procedure standardizes cocaine consumption across animals, removing variations in cocaine history as a confound and thus decreasing subsequent variability in neurobiological outcomes. This is the first demonstration that inhibition of DA release by KOR activation in the NAc is augmented by cocaine self-administration in rats. Additionally, unlike expected compensatory adaptations between receptor and ligand, in which receptor levels are reduced in the presence of excess ligand, we show here that both *Pdyn* and *Oprk* mRNA levels were elevated in the NAc following cocaine self-administration. Together, these findings suggest heightened activity of the Dyn/KOR system after cocaine self-administration in rats, which could contribute to hypodopaminergia and thereby promote the progression of addiction-like behaviors.

Dynorphin-containing neurons and KORs are widely distributed in the limbic system, including the NAc and VTA, among other regions

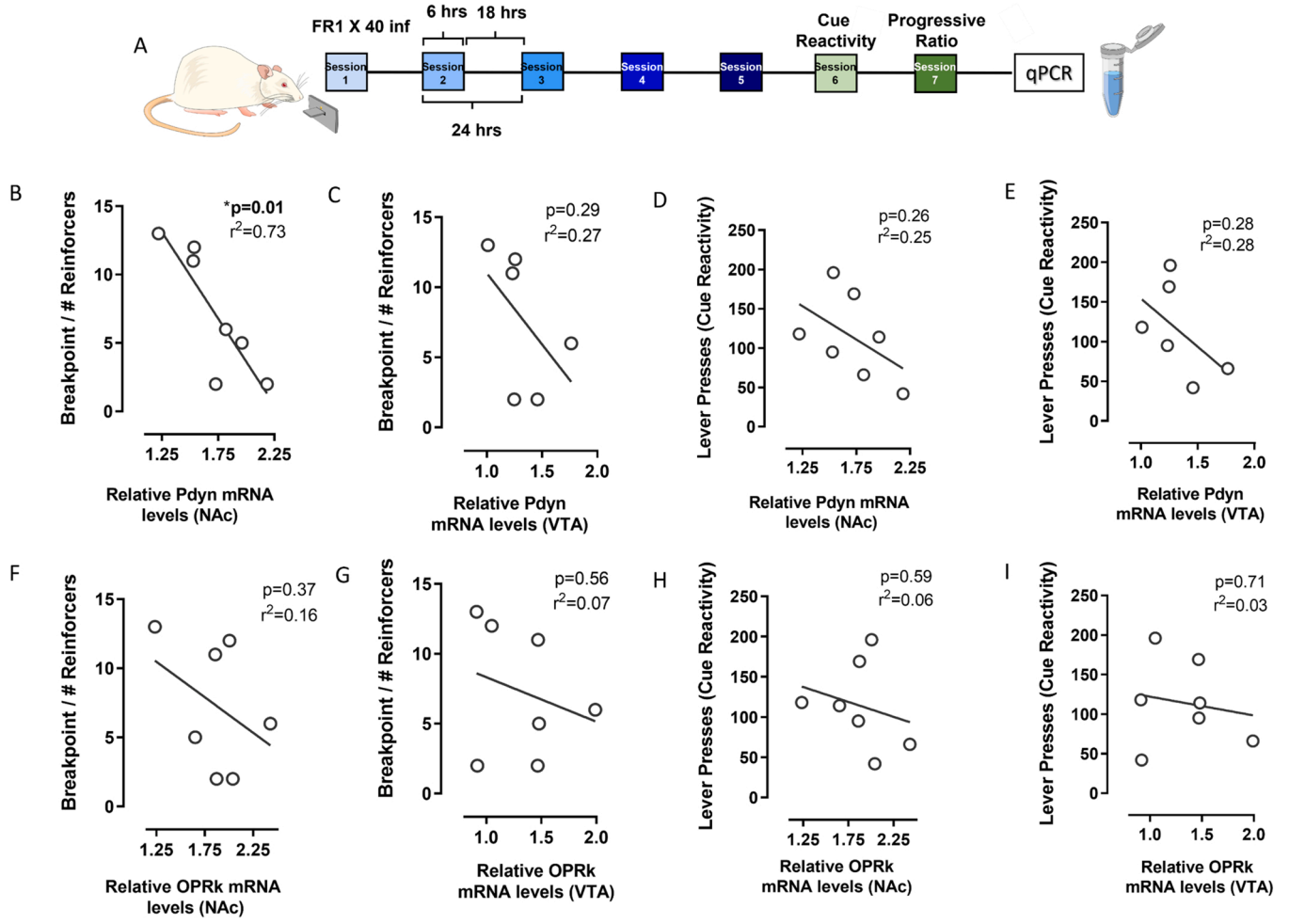

Fig. 4. Correlations between *Pdyn* and *Oprk* mRNA levels and operant self-administration behaviors. A) Schematic of experimental timeline. B, C) Relative *Pdyn* mRNA levels in the NAc but not the VTA correlate with cocaine breakpoints on a progressive ratio schedule of reinforcement (0.1875 mg/kg/inf). D, E) Relative *Pdyn* mRNA levels in the NAc or VTA do not correlate with cue reactivity responses. F-I) Relative *Oprk* mRNA levels in the NAc or VTA do not correlate with breakpoints or cue reactivity responses. *p < 0.05. mRNA levels were normalized to *Gapdh* gene. FR1, fixed-ratio one; inf, infusion; qPCR, quantitative real-time PCR; NAc, nucleus accumbens; VTA, ventral tegmental area.

(Meng et al., 1993; Simonin et al., 1995; Al-Hasani et al., 2015). The Dyn/KOR and DA systems interact with one another, especially within the mesolimbic pathway (Heidbreder et al., 1998; Chefer et al., 2013; Ehrich et al., 2014, 2015). Pharmacological activation of KORs inhibits DA release and decreases extracellular DA levels, as measured by microdialysis (Di Chiara and Imperato, 1988; Spanagel et al., 1992; Zhang et al., 2004; Chefer et al., 2006; Fuentealba et al., 2006; Gehrke et al., 2008; Karkhanis et al., 2016). Upregulation of the Dyn/KOR system after cocaine exposure is thought to be part of a compensatory mechanism to reduce DA levels in response to chronic cocaine-mediated increased dopaminergic activity in the mesolimbic pathway (for review, see Koob 2008). Here we showed that Dyn/KOR activity was heightened after cocaine self-administration by measuring KOR agonist-induced inhibition of electrically-stimulated DA release using FSCV. While we were able to show