JPET Fast Forward. Published on December 6, 2022 as DOI: 10.1124/jpet.122.001363
This article has not been copyedited and formatted. The final version may differ from this version.

THE EFFECTS OF THE DOPAMINE TRANSPORTER LIGANDS JJC8-088 AND JJC8-091 ON COCAINE VS. FOOD CHOICE IN RHESUS MONKEYS

Omeed Rahimi[ad], Jianjing Cao[b], Jenny Lam[bc], Steven R. Childers[ad], Rana Rais[c], Linda J. Porrino[a], Amy Hauck Newman[b], Michael A. Nader[a]

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

[b]Medicinal Chemistry Section, Molecular Targets and Medications Discovery Branch, National Institute on Drug Abuse Intramural Research Program, National Institutes of Health, Baltimore, MD, USA

[c]Department of Neurology, Johns Hopkins Drug Discovery, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

[d]EncepHeal Therapeutics, Inc. Winston-Salem, NC, USA

JPET Fast Forward. Published on December 6, 2022 as DOI: 10.1124/jpet.122.001363
This article has not been copyedited and formatted. The final version may differ from this version.

a) Running Title: DAT ligands on Cocaine-Food Choice in Monkeys

b) Address correspondence to:

Michael A. Nader, Ph.D.
Department of Physiology and Pharmacology
Wake Forest University School of Medicine
Medical Center Blvd, NRC 546
Winston-Salem, NC 27157-1083
mnader@wakehealth.edu

c) Contents

Text pages: 16

Tables: 2

Figures: 6

References: 43

Words in Abstract: 250 words

Words in Introduction: 736 words

Words in Discussion: 1280 words

d) Nonstandard Abbreviations: DA, dopamine; DAT, dopamine transporter; FR, fixed-ratio; NHP, nonhuman primate

e) Recommended Section Assignment: **Behavioral Pharmacology**

JPET Fast Forward. Published on December 6, 2022 as DOI: 10.1124/jpet.122.001363
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

While there are no FDA-approved treatments for cocaine use disorder (CUD), several modafinil analogs have demonstrated promise in reducing cocaine self-administration and reinstatement in rats. Furthermore, the range of dopamine transporter (DAT) compounds provides an opportunity to develop pharmacotherapeutics without abuse liability. This study extended the comparison of JJC8-088 and JJC8-091, the former compound having higher DAT affinity and predicted abuse liability, to rhesus monkeys using a concurrent cocaine-food schedule of reinforcement. First, binding to striatal DAT was examined in cocaine-naïve monkey tissue. Next, i.v. pharmacokinetics of both JJC compounds were evaluated in cocaine-experienced male monkeys (n=3/drug). In behavioral studies, acute and chronic administration of both compounds were evaluated in these same monkeys responding under a concurrent food vs. cocaine (0, 0.003-0.1 mg/kg/injection) schedule of reinforcement. In nonhuman primate striatum, JJC8-088 had higher DAT affinity compared to JJC8-091 (14.4 ± 9 vs. 2,730 ± 1,270 nM, respectively). Both JJC compounds had favorable plasma pharmacokinetics for behavioral assessments, with half-lives ($t_{1/2}$) of 1.1 h and 3.5 h for JJC8-088 (0.7 mg/kg, i.v.) and JJC8-091 (1.9 mg/kg, i.v.), respectively. Acute treatment with both compounds shifted the cocaine dose-response curve to the left. Chronic treatment with JJC8-088 decreased cocaine choice in two of the three monkeys, while JJC8-091 only modestly reduced cocaine allocation in one monkey. Differences in affinities of JJC8-091 DAT binding in monkeys compared to rat, may account for the poor rodent-to-monkey translation. Future studies should evaluate atypical DAT blockers in combination with behavioral interventions that may further decrease cocaine choice.

Significance Statement: Cocaine use disorder (CUD) remains a significant public health problem with no FDA-approved treatments. The ability of drugs that act in the brain in a similar manner to cocaine, but with lower abuse liability, has clinical implications for a treatment of CUD.

INTRODUCTION

Cocaine use disorder (CUD) is a serious condition with profound consequences for individuals and society. The current public health epidemic associated with opioid use disorder and overdose is well known. However, deaths associated with stimulant use (i.e., cocaine and methamphetamine) have progressed in parallel with those from opioids and are increasing at an alarming rate (Jalal *et al.*, 2018; Kariisa *et al.*, 2019). Medications and treatment options are available for opioid use disorder and overdose. In contrast, no FDA-approved pharmacotherapeutics currently exist for stimulant use disorders and overdose. Hence, treatment clinics are focused on behavioral modification strategies that do not produce long-lasting abstinence in most patients (Kampman, 2019). Numerous different approaches are being explored for drug development in this area (Czoty *et al.*, 2016).

Indirect dopamine (DA) agonists, such as *d*-amphetamine, have been shown to decrease cocaine self-administration in several preclinical animal models (Negus, 2003; Chiodo *et al.*, 2008; Chiodo and Roberts, 2009; Czoty *et al.*, 2010, 2011). However, these compounds have abuse liability and are therefore predict to have poor outcomes for pharmacotherapy development (Carroll *et al.*, 2006; Reith *et al.*, 2015). One widely studied candidate for CUD treatment is modafinil (Provigil®), an FDA-approved wake-promoting prescription medication for treating narcolepsy and other sleep disorders, which has been shown to have low abuse potential (Jasinski, 2000; Mereu *et al.*, 2013, 2020; Hersey *et al.*, 2021). Modafinil has been promoted as a potential treatment for psychostimulant dependence due to its atypical binding to DA transporter (DAT) compared to cocaine (Loland *et al.*, 2012). Early clinical trials reported that modafinil successfully reduced cocaine intake and prevented relapse (Dackis *et al.*, 2005; Hart *et al.*, 2008; Kampman *et al.*, 2015). Yet, larger multi-center trials reported only modest efficacy of modafinil in promoting cocaine abstinence (Anderson *et al.*, 2009; Dackis *et al.*, 2012). Thus, its weak potency, poor pharmacokinetics and low efficacy in reducing cocaine intake make modafinil an unlikely pharmacotherapy to treat CUDs.