augmented KOR-induced inhibition of DA release after cocaine self-administration, as well as elevated *Pdyn* and *Oprk* mRNA levels in the NAc, we do not know the location of the increased KORs in the NAc. KORs are located not only on the axonal DA projections from the VTA to the NAc (Svingos et al., 1999), but also on various pre-and post-synaptic sites, including but not limited to cholinergic interneurons as well as serotonergic (Fontaine et al., 2022) and glutamatergic (Svingos et al., 1999; Coleman et al., 2021) afferents.

Previous studies showed that repeated non-contingent cocaine injections increased *Pdyn* and *Oprk* mRNA levels in several mesolimbic brain regions (Mathieu-Kia and Besson, 1998; Turchan et al., 1999), though these results have not been consistently found (Daunais and McGinty, 1995; Rosin et al., 1999; Bailey et al., 2005). The majority of prior studies looked at non-contingent cocaine exposure; therefore, our study aimed to understand potential mechanisms for upregulation of the Dyn/KOR system after cocaine self-administration to better reflect human drug taking-behaviors (Panlilio and Goldberg, 2007) and remove the severe stressor of non-contingent administration (Ploense et al., 2018). Prior studies have also shown that, compared to cocaine self-administration, non-contingent cocaine exposure produces differential alterations in gene expression, protein expression, and DA activity (Stefański et al., 2007; Fumagalli et al., 2013; Caffino et al., 2014; Hemby et al., 1997; Stuber et al., 2005). Since cocaine self-administration itself is a physiological stressor, in addition to daily withdrawal periods, we cannot exclude that the enhanced KOR system activity seen in our study may be due to both cocaine exposure and stress. Moreover, it is possible that stress-induced release of dynorphin due to cocaine is a potential mechanism for the mRNA changes seen in our study. Nevertheless, self-administration is likely less stressful than non-contingent methods, given that the animal anticipates and controls drug exposure, making these findings a better approximation of cocaine-induced gene expression changes.

Additionally, we were interested in the co-regulation of *Pdyn* and *Oprk* in the VTA and NAc to better understand the influence of Dyn/KOR

on the DA system in both the cell bodies and terminals of dopaminergic neurons, since these regions are often differentially regulated. Examining both *Pdyn* and Oprk also allowed us to examine changes in both the ligand and receptor. Prior to this study, *Pdyn* and *Oprk* expression changes in the NAc and VTA after cocaine self-administration had not been measured in the same animals.

In several prior studies, contingent cocaine self-administration in rats showed increased *Pdyn* levels in the caudate and/or NAc (Hurd et al., 1992; Daunais et al., 1993; Ziółkowska et al., 2006; Valenza et al., 2016), consistent with our findings. The same *Pdyn* increase was also found in non-human primate and human studies (Hurd and Herkenham, 1993; Fagergren et al., 2003; Frankel et al., 2008). However, there has only been one study examining *Oprk* mRNA levels after contingent cocaine self-administration, where the MJ Kreek laboratory found that the results differed depending on the brain region and the strain of rats used. *Oprk* mRNA was increased in the dorsal striatum of Lewis but not Fischer rats, and no changes were found in the NAc (Valenza et al., 2016). There were, however, several experimental differences between the present study and the one by Valenza and colleagues (2016), aside from our study using Sprague Dawley rats. Their rats were allowed unlimited access to cocaine for 14 days, 18 h per day, and the doses were chosen individually by each rat ("subject-controlled unit dose selection"), varying from 0.2 to 2.5 mg/kg/inf. In our study, we used 6-hour access sessions with a cocaine dose of 1.5 mg/kg/inf for 5 days, and we limited consumption so that each rat took the same amount every day (40 inf or 60 mg/kg), dramatically reducing between-subject variability compared to Valenza et al. (2016). Together, these studies suggest that results can vary depending on strain, schedule and total consumption of cocaine.

To further understand the impact of alterations in KORs and Dyn on behavioral outcomes, cocaine reinforcement and seeking behaviors were examined. While KOR antagonists consistently decrease cocaine's behavioral effects (for review see Reed et al., 2020, but see Beardsley et al., 2005), activating the KOR system can either augment or inhibit cocaine's effects, in a complex time-, schedule- and context-sensitive manner (for review see Escobar et al., 2020). One potential mechanism that could result in paradoxical effects is cocaine's regulation of CREB-mediated transcription of dynorphin in the NAc, which is known to influence the rewarding properties of cocaine. Aligning with the work of Carlezon and colleagues, cocaine could induce CREB overexpression, elevating Pdyn levels and decreasing cocaine breakpoints, suggesting reduced reinforcing effects of cocaine (Carlezon et al., 1998).

The NAc is a key region involved in motivated behaviors and thought to be involved in symptoms of major depressive disorder such as anhedonia, where there is loss of reward function (Russo and Nestler, 2013). As discussed previously, the KOR/Dyn system is a known neuromodulator of the NAc. Interestingly, there are subpopulations of dynorphin-containing neurons in the NAc shell that are involved in aversive (ventral shell) vs reinforcing behaviors (dorsal shell)(Al-Hasani et al., 2015). The mRNA data presented here from the NAc contains tissue from both the shell and core, which may have influenced our correlation results between motivated behaviors and mRNA levels. Further investigation will be necessary to understand the complex relationships between *Pdyn*, *Oprk* and cocaine's behavioral effects.

Understanding how the interaction between Dyn/KOR and DA systems is altered following stress and drug exposure is an expanding field, and additional mechanistic studies are necessary to further examine co-regulation of these two systems. For example, monitoring extracellular levels of dynorphin in real time would add to the current state of the literature, but methodologies are limited. The Kennedy laboratory is making headway in analytical techniques to allow rapid measurements of dynorphin using microdialysis and liquid chromatography/mass spectrometry, but these methods are not currently widely available (Li et al., 2009; Zhou et al., 2013; Al-Hasani et al., 2018). The Chavkin laboratory has recently used the sensor kLight1.2a to detect both pharmacological and endogenously released KOR ligands in mice (Abraham et al., 2021), which would be useful to determine if there is upregulation of DYN after cocaine self-administration. Though several KOR antibodies have been created, they may lack specificity, as is often the case with antibodies against G-protein coupled receptors (for review, see Michel et al., 2009). A new knock-in mouse line was recently created that has a fluorescent protein fused to the KOR, bypassing some of the issues with KOR antibodies (Chen et al., 2020).