Newly developed analogs exploit modafinil's atypical structure and improve its low affinity and bioavailability shortcomings (Okunola-Bakare *et al.*, 2014; Cao *et al.*, 2016). The prototype atypical DAT analog, JJC8-016, was more potent than modafinil in binding to DAT, blocked cocaine self-administration in rats responding under a fixed-ratio (FR) schedule of reinforcement, did not maintain self-administration on its own, and produced little locomotor stimulation (Zhang *et al.*, 2017). However, JJC8-016 failed cardiac safety tests by exhibiting relatively high affinity at hERG channels; thus, this analog was abandoned from further development. Recently, Newman and colleagues (Cao *et al.*, 2016; Zhang *et al.*, 2017; Newman *et al.*, 2019, 2021; Tanda *et al.*, 2021) developed a series of modafinil analogs that bind to DAT in a manner characterized as 'typical vs. atypical'. Two of these compounds, JJC8-088 and JJC8-091, have been evaluated in several rodent models of CUD (Tunstall *et al.*, 2018; Newman *et al.*, 2019). In these studies, JJC8-088 appeared to be cocaine-like or a “typical” DAT inhibitor, reducing cocaine self-administration in rats responding under an FR schedule of reinforcement. However, JJC8-088 maintained self-administration when substituted for cocaine, suggesting abuse potential. In contrast, JJC8-091, displayed an atypical DAT blocker profile, reducing cocaine breakpoints under a progressive-ratio schedule of reinforcement, and not functioning as a reinforcer when available on an FR schedule (Newman *et al.*, 2019).

A major goal of this study was to extend the findings of the effects of JJC8-088 and JJC8-091 on cocaine self-administration from rodent models to nonhuman primates (NHPs). Old World macaques resemble humans in neuroanatomy and neurochemistry (Berger et al., 1991; Joel and Weiner, 2000), and their extensive cocaine histories represent patients with years of cocaine use. It has been hypothesized that cocaine use represents a choice to procure cocaine over behaviors leading to alternative reinforcers in the environment (Brutcher *et al.*, 2016; Moerke *et al.*, 2017; Negus and Banks, 2018, 2021; Lile *et al.*, 2020). Thus, a second goal of this study was to evaluate JJC8-088 and JJC8-091 on cocaine self-administration under a concurrent schedule with food choice as the alternative. To evaluate the effects of chronic

drug treatments on cocaine self-administration, we have incorporated a highly translational cocaine-food choice paradigm, developed by Negus (Negus, 2003), that allows for complete cocaine dose-response curves to be determined in each session (Czoty and Nader, 2013). We aimed to test the hypothesis posed by Newman *et al*. (Newman *et al.*, 2019) that atypical DAT inhibitors might effectively decrease cocaine self-administration and/or prevent relapse to cocaine seeking.

METHODS

Animals: Four adult male rhesus macaques (*Macaca mulatta*) were prepared with a chronic indwelling venous catheter and subcutaneous vascular access port (VAP; Access Technologies, Skokie, IL, USA) as described previously (Czoty *et al.*, 2010). All monkeys had self-administered cocaine for more than two years before the start of the study. The monkeys were housed in stainless-steel cages (Allentown Caging Equipment, Allentown, NJ, USA) within an environmentally controlled room, with visual and auditory contact with each other. Each monkey was fitted with an aluminum collar (Primate Products, Redwood City, CA, USA) and trained to sit in a primate chair (Primate Products). Monkeys were weighed weekly and fed enough food (Purina LabDiet 5045, St Louis, MO, USA) and fresh fruit and vegetables daily to maintain healthy body weights. Water was available *ad libitum* in the home cage. All monkeys were provided with environmental enrichment as outlined in the Animal Care and Use Committee of Wake Forest University Nonhuman Primate Environmental Enrichment Plan. All procedures were performed in accordance with the 2011 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and were approved by the Wake Forest University Animal Care and Use Committee.

Experiment 1: Binding of JJC8-088 and JJC8-091 in NHP striatum

[^{3}H]WIN 35428 binding at DAT in NHP striatum was adapted from a previous study in rat brain (Loland *et al.*, 2012; Cao *et al.*, 2016). In brief, midbrain caudate nucleus tissues were

dissected from cocaine-naïve *Macaca mulatta* frozen brains provided by Drs. Michael Nader and Linda Porrino (Wake Forest University School of Medicine). Affinities of compounds at DAT were determined by the displacement of [^{3}H]WIN 35,428 (NET1033, Perkin Elmer, Waltham, MA) binding in membranes from NHP caudate nucleus. Frozen NHP caudates were dissected on ice, then homogenized in 20 volumes (w/v) of ice-cold modified sucrose phosphate buffer (0.32 M sucrose, 7.74 mM Na_2HPO_4, 2.26 mM NaH_2PO_4, pH 7.4) using a Brinkman Polytron (Setting 6 for 20 sec) and centrifuged at 20,000 RPM for 10 min at 4°C. The resulting pellet was re-suspended in buffer, re-centrifuged, and suspended in buffer again to a concentration of 10 mg/mL, original wet weight (OWW). Experiments were conducted in assay tubes containing 0.5 mL sucrose phosphate buffer, 0.5 nM [^{3}H]WIN 35,428 (84 Ci/mmol), 1.0 mg of tissue OWW, and various concentrations of inhibitor. The reaction was started with the addition of tissue, and tubes were incubated for 120 min at 25°C. Nonspecific binding was determined using 2 μM GBR 12909. IC_{50} and K_i values were determined from displacement curves using seven concentrations of inhibitor using a K_D value of 5.5 nM for [^{3}H]WIN 35,428. K_i values are averages from three separate experiments.

Experiment 2: Pharmacokinetics of JJC8-088 and JJC8-091 in cocaine-experienced NHPs

Procedure: Blood was collected in the same monkeys used in behavioral studies (Experiment 3) to analyze plasma concentration levels over 24 h of the two JJC compounds to be evaluated on cocaine self-administration. JJC8-088 (0.7 mg/kg) and JJC8-091 (1.9 mg/kg) were delivered intravenously followed by a heparinized saline flush at time 0, and blood was collected at 5-, 15-, and 30-min, 1-, 2-, 4-, 6-, and 24-h post injection through the saphenous vein on either leg; blood was placed into tubes containing K3-EDTA and stored briefly on ice. Samples were then centrifuged for 15 min, and the supernatant was transferred to a 1.5-ml storage tube and frozen at -80°C.