Due to the limitations of utilizing KOR antibodies, we focused on changes in mRNA levels; future work will be necessary to examine if these changes translate to alterations in protein levels. However, in the present study we examined the function of KORs, which is arguably the most important measure of change in a system. Using FSCV, we documented an increase in the ability of the KOR agonist, U50,488 to inhibit DA release in the NAc, with a greater maximal effect in the cocaine group. More studies are necessary to determine if there is a difference in receptor number and/or intrinsic activity of the receptor underlying these functional changes. Albeit not addressed in this study, we are very interested in the potential sex differences of the Dyn/KOR system, and how these sex differences may alter dopamine release and impact subsequent behavior. Although *Oprk* and *Pdyn* mRNA levels do not differ between male and female rats, KOR-mediated inhibition of dopamine release was shown to be blunted in female rats (Conway et al., 2019). A review by Chartoff and Mavrikaki (2015) explored the sex differences in the Dyn/KOR system and its impact on analgesia, affect, and addiction in both clinical and pre-clinical models. Further research comparing the Dyn/KOR system and its interactions with the dopamine system between males and females will hopefully be insightful into why addiction is differentially experienced between sexes.

In conclusion, our results show that the Dyn/KOR system is upregulated in rats after binge-like cocaine self-administration. In addition to *Oprk* and *Pdyn* mRNA levels being elevated in the NAc, we showed that KOR activity in the NAc is also augmented. While we only see a trend for an increase in *Oprk* in the VTA, a significant effect may be masked by the low sample size. Additional studies will be necessary to fully understand the relationship between KOR and DYN expression and how this relates to behavioral outcomes. Nonetheless, these results provide support to continue examining the Dyn/KOR system in relation to the DA system, and how these two systems can be targeted to treat cocaine use disorder.

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CRediT authorship contribution statement

Paige M. Estave: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, **Haiguo Sun:** Methodology, Investigation, Formal analysis, **Emily G. Peck:** Investigation, **Katherine M. Holleran:** Formal analysis, Writing – review & editing, Visualization, Supervision, **Rong Chen:** Methodology, Formal analysis, Writing – review & editing. **Sara R. Jones:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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References

Abraham, A.D., Casello, S.M., Schattauer, S.S., Wong, B.A., Mizuno, G.O., Mahe, K., Tian, L., Land, B.B., Chavkin, C., 2021. Release of endogenous dynorphin opioids in the prefrontal cortex disrupts cognition. Neuropsychopharmacol. Publ. Am. Coll. Neuropsychopharmacol. 46, 2330–2339.

Al-Hasani, R., McCall, J.G., Shin, G., Gomez, A.M., Schmitz, G.P., Bernardi, J.M., Pyo, C.-O., Park, S.I., Marcinkiewcz, C.M., Crowley, N.A., Krashes, M.J., Lowell, B.B., Kash, T.L., Rogers, J.A., Bruchas, M.R., 2015. Distinct subpopulations of nucleus accumbens dynorphin neurons drive aversion and reward. Neuron 87, 1063–1077.

Al-Hasani R., Wong J.-M.T., Mabrouk O.S., McCall J.G., Schmitz G.P., Porter-Stransky K. A., Aragona B.J., Kennedy R.T., Bruchas M.R. , 2018 In vivo detection of optically-evoked opioid peptide release Kauer J.A., Dulac C., Kauer J.A., Evans C., Mains R.E., eds. eLife 7:e36520.

Bailey, A., Yuferov, V., Bendor, J., Schlussman, S.D., Zhou, Y., Ho, A., Kreek, M.J., 2005. Immediate withdrawal from chronic "binge" cocaine administration increases μ-opioid receptor mRNA levels in rat frontal cortex. Mol. Brain Res 137, 258–262.

Beardsley, P.M., Howard, J.L., Shelton, K.L., Carroll, F.I., 2005. Differential effects of the novel kappa opioid receptor antagonist, JDTic, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats. Psychopharmacology 183, 118–126.

Caffino, L., Cassina, C., Giannotti, G., Orrù, A., Moro, F., Di Clemente, A., Racagni, G., Fumagalli, F., Cervo, L., 2014. Short-term abstinence from cocaine self-administration, but not passive cocaine infusion, elevates αCaMKII autophosphorylation in the rat nucleus accumbens and medial prefrontal cortex. Int J. Neuropsychopharmacol. 17, 323–329.

Calipari, E.S., Ferris, M.J., Jones, S.R., 2014. Extended access cocaine self-administration results in tolerance to the dopamine-elevating and locomotor-stimulating effects of cocaine. J. Neurochem 128, 224–232.

Calipari, E.S., Ferris, M.J., Zimmer, B.A., Roberts, D.C.S., Jones, S.R., 2013. Temporal pattern of cocaine intake determines tolerance vs sensitization of cocaine effects at the dopamine transporter. Neuropsychopharmacol. Publ. Am. Coll. Neuropsychopharmacol. 38, 2385–2392.

Carlezon, W.A., Thome, J., Olson, V.G., Lane-Ladd, S.B., Brodkin, E.S., Hiroi, N., Duman, R.S., Neve, R.L., Nestler, E.J., 1998. Regulation of cocaine reward by CREB. Science 282, 2272–2275.

Chartoff, E.H., Mavrikaki, M., 2015. Sex differences in kappa opioid receptor function and their potential impact on addiction. Front Neurosci. 9, 466.

Chavkin, C., Koob, G.F., 2016. Dynorphin, dysphoria, and dependence: the stress of addiction. Neuropsychopharmacology 41, 373–374.

Chefer, V.I., Bäckman, C.M., Gigante, E.D., Shippenberg, T.S., 2013. Kappa Opioid Receptors on dopaminergic neurons are necessary for kappa-mediated place aversion. Neuropsychopharmacology 38, 2623–2631.

Chefer, V.I., Zapata, A., Shippenberg, T.S., Bungay, P.M., 2006. Quantitative no-net-flux microdialysis permits detection of increases and decreases in dopamine uptake in mouse nucleus accumbens. J. Neurosci. Methods 155, 187–193.

Chen C., Willhouse A.H., Huang P., Ko N., Wang Y., Xu B., Huang L.H.M., Kieffer B., Barbe M.F., Liu-Chen L.-Y. (2020) Characterization of a Knock-In Mouse Line Expressing a Fusion Protein of κ Opioid Receptor Conjugated with tdTomato: 3-Dimensional Brain Imaging via CLARITY. eNeuro 7 Available at: ⟨https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7385665/⟩ [Accessed April 2, 2021].

Coleman, B.C., Manz, K.M., Grueter, B.A., 2021. Kappa opioid receptor modulation of excitatory drive onto nucleus accumbens fast-spiking interneurons. Neuropsychopharmacol. Publ. Am. Coll. Neuropsychopharmacol. 46, 2340–2349.

Conway, S.M., Puttick, D., Russell, S., Potter, D., Roitman, M.F., Chartoff, E.H., 2019. Females are less sensitive than males to the motivational- and dopamine-suppressing effects of kappa opioid receptor activation. Neuropharmacology 146, 231–241.

Daunais, J.B., McGinty, J.F., 1995. Cocaine binges differentially alter striatal preprodynorphin and zif/268 mRNAs. Mol. Brain Res 29, 201–210.

Daunais, J.B., Roberts, D.C., McGinty, J.F., 1995. Short-term cocaine self administration alters striatal gene expression. Brain Res Bull. 37, 523–527.

Daunais, J.B., Roberts, D.C.S., McGinty, J.F., 1993. Cocaine self-administration increases preprodynorphin, but not c-fos, mRNA in rat striatum. NeuroReport 4, 543–546.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85, 5274–5278.

Ehrich, J.M., Messinger, D.I., Knakal, C.R., Kuhar, J.R., Schattauer, S.S., Bruchas, M.R., Zweifel, L.S., Kieffer, B.L., Phillips, P.E.M., Chavkin, C., 2015. Kappa opioid receptor-induced aversion requires p38 MAPK activation in VTA dopamine neurons. J. Neurosci. J. Soc. Neurosci. 35, 12917–12931.

Ehrich, J.M., Phillips, P.E.M., Chavkin, C., 2014. Kappa opioid receptor activation potentiates the cocaine-induced increase in evoked dopamine release recorded in vivo in the mouse nucleus accumbens. Neuropsychopharmacology 39, 3036–3048.

Estave, P.M., Spodnick, M.B., Karkhanis, A.N., 2020. KOR control over addiction processing: an exploration of the mesolimbic dopamine pathway. Handb. Exp. Pharm.

Fagergren, P., Smith, H.R., Daunais, J.B., Nader, M.A., Porrino, L.J., Hurd, Y.L., 2003. Temporal upregulation of prodynorphin mRNA in the primate striatum after cocaine self-administration. Eur. J. Neurosci. 17, 2212–2218.