Plasma samples were analyzed as described previously (Rais *et al.*, 2014, 2015) using protein precipitation and subsequently processed for analysis by LC/MS/MS. Briefly, prior to extraction, frozen samples were thawed on ice. The calibration curves were developed using plasma from naive male rhesus nonhuman primates as a matrix. Plasma samples (50 µl) were processed using a one-step protein precipitation method by the addition of 150 µl of acetonitrile with the internal standard, followed by mixing by vortex for 30 s and then centrifugation at 12000 x *g* for 10 min. Aliquots (50 µl) were extracted with 100 µl acetonitrile containing 1.5 µM losartan as the internal standard. The extracts were centrifuged at 16000 x g at 4°C for 10 min. The supernatants were transferred to 250 µl polypropylene autosampler vials that were sealed with a Teflon cap. A 5 µl volume was injected in the ultra-performance liquid chromatography (UPLC) instrument for quantitative analysis by LC/MS/MS.

The chromatographic analysis was performed using an Accel ultra-high-performance system consisting of an analytical pump and an autosampler coupled to a TSQ Vantage mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The analyte was separated at ambient temperature using an Agilent Eclipse Plus column (100 x 2.1 mm inner diameter) packed with a 1.8 µm C18 stationary phase. The mobile phase was composed of 0.1% formic acid in acetonitrile and 0.1% formic acid in H_2O with gradient elution. The total run time for each analyte was 4.5 min. The [M+H]+ ion transitions of JJC8-088 (m/z 499.202>233.177, 296.184), JJC8-091 (m/z 423.193>157.17, 220.167), and the internal standard losartan (m/z 422.977>180.072, 207.090) were used for analysis.

Calibration curves for JJC8-088 and JJC8-091 were computed using the ratio of the peak area of analyte to the internal standard by using a quadratic regression with 1/x weighting. The parameters of each calibration curve were used to back-calculate concentrations and to obtain values for the QC samples and unknown samples by interpolation. Correlation coefficient of greater than 0.99 was obtained in all analytical runs.

JPET Fast Forward. Published on December 6, 2022 as DOI: 10.1124/jpet.122.001363
This article has not been copyedited and formatted. The final version may differ from this version.

Experiment 3: Effects of JJC8-088 and JJC8-091 on cocaine vs. food choice

Apparatus: All behavioral studies were conducted in ventilated, sound-attenuating chambers (MED-600-B1; Med Associates, St. Albans, VT, USA). The testing chambers were equipped with an operant conditioning panel that contained two photo-optic switches (Model 117–1007; Stewart Ergonomics, Inc., Furlong, PA, USA) located on each side of the panel with a horizontal row of four stimulus lights above each switch. The primate chair was positioned to be within easy reach of the monkey to operate both switches. A food receptacle was located between the photo-optic switches and connected with a Tygon tube to a pellet dispenser (Med Associates) located on the top of the chamber to deliver 1-g banana-flavored food pellets (Bio-Serv, Frenchtown, NJ, USA). A peristaltic infusion pump (Cole-Parmer Instrument Co., Niles, IL, USA), located on top of the chamber, was used for delivering cocaine.

Procedure: The sessions began at approximately noon each day. Before each session, the area on the back of the animal containing the vascular access port was cleaned with chlorhexidine and isopropyl alcohol swabs (Prevantics™, Orangeburg, NY, USA). Approximately 1 min prior to the start of the session, a dose of JJC8-088 or JJC8-091 was given intravenously outside the chamber. A 22-gauge Huber Point Needle (Access Technologies, Skokie, IL, USA) was inserted into the port, which connected the venous catheter to the infusion pump. The pump was operated for approximately 3 seconds to fill the port with the concentration of cocaine prior to the start of self-administration. After each session, catheters were flushed with heparinized saline (100 U/ml) to prevent clotting. The cocaine-food choice model has previously described (John *et al.*, 2015). Briefly, food availability was signaled by illumination of a green light above one of the two switches, while varying doses of cocaine were signaled by illumination of different discriminative stimuli above the other switch (see John *et al.*, 2015 for details). Each daily session consisted of five components in which monkeys chose between food pellets and ascending unit doses of cocaine (i.e., no injection, 0.003, 0.01, 0.03 and 0.1 mg/kg per injection); the drug dose was varied by manipulating the pump duration and,

consequently, the volume delivered. Thus, a complete cocaine dose-response curve was determined in each monkey each session. Each component ended when ten total reinforcers had been earned (food and cocaine injections) or 20 min had elapsed, whichever came first; components were separated by a 5-min timeout (TO) period. The first switch's response requirement was reset if a response was emitted on the alternate switch before an FR was completed. Delivery of cocaine was accompanied by illumination of the red light in the center of the response panel. Following the delivery of each reinforcer (food or drug), there was a 30-s TO during which all lights remained off and responding had no scheduled consequences.

To generate cocaine dose-response curves that were sigmoidal, the ratio requirements for food and drug were adjusted for each monkey such that allocation of responding to the drug switch increased over the session as the available dose of cocaine increased. Food/Drug FR values for each monkey were: R-1688 = 10/40; R-1690 = 5/40; R-1691 = 10/70; R-1710 = 20/10. Responding was considered stable when ≤20 % of reinforcers were earned on the drug switch when the alternative to food was no injection (component 1) or 0.003 mg/kg per injection cocaine (component 2) and ≥80 % of cocaine reinforcers were earned on the drug switch when the alternative to food was 0.1 mg/kg per injection cocaine (component 5). An additional criterion of stability was an observation of a dose-related increase in drug choice in which the % cocaine reinforcers for 0.01 mg/kg per injection cocaine (component 3) was lower than 0.03 mg/kg per injection cocaine (component 4). This criterion had to be met for three consecutive days for an animal to be considered stable in responding before acute drug treatment was administered or the chronic treatment was initiated.

Drug treatments: An individualized drug treatment regimen was used for each monkey. The JJC compounds pretreatment time and dose ranges were based on pharmacokinetic data collected in NHPs at the beginning of this study (Experiment 2) and previously published plasma and brain kinetics and behavioral data in rats (Tunstall *et al.*, 2018; Newman *et al.*, 2019). A crossover design was used where two monkeys were completely evaluated (acute then chronic)

with JJC8-091 (R-1691 and R-1710) and the other two monkeys were initially tested with JJC8-088 (R-1688 and R-1690). First, acute dose-response curves were determined for JJC8-088 (0.7-3.0 mg/kg, i.v.) and JJC8-091 (3.6-17.0 mg/kg, i.v.), administered 1 min before the self-administration session (n=3/group). The minimum acute treatment dose that shifted the cocaine-food choice ED50 curve was used as the initial dose for chronic treatment. While the starting dose for each monkey was determined individually, the data (not shown) suggested that each monkey started with the lowest dose, 0.7 mg/kg JJC8-088 and 3.6 mg/kg JJC8-091. Because of the resources required to generate the large quantities of JJC8-091 needed for these experiments, a higher starting dose of JJC8-091 was selected for R-1688. Each day before, during, and after JJC compound drug treatment, laboratory personnel noted occurrences of any behaviors that were uncharacteristic for each monkey, with a focus on behaviors that have been observed during treatment with other dopaminergic drugs, such as locomotor activation, agitation, stereotypies or other unconditioned behavioral effects.

Once responding was stable following the acute treatment assessment, chronic drug treatments began and were conducted using a 5-day treatment regimen over consecutive one-week periods in which the same dose of the JJC compound was administered for five consecutive daily sessions. The 5-day treatment paradigm was broken into two segments of components tested during a session; during Days 1-4, only component 1 (food only) was available, and on Day 5, all five components were available (**Figure 1**). After a 5-day treatment, each monkey's cocaine ED50 (i.e., the cocaine dose resulting in 50% cocaine choice vs. food) was determined, and one of three adjustments were made based on the outcome: (1) if there was no effect on food reinforcement (Days 1-4) and no change in the cocaine ED50 (Day 5), the JJC dose was increased by one-half log-units; (2) if food choice was disrupted and the cocaine ED50 was reduced, the JJC dose was lowered by one-half log-units; (3) if there was no effect on food reinforcement and an increase in the cocaine ED50 (i.e., a positive outcome), the JJC dose remained the same in order to determine if tolerance developed to those effects. After fully

JPET Fast Forward. Published on December 6, 2022 as DOI: 10.1124/jpet.122.001363
This article has not been copyedited and formatted. The final version may differ from this version.

evaluating one of the JJC compounds, all treatment was discontinued for at least a one-week washout and a return to baseline cocaine-food choice before testing the other JJC compound, beginning with acute testing. There were two exceptions to this strategy. R-1690 and R-1688 were not given access to the cocaine-food choice paradigm during the washout period. R-1690 had a persistent drop in food-maintained responding during treatment, to which no tolerance developed and did not subside following a reduction in JJC8-088 dose. In between testing of JJC8-088 and JJC8-091, R-1688 was evaluated with chronic *d*-amphetamine (0.1-0.56 mg/kg, i.v.) and these data are described in the **Supplemental Files**.

Data analysis: Mean concentration−time data were used for pharmacokinetic analysis (Experiment 2). Noncompartmental-analysis module in WinNonlin (version 8.2) was used to assess pharmacokinetic parameters of JJC8-088 and JJC8-091. Peak plasma concentrations (Cmax) and time to Cmax (Tmax) were the observed values. Area under the curve (AUC) was calculated by log−linear trapezoidal rule to the end of sample collection (AUClast).

For cocaine-food choice (Experiment 3), the primary dependent variable was percent cocaine choice defined as (number of FRs completed on the drug-associated switch ÷ total number of FRs completed) × 100. Additional dependent variables collected during each session included the number of injections, food reinforcers, and total reinforcers per component. Also included was number of food reinforcers in Component 1 during chronic treatment. These data are presented as mean (± SD) for the first 4 days of treatment each week. For % cocaine choice, cocaine ED50 values were determined using the linear portion of the cocaine choice dose-effect curve that crossed 50% choice. The linear ED50 values of treatment Day 5 were annotated to indicate a rightward shift in ED50 of the cocaine vs. food dose-response curve. In some cases, choice curve ED50 values were estimated based on a limited data set because cocaine was less than 50% for all doses that were available during the session; these cases are indicated with ** in **Table 1** for individual monkeys.

For the effects of each JJC compound on percent cocaine choice (**Table 1**), ED50 values for each monkey were compared using one-way ANOVAs. All analyses were conducted with Prism 6 software (Graphpad Software, Inc., San Diego, CA, USA). Food ED50 values were determined using the linear portion of the dose-response curve that reduced food reinforcers by 50% during the food-only component of the last three days of a treatment week. In some cases, only a single dose of treatment drug was tested (JJC8-091 in R-1688) or no change in food-maintained responding was observed in the last 3 days of treatment week and JJC8-091 in R-1691), the food ED50 curve was determined to be "Flat" (**Table 2**).

Drugs: (-)Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in sterile 0.9% saline for self-administration studies. Changing the self-administered cocaine dose was accomplished by changing the time (seconds; 1-30 sec) of cocaine delivered over a single concentration of cocaine. The positive control, *d*-amphetamine (Sigma-Aldrich, St Louis, MO, USA), was dissolved in sterile 0.9% saline. JJC8-088 and JJC8-091 (fumarate salts) were synthesized at the National Institute on Drug Abuse (NIDA)-Intramural Research Program, Baltimore, MD, USA using published procedures (Cao *et al.*, 2016). The JJC compounds were dissolved in a sterile 0.9% saline. Heat and sonication were used to solvate JJC compounds into solution. All drug solutions used for intravenous (i.v.) administration were passed through a sterile 0.2-µm filter (Millipore Inc, Burlington, MA, USA).