Ferris, M.J., Calipari, E.S., Mateo, Y., Melchior, J.R., Roberts, D.C.S., Jones, S.R., 2012. Cocaine self-administration produces pharmacodynamic tolerance: differential effects on the potency of dopamine transporter blockers, releasers, and methylphenidate. Neuropsychopharmacol. Publ. Am. Coll. Neuropsychopharmacol. 37, 1708–1716.

Ferris, M.J., Mateo, Y., Roberts, D.C.S., Jones, S.R., 2011. Cocaine-insensitive dopamine transporters with intact substrate transport produced by self-administration. Biol. Psychiatry 69, 201–207.

Fontaine, H.M., Silva, P.R., Neiswanger, C., Tran, R., Abraham, A.D., Land, B.B., Neumaier, J.F., Chavkin, C., 2022. Stress decreases serotonin tone in the nucleus accumbens in male mice to promote aversion and potentiate cocaine preference via decreased stimulation of 5-HT1B receptors. Neuropsychopharmacology 47, 891–901.

Frankel, P.S., Alburges, M.E., Bush, L., Hanson, G.R., Kish, S.J., 2008. Striatal and ventral pallidum dynorphin concentrations are markedly increased in human chronic cocaine users. Neuropharmacology 55, 41–46.

Fuentealba, J.A., Gysling, K., Magendzo, K., Andrés, M.E., 2006. Repeated administration of the selective kappa-opioid receptor agonist U-69593 increases stimulated dopamine extracellular levels in the rat nucleus accumbens. J. Neurosci. Res 84, 450–459.

Fumagalli, F., Moro, F., Caffino, L., Orrù, A., Cassina, C., Giannotti, G., Di Clemente, A., Racagni, G., Riva, M.A., Cervo, L., 2013. Region-specific effects on BDNF expression after contingent or non-contingent cocaine i.v. self-administration in rats. Int J. Neuropsychopharmacol. 16, 913–918.

Gehrke, B.J., Chefer, V.I., Shippenberg, T.S., 2008. Effects of acute and repeated administration of salvinorin A on dopamine function in the rat dorsal striatum. Psychopharmacol. (Berl.) 197, 509–517.

Heidbreder, C.A., Schenk, S., Partridge, B., Shippenberg, T.S., 1998. Increased responsiveness of mesolimbic and mesostriatal dopamine neurons to cocaine following repeated administration of a selective kappa-opioid receptor agonist. Synap. N. Y N. 30, 255–262.

Hemby, S.E., Co, C., Koves, T.R., Smith, J.E., Dworkin, S.I., 1997. Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. Psychopharmacol. (Berl.) 133, 7–16.

Hurd, Y.L., Brown, E.E., Finlay, J.M., Fibiger, H.C., Gerfen, C.R., 1992. Cocaine self-administration differentially alters mRNA expression of striatal peptides. Mol. Brain Res 13, 165–170.

Hurd, Y.L., Herkenham, M., 1993. Molecular alterations in the neostriatum of human cocaine addicts. Synapse 13, 357–369.

Karkhanis, A.N., Rose, J.H., Weiner, J.L., Jones, S.R., 2016. Early-life social isolation stress increases kappa opioid receptor responsiveness and downregulates the dopamine system. Neuropsychopharmacology 41, 2263–2274.

Koob, G.F., 2021. Drug addiction: hyperkatifeia/negative reinforcement as a framework for medications development. Pharm. Rev. 73, 163–201.

Koob, G.F., Buck, C.L., Cohen, A., Edwards, S., Park, P.E., Schlosburg, J.E., Schmeichel, B., Vendruscolo, L.F., Wade, C.L., Whitfield, T.W., George, O., 2014. Addiction as a stress surfeit disorder. Neuropharmacology 76 Pt B, 370–382.

Li, Q., Zubieta, J.-K., Kennedy, R.T., 2009. Practical aspects of in vivo detection of neuropeptides by microdialysis coupled off-line to capillary LC with multistage MS. Anal. Chem. 81, 2242–2250.

Liu, Y., Morgan, D., Roberts, D.C.S., 2007. Cross-sensitization of the reinforcing effects of cocaine and amphetamine in rats. Psychopharmacol. (Berl.) 195, 369–375.