RESULTS

Experiment 1: Binding of JJC8-088 and JJC8-091 in NHP striatum

Binding affinity (K_i) at DAT was assessed by the displacement of [^{3}H]WIN 35428 in prepared frozen caudate nucleus from cocaine-naïve NHPs as previously described in rats (Cao *et al.*, 2016). JJC8-088 bound to NHP DAT at 14.4 ± 9 (nM), whereas JJC8-091 bound at 2,730 ± 1,270 (nM). In rat DAT, JJC8-088 and JJC8-091 affinities have previously been reported (Cao *et al.*, 2016) as 2.53 ± 0.25 (nM) and 289 ± 43 (nM) respectively.

JPET Fast Forward. Published on December 6, 2022 as DOI: 10.1124/jpet.122.001363
This article has not been copyedited and formatted. The final version may differ from this version.

Experiment 2: Pharmacokinetics of JJC8-088 and JJC8-091 in cocaine-experienced NHPs

Pharmacokinetic parameters of JJC8-088 (0.7 mg/kg) and JJC8-091 (1.9 mg/kg) by i.v. administration were determined in nonhuman primates. **Figure 2** and **Supplemental Table S1** show the 24-hour time course of JJC8-088 and JJC8-091 plasma levels. At the 24-hour time point, the plasma levels of JJC8-088 in all NHPs were undetectable (**Figure 2A**), while JJC8-091 produced quantifiable plasma levels over the 24-hour time frame (**Figure 2B**). The pharmacokinetic parameters for both compounds are presented in **Figure 2C**. Following i.v. administration, JJC8-088 provided high plasma exposures with $AUC_{0\text{-}t}$ of 2921±476 pmol·h/mL, half-life ($t_{1/2}$) = 1.1 h and a moderate volume of distribution (Vd)=0.96 L/kg. In contrast, JJC8-091 administration at a 3-fold higher dose exhibited similar plasma exposure with $AUC_{0\text{-}t}$ of 3509±592 pmol·h/mL, but a longer $t_{1/2}$ = 3.5 h, as well as greater Vd =7.9 L/kg. In general, these data were used to determine the initial doses of JJC compounds for cocaine-food choice and helped establish an initial PK/PD relationship.

Experiment 3: Effects of JJC8-088 and JJC8-091 on cocaine vs. food choice

Baseline cocaine-food choice: Under baseline conditions (**Figures 3** - **6**, open circles), monkeys exclusively chose food when no other choice was available (component 1) or when the alternative was a low dose of cocaine (0.003 – 0.01 mg/kg/injection). All monkeys exclusively reallocated their choice to cocaine when higher doses (0.03 - 0.1 mg/kg) were available. Under baseline conditions, total trials completed per component were generally 10 (the maximum), except in the 5th component, which was associated with the highest cocaine dose (**Supplemental Figures S1** and **S2**, panel **A**). In the early components, food was chosen almost exclusively and declined to 0 when higher cocaine doses were available (**Supplemental Figures S1** and **S2**, panel **B**). When 0 or low cocaine doses were available, the number of injections was near zero and increased to 10 per component at the higher cocaine doses (**Supplemental Figures S1** and **S2**, panel **C**).

JPET Fast Forward. Published on December 6, 2022 as DOI: 10.1124/jpet.122.001363
This article has not been copyedited and formatted. The final version may differ from this version.

Effects of chronic JJC8-088: Prior to chronic treatment, acute effects of JJC8-088 were determined (**Figure 3**). In all three monkeys, JJC8-088 shifted the cocaine dose-response curve to the left. Based on the acute dose-response curve data, chronic treatment began with 0.7 mg/kg JJC8-088. During chronic dosing, only component 1 (food reinforcement) was available for four consecutive days, and all components were available on Day 5, which allowed for the determination of a cocaine dose-response curve. In all monkeys, one week of treatment with chronic 0.7 mg/kg JJC8-088 increased cocaine choice when the alternative was low-dose cocaine (**Figure 3**, top panel). **Table 1** shows this leftward shift from baseline in the cocaine-food choice ED50 persisted for five weeks in R-1690 and six weeks in R-1688, whereas R-1710 developed tolerance to JJC8-088-induced increases in cocaine choice after one week. By week 5, R-1690 showed no appreciable reallocation of cocaine choice to food pellets from baseline during the final month of chronic JJC8-088 treatment (**Table 1** and **Figure 5**). The other two monkeys continued treatment with JJC8-088 resulted in rightward shifts in the % choice cocaine dose-response curve. **Table 1** shows chronic treatment with JJC8-088 in R-1710 produced five consecutive weeks of a rightward shift in cocaine-food choice ED50 after week 1. In R-1688, chronic JJC8-088 produced a persistent leftward shift in cocaine choice (**Table 1**), in which by week 6 at 1.7 mg/kg, tolerance developed to the JJC8-088-induced increase in cocaine choice. An increase in JJC8-088 dose to 3.0 mg/kg for weeks 7 and 8 produced a marked rightward shift in the cocaine-food choice curve at week 7, but this effect reversed by week 8 and persisted as a leftward shift in cocaine choice even following a decrease to chronic 1.7 mg/kg JJC8-088 during week 9 (**Figure 5**).

Consistent with the rightward shift in the cocaine choice dose-response curve, continued treatment with JJC8-088 resulted in increases in the number of food reinforcers (**Supplemental Figure S1B**) and decreases in the number of cocaine injections per component (**Supplemental Figure S1C**). In two of three monkeys, chronic JJC8-088 treatment resulted in an increase in trials completed when the highest cocaine dose was available (**Supplemental Figure S1A**),

which was due to increases in food choices. During the first 4 days of each week of chronic administration of JJC8-088, only the first component (Food only) was available. Chronic JJC8-088 treatment resulted in disruptions in food-maintained responding in two of three monkeys, with no evidence of tolerance to these effects (**Table 2**).

Effects of chronic JJC8-091: Prior to chronic treatment, acute effects of JJC8-091 were determined (**Figure 4**). In two of three monkeys, JJC8-091 shifted the cocaine dose-response curve to the left. Based on the acute dose-response curve data, chronic treatment began with 3.6 mg/kg JJC8-091 in monkeys R-1691 and R-1710; due to supply issues, JJC8-091 treatment for monkey R-1688 began at 17 mg/kg (**Figure 6**). For Monkey R-1691, chronic treatment with JJC8-091 resulted in primarily leftward shifts in the cocaine dose-response curves, with the exception of week 2, in which 3.6 mg/kg JJC8-091 produced a rightward shift (**Figure 6**, **Table 1**); these increases in cocaine choice were accompanied by dose-dependent decreases in food reinforcers (**Supplemental Figure S2B**) and increases in cocaine injections (**Supplemental Figure S2C**). For monkey R-1710, chronic treatment with 5.6 mg/kg JJC8-091 produced a rightward shift in cocaine choice by week 4 and a dramatic reallocation of cocaine choice below 25% for all doses except 0.1 mg/kg/injection at week 5 (**Figure 6**); this treatment resulted in a large increase in the ED50 for cocaine choice (**Table 1**). During the first week of treatment, JJC8-091 produced a leftward shift in the cocaine dose-response curve, with tolerance developing to that effect by week 2, resulting in a slight rightward shift during week 3 (**Table 1**, **Figure 6**). In both of these monkeys, JJC8-091-induced rightward shifts in the cocaine dose-response curves were accompanied by rightward shifts in the frequency of food choices (**Supplemental Figure S2B**) and number of cocaine injections (**Supplemental Figure S2C**). The effects of JJC8-091 on cocaine choice occurred at doses that did not affect total reinforcers earned in the session (**Supplemental Figure S2A**). Chronic JJC8-091 treatment did not disrupt food-maintained responding in two of the three monkeys (**Table 2**). In R-1688, tolerance developed to the JJC8-091-induced disruptions of food-maintained responding. As a positive

control, the effects of *d*-amphetamine were examined on cocaine-food choice in R-1688 and showed predicted rightward shifts in the cocaine choice dose-response curve (**Supplemental Files and Table S2**).

DISCUSSION

The present study evaluated the pharmacodynamic, pharmacokinetic, and behavioral efficacy of two novel modafinil analogs, the more cocaine-like compound JJC8-088, and the atypical DAT inhibitor JJC8-091, in rhesus monkeys self-administering cocaine under a concurrent food vs. cocaine reinforcement schedule. Similar to previous studies in rat striatal membranes (Cao *et al.*, 2016), in monkey tissue, JJC8-088 had substantially higher affinity for the DAT compared with JJC8-091. Consistent with previous studies in rats showing both JJC8-088 and JJC8-091 produced good plasma exposure following i.p. administration (Tunstall *et al.*, 2018), a single bolus dose in monkeys resulted in stable plasma kinetics with no evidence of rapid clearance. While chronic oral treatment is ultimately desired, these results demonstrated that bolus i.v. administration of both JJC compounds were sufficient for evaluating in cocaine vs. food choice prior to more time- and resource-intensive chronic repeated oral administration studies. There was evidence that chronic treatment with both JJC8-088 and JJC8-091 decreased cocaine choice, relative to the alternative food reinforcer, in most, but not all monkeys.

The disparate mechanisms of action at the DAT between JJC8-088 (typical) and JJC8-091 (atypical) may underpin the differences in effect on cocaine vs. food choice between compounds. Newman *et al*. (2019) showed that typical and atypical DAT compounds reduced cocaine self-administration, albeit affecting different aspects of self-administration. The atypical compound, JJC8-091, given at 30 mg/kg i.p., significantly reduced breakpoint on a progressive-ratio (PR) schedule of reinforcement, a model of reinforcing strength of cocaine, but not when cocaine was available under a fixed-ratio schedule of reinforcement. In contrast, the typical DAT

compound JJC8-088, at 10 mg/kg i.p., had no significant effect on cocaine self-administration under a PR schedule of reinforcement but significantly reduced the number of cocaine injections on a FR schedule of reinforcement (Arnold and Roberts, 1997). The results from these rodent studies suggest the atypical compound, JJC8-091, reduced motivation to seek cocaine, whereas the typical DAT inhibitor, JJC8-088, maybe substituting for the reinforcing effects of cocaine. This observation is important because the atypical DAT blocker, JJC8-091, is believed to have lower reinforcing effects compared with the typical DAT blocker, JJC8-088 (Newman *et al.*, 2019).

Repeated drug effects on behavior are most relevant to a compound's ultimate clinical utility, allowing for the assessment of whether tolerance or sensitization develops to the behavioral effects of the compound. Therefore, prior to chronic administration, in each monkey, we determined an acute dose-response for each JJC compound and used the minimum dose that produced a shift in cocaine vs. food choice ED50 as the starting dose for chronic administration in that monkey. Consistent with rodent studies (Tunstall *et al.*, 2018), JJC8-088, which a higher affinity at DAT than JJC8-091, had a lower starting dose of 0.7 mg/kg JJC8-088 in all three monkeys, compared with doses of 3.6-17 mg/kg JJC8-091 used to begin chronic drug treatments.

Chronic i.v. administration of JJC8-088 in monkeys demonstrated classic dopaminergic compound characteristics on cocaine vs. food choice behavior (Thomsen *et al.*, 2008, 2013; John *et al.*, 2015). Early in treatment, chronic JJC8-088 produced transient leftward shifts in the cocaine choice dose-response curves in all three monkeys. At some point in the 9 weeks of chronic treatment, there was evidence of rightward shifts; for R-1710, this shift was only to return to baseline cocaine vs. food choice. In this monkey, one possible reason for the slight rightward shift in the cocaine choice dose-response curve, may involve tolerance to the rate-decreasing effects of JJC8-088 early in each session, as indicated by significant reductions in food reinforcement early in the treatment (week 1) that were absent by week 5. For the other

two monkeys (R-1690 and R-1688), there was early evidence of JJC8-088-induced appetite suppressant effects; however, these effects persisted for only a few sessions before tolerance developed. At chronic high-dose administration, the efficacy of JJC8-088 in reducing cocaine choice was accompanied by increased stereotypy and a sharp reduction in the number of reinforcers earned in all monkeys tested. This suggests a limitation to the use of this compound as a medication, even when decreases in cocaine choice were apparent.