Liu, Y., Roberts, D.C.S., Morgan, D., 2005. Sensitization of the reinforcing effects of self-administered cocaine in rats: effects of dose and intravenous injection speed. Eur. J. Neurosci. 22, 195–200.

Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H., Watson, S.J., 1988. Anatomy of CNS opioid receptors. Trends Neurosci. 11, 308–314.

Margolis, E.B., Karkhanis, A.N., 2019. Dopaminergic cellular and circuit contributions to kappa opioid receptor mediated aversion. Neurochem Int 129, 104504.

Martinez, D., Slifstein, M., Matuskey, D., Nabulsi, N., Zheng, M.-Q., Lin, S., Ropchan, J., Urban, N., Grassetti, A., Chang, D., Salling, M., Foltin, R., Carson, R.E., Huang, Y., 2019. Kappa-opioid receptors, dynorphin, and cocaine addiction: a positron emission tomography study. Neuropsychopharmacology 44, 1720–1727.

Mash, D.C., Staley, J.K., 1999. D3 dopamine and kappa opioid receptor alterations in human brain of cocaine-overdose victims. Ann. N. Y Acad. Sci. 877, 507–522.

Mathieu-Kia, A.-M., Besson, M.-J., 1998. Repeated administration of cocaine, nicotine and ethanol: effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. Mol. Brain Res 54, 141–151.

Meng, F., Xie, G.X., Thompson, R.C., Mansour, A., Goldstein, A., Watson, S.J., Akil, H., 1993. Cloning and pharmacological characterization of a rat kappa opioid receptor. Proc. Natl. Acad. Sci. 90, 9954–9958.

Michel, M.C., Wieland, T., Tsujimoto, G., 2009. How reliable are G-protein-coupled receptor antibodies? Naunyn Schmiede Arch. Pharm. 379, 385–388.

Morgan, D., Liu, Y., Roberts, D.C.S., 2006. Rapid and persistent sensitization to the reinforcing effects of cocaine. Neuropsychopharmacology 31, 121–128.

Panlilio, L.V., Goldberg, S.R., 2007. Self-administration of drugs in animals and humans as a model and an investigative tool. Addict. Abingdon Engl. 102, 1863–1870.

Paton, K.F., Atigari, D.V., Kaska, S., Prisinzano, T., Kivell, B.M., 2020. Strategies for developing κ opioid receptor agonists for the treatment of pain with fewer side effects. J. Pharm. Exp. Ther. 375, 332–348.

Ploense, K.L., Vieira, P., Bubalo, L., Olivarria, G., Carr, A.E., Szumlinski, K.K., Kippin, T. E., 2018. Contributions of prolonged contingent and non-contingent cocaine exposure to escalation of cocaine intake and glutamatergic gene expression. Psychopharmacol. (Berl.) 235, 1347–1359.