The atypical compound, JJC8-091, produced less robust efficacy, but a lower incidence of undesirable effects when administered chronically. With regard to this latter point, two of the three monkeys were tested with the highest dose available, 17 mg/kg, and one monkey, R-1710, did show decreases in food choice, accompanied by a decrease in post-session chow consumption following week 7 treatment with 10 mg/kg, so higher doses were not tested. Interestingly, this is the only monkey that had two consecutive weeks of decreases in cocaine choice (weeks 4 and 5), with a return to baseline for the remainder of his treatment. This monkey, along with R-1688, were tested with both JJC compounds; R-1710 was tested with JJC8-091 before JJC8-088, while the reverse was true for R-1688, who also received *d*-amphetamine treatments between evaluation of both JJC compounds (see **Supplemental File**). Thus, the efficacy of JJC8-088 relative to JJC8-091 is not due to order of testing. Importantly, chronic *d*-amphetamine was tested in this cocaine-food choice paradigm as a positive control, and showed prolonged rightward shifts in the cocaine choice dose-response curve in the one monkey tested, supporting targeting the DAT for treatment of CUD.

In rodents, JJC8-091 reduced cocaine breakpoints under a PR schedule of reinforcement (Newman *et al.*, 2019), but was only modestly effective on cocaine choice in rhesus monkeys. In comparison, JJC8-088 was more effective in decreasing cocaine responses in rodents (Newman *et al.*, 2019) and cocaine choice in NHPs. In addition to potential species differences, another possible explanation is the different schedules of reinforcement used between the studies. Importantly, in the present study, the difference in bioavailability of JJC8-

088 and JJC8-091, with JJC8-091 having a longer half-life than JJC8-088, cannot explain the difference in efficacy of each compound on cocaine choice. The pharmacokinetic data in the present study and previous studies in rats (Tunstall *et al.*, 2018) suggest that both plasma and brain exposure were ample to reach the occupancy necessary to elicit a biochemical response. Therefore, the potency of the JJC compounds at monkey DAT was investigated and provides a basis for potential differences in efficacy in cocaine choice. Radioligand binding with drug-naïve rhesus monkey caudate tissue was conducted to assess the potency of JJC8-088 and JJC8-091 at the DAT. Our results showed that while similar DAT affinities were noted for JJC8-088 in monkey and rodent tissue, the affinity of JJC8-091 for DAT in monkeys was markedly lower than previously published data (Newman *et al.*, 2019). Therefore, it is possible that the affinity of JJC8-091 for the monkey DAT may underlie the disparate results between rat and NHP cocaine self-administration studies.

Altogether the results of this study indicate that both JJC compounds produced adequate bioavailability following i.v. administration in rhesus monkeys. Further, it was demonstrated that modafinil analogs may have the potential to be viable targets for the treatment of CUD due to the appreciable decreases in cocaine choice following chronic administration in cocaine-experienced monkeys. In comparison to JJC8-088, JJC8-091 presented fewer observed adverse events. Furthermore, this study demonstrated the potential of this preclinical chronic drug treatment on cocaine vs. food choice paradigm in monkeys to provide insights into real-world translatability to long-term outpatient treatment (Negus and Banks, 2021). While it is well known clinically that chronic treatment with dopaminergic-acting compounds can produce appetite suppression (Berridge, 2007), there was evidence that tolerance to disruptive effects of both JJC compounds on food-maintained responding was observed in the present study (**Table 2**). Going forward, it would be pertinent to continue studying the differences in the efficacy of typical and atypical DAT blockers in combination with behavioral interventions that may further decrease cocaine choice.

Acknowledgments:

We thank Ruoyu Xiao, Michael Coller, Jillian Odom, and Chrystal Bragg for their outstanding technical support.

Authorship contribution.

Participated in research design: Rahimi, Newman, Nader.

Conducted experiments: Rahimi, Cao, Lam, Rais, Childers, Porrino.

Contributed new reagents or analytic tools: not applicable.

Performed data analysis: Rahimi.

Wrote or contributed to the writing of the manuscript: Rahimi, Newman, Nader.

REFERENCES

Anderson AL, Reid MS, Li S-H, Holmes T, Shemanski L, Slee A, Smith EV, Kahn R, Chiang N, Vocci F, Ciraulo D, Dackis C, Roache JD, Salloum IM, Somoza E, Urschel HC, and Elkashef AM (2009) Modafinil for the treatment of cocaine dependence. *Drug Alcohol Depend* **104**:133–139.

Arnold JM, and Roberts DC (1997) A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav* **57**:441–447.

Berger B, Gaspar P, Verney C. Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates. Trends Neurosci 14: 21–27, 1991.

Berridge KC (2007) The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl)* **191**:391–431.

Brutcher RE, Nader SH, and Nader MA (2016) Evaluation of the Reinforcing Effect of Quetiapine, Alone and in Combination with Cocaine, in Rhesus Monkeys. *J Pharmacol Exp Ther* **356**:244–250.

Cao J, Slack RD, Bakare OM, Burzynski C, Rais R, Slusher BS, Kopajtic T, Bonifazi A, Ellenberger MP, Yano H, He Y, Bi G-H, Xi Z-X, Loland CJ, and Newman AH (2016) Novel and High Affinity 2-[(Diphenylmethyl)sulfinyl]acetamide (Modafinil) Analogues as Atypical Dopamine Transporter Inhibitors. *J Med Chem* **59**:10676–10691.

Carroll FI, Howard JL, Howell LL, Fox BS, and Kuhar MJ (2006) Development of the dopamine transporter selective RTI-336 as a pharmacotherapy for cocaine abuse. *AAPS J* **8**:E196-203.

Chiodo KA, Läck CM, and Roberts DCS (2008) Cocaine self-administration reinforced on a progressive ratio schedule decreases with continuous D-amphetamine treatment in rats. *Psychopharmacology (Berl)* **200**:465–473.

Chiodo KA, and Roberts DCS (2009) Decreased reinforcing effects of cocaine following 2 weeks of continuous D-amphetamine treatment in rats. *Psychopharmacology (Berl)* **206**:447–456.

Czoty PW, Gould RW, Martelle JL, and Nader MA (2011) Prolonged attenuation of the reinforcing strength of cocaine by chronic d-amphetamine in rhesus monkeys. *Neuropsychopharmacology* **36**:539–547.

Czoty PW, Martelle JL, and Nader MA (2010) Effects of chronic d-amphetamine administration on the reinforcing strength of cocaine in rhesus monkeys. *Psychopharmacology (Berl)* **209**:375–382.

Czoty PW, and Nader MA (2013) Effects of dopamine D2/D3 receptor ligands on food-cocaine choice in socially housed male cynomolgus monkeys. *J Pharmacol Exp Ther* **344**:329–338.

Czoty PW, Stoops WW, and Rush CR (2016) Evaluation of the “Pipeline” for Development of Medications for Cocaine Use Disorder: A Review of Translational Preclinical, Human Laboratory, and Clinical Trial Research. *Pharmacol Rev* **68**:533–562.

Dackis CA, Kampman KM, Lynch KG, Pettinati HM, and O’Brien CP (2005) A double-blind, placebo-controlled trial of modafinil for cocaine dependence. *Neuropsychopharmacology* **30**:205–211.

Dackis CA, Kampman KM, Lynch KG, Plebani JG, Pettinati HM, Sparkman T, and O’Brien CP (2012) A double-blind, placebo-controlled trial of modafinil for cocaine dependence. *J Subst Abuse Treat* **43**:303–312.

Hart CL, Haney M, Vosburg SK, Rubin E, and Foltin RW (2008) Smoked cocaine self-administration is decreased by modafinil. *Neuropsychopharmacology* **33**:761–768.

Hersey M, Bacon AK, Bailey LG, Coggiano MA, Newman AH, Leggio L, and Tanda G (2021) Psychostimulant Use Disorder, an Unmet Therapeutic Goal: Can Modafinil Narrow the Gap? *Front Neurosci* **15**:656475.

Jalal H, Buchanich JM, Roberts MS, Balmert LC, Zhang K, and Burke DS (2018) Changing dynamics of the drug overdose epidemic in the United States from 1979 through 2016. *Science* **361**:eaau1184.

Jasinski DR (2000) An evaluation of the abuse potential of modafinil using methylphenidate as a reference. *J Psychopharmacol* **14**:53–60.

Joel D, and Weiner I (2000) The connections of the dopaminergic system with the striatum in rats and primates: an analysis with respect to the functional and compartmental organization of the striatum. *Neuroscience* **96**:451–474.

John WS, Banala AK, Newman AH, and Nader MA (2015) Effects of buspirone and the dopamine D3 receptor compound PG619 on cocaine and methamphetamine self-administration in rhesus monkeys using a food-drug choice paradigm. *Psychopharmacology (Berl)* **232**:1279–1289.

Kampman KM (2019) The treatment of cocaine use disorder. *Sci Adv* **5**:eaax1532.

Kampman KM, Lynch KG, Pettinati HM, Spratt K, Wierzbicki MR, Dackis C, and O'Brien CP (2015) A double blind, placebo controlled trial of modafinil for the treatment of cocaine dependence without co-morbid alcohol dependence. *Drug Alcohol Depend* **155**:105–110.

Kariisa M, Scholl L, Wilson N, Seth P, and Hoots B (2019) Drug Overdose Deaths Involving Cocaine and Psychostimulants with Abuse Potential - United States, 2003-2017. *MMWR Morb Mortal Wkly Rep* **68**:388–395.

Lile JA, Johnson AR, Banks ML, Hatton KW, Hays LR, Nicholson KL, Poklis JL, Rayapati AO, Rush CR, Stoops WW, and Negus SS (2020) Pharmacological validation of a translational model of cocaine use disorder: Effects of d-amphetamine maintenance on choice between intravenous cocaine and a nondrug alternative in humans and rhesus monkeys. *Exp Clin Psychopharmacol* **28**:169–180.

Loland CJ, Mereu M, Okunola OM, Cao J, Prisinzano TE, Mazier S, Kopajtic T, Shi L, Katz JL, Tanda G, and Newman AH (2012) R-modafinil (armodafinil): a unique dopamine uptake inhibitor and potential medication for psychostimulant abuse. *Biol Psychiatry* **72**:405–413.

Mereu M, Bonci A, Newman AH, and Tanda G (2013) The neurobiology of modafinil as an enhancer of cognitive performance and a potential treatment for substance use disorders. *Psychopharmacology (Berl)* **229**:415–434.

Mereu M, Hiranita T, Jordan CJ, Chun LE, Lopez JP, Coggiano MA, Quarterman JC, Bi G-H, Keighron JD, Xi Z-X, Newman AH, Katz JL, and Tanda G (2020) Modafinil potentiates cocaine self-administration by a dopamine-independent mechanism: possible involvement of gap junctions. *Neuropsychopharmacology* **45**:1518–1526.

Moerke MJ, Banks ML, Cheng K, Rice KC, and Negus SS (2017) Maintenance on naltrexone+amphetamine decreases cocaine-vs.-food choice in male rhesus monkeys. *Drug Alcohol Depend* **181**:85–93.

Negus SS (2003) Rapid assessment of choice between cocaine and food in rhesus monkeys: effects of environmental manipulations and treatment with d-amphetamine and flupenthixol. *Neuropsychopharmacology* **28**:919–931.

Negus SS, and Banks ML (2021) Confronting the challenge of failed translation in medications development for substance use disorders. *Pharmacol Biochem Behav* **210**:173264.

Negus SS, and Banks ML (2018) Modulation of drug choice by extended drug access and withdrawal in rhesus monkeys: Implications for negative reinforcement as a driver of addiction and target for medications development. *Pharmacol Biochem Behav* **164**:32–39.

Newman AH, Cao J, Keighron JD, Jordan CJ, Bi G-H, Liang Y, Abramyan AM, Avelar AJ, Tschumi CW, Beckstead MJ, Shi L, Tanda G, and Xi Z-X (2019) Translating the atypical

dopamine uptake inhibitor hypothesis toward therapeutics for treatment of psychostimulant use disorders. *Neuropsychopharmacology* **44**:1435–1444.

Newman AH, Ku T, Jordan CJ, Bonifazi A, and Xi Z-X (2021) New Drugs