Reed, B., Butelman, E.R., Kreek, M.J., 2020. Kappa Opioid Receptor antagonists as potential therapeutics for mood and substance use disorders. Handb. Exp. Pharm.
Richardson, N.R., Roberts, D.C., 1996. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J. Neurosci. Methods 66, 1–11.
Rosin, Å., Lindholm, S., Franck, J., Georgieva, J., 1999. Downregulation of kappa opioid receptor mRNA levels by chronic ethanol and repetitive cocaine in rat ventral tegmentum and nucleus accumbens. Neurosci. Lett. 275, 1–4.
Russo, S.J., Nestler, E.J., 2013. The brain reward circuitry in mood disorders. Nat. Rev. Neurosci. 14, 609–625.
Shippenberg, T.S., Zapata, A., Chefer, V.I., 2007. Dynorphin and the pathophysiology of drug addiction. Pharm. Ther. 116, 306–321.
Siciliano, C.A., Mauterer, M.I., Fordahl, S.C., Jones, S.R., 2019. Modulation of striatal dopamine dynamics by cocaine self-administration and amphetamine treatment in female rats. Eur. J. Neurosci. 50, 2740–2749.
Simonin, F., Gavériaux-Ruff, C., Befort, K., Matthes, H., Lannes, B., Micheletti, G., Mattéi, M.G., Charron, G., Bloch, B., Kieffer, B., 1995. kappa-Opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology, and expression pattern in the central nervous system. Proc. Natl. Acad. Sci. 92, 7006–7010.
Slowe, S.J., Simonin, F., Kieffer, B., Kitchen, I., 1999. Quantitative autoradiography of mu-,delta- and kappa1 opioid receptors in kappa-opioid receptor knockout mice. Brain Res. 818, 335–345.
Spanagel, R., Herz, A., Shippenberg, T.S., 1992. Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc. Natl. Acad. Sci. USA 89, 2046–2050.
Spangler, R., Ho, A., Zhou, Y., Maggos, C.E., Yuferov, V., Kreek, M.J., 1996. Regulation of kappa opioid receptor mRNA in the rat brain by 'binge' pattern cocaine administration and correlation with preprodynorphin mRNA. Mol. Brain Res 38, 71–76.
Staley, J.K., Rothman, R.B., Rice, K.C., Partilla, J., Mash, D.C., 1997. Kappa2 opioid receptors in limbic areas of the human brain are upregulated by cocaine in fatal overdose victims. J. Neurosci. J. Soc. Neurosci. 17, 8225–8233.
Stefański, R., Ziółkowska, B., Kuśmider, M., Mierzejewski, P., Wyszogrodzka, E., Kołomańska, P., Dziedzicka-Wasylewska, M., Przewłocki, R., Kostowski, W., 2007. Active versus passive cocaine administration: differences in the neuroadaptive changes in the brain dopaminergic system. Brain Res 1157, 1–10.
Stuber, G.D., Roitman, M.F., Phillips, P.E.M., Carelli, R.M., Wightman, R.M., 2005. Rapid dopamine signaling in the nucleus accumbens during contingent and noncontingent cocaine administration. Neuropsychopharmacology 30, 853–863.
Svingos, A.L., Colago, E.E., Pickel, V.M., 1999. Cellular sites for dynorphin activation of kappa-opioid receptors in the rat nucleus accumbens shell. J. Neurosci. J. Soc. Neurosci. 19, 1804–1813.
Trifilieff, P., Martinez, D., 2013. Kappa-opioid receptor signaling in the striatum as a potential modulator of dopamine transmission in cocaine dependence. Front Psychiatry 4, 44.
Turchan, J., Przewłocka, B., Toth, G., Lasoń, W., Borsodi, A., Przewłocki, R., 1999. The effect of repeated administration of morphine, cocaine and ethanol on mu and delta opioid receptor density in the nucleus accumbens and striatum of the rat. Neuroscience 91, 971–977.
Unterwald, E.M., Rubenfeld, J.M., Kreek, M.J., 1994. Repeated cocaine administration upregulates kappa and mu, but not delta, opioid receptors. Neuroreport 5, 1613–1616.
Valenza, M., Picetti, R., Yuferov, V., Butelman, E.R., Kreek, M.J., 2016. Strain and cocaine-induced differential opioid gene expression may predispose Lewis but not Fischer rats to escalate cocaine self-administration. Neuropharmacology 105, 639–650.
Wee, S., Koob, G.F., 2010. The role of the dynorphin-kappa opioid system in the reinforcing effects of drugs of abuse. Psychopharmacol. (Berl.) 210, 121–135.
Wee, S., Orio, L., Ghirmai, S., Cashman, J.R., Koob, G.F., 2009. Inhibition of kappa opioid receptors attenuated increased cocaine intake in rats with extended access to cocaine. Psychopharmacol. (Berl.) 205, 565–575.
Yorgason, J.T., España, R.A., Jones, S.R., 2011. Demon voltammetry and analysis software: analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. J. Neurosci. Methods 202, 158–164.
Zhang, Y., Butelman, E.R., Schlussman, S.D., Ho, A., Kreek, M.J., 2004. Effect of the kappa opioid agonist R-84760 on cocaine-induced increases in striatal dopamine levels and cocaine-induced place preference in C57BL/6J mice. Psychopharmacol. (Berl.) 173, 146–152.
Zhou, Y., Mabrouk, O.S., Kennedy, R.T., 2013. Rapid preconcentration for liquid chromatography-mass spectrometry assay of trace level neuropeptides. J. Am. Soc. Mass Spectrom. 24, 1700–1709.
Ziółkowska, B., Stefański, R., Mierzejewski, P., Zapart, G., Kostowski, W., Przewłocki, R., 2006. Contingency does not contribute to the effects of cocaine self-administration on prodynorphin and proenkephalin gene expression in the rat forebrain. Brain Res 1069, 1–9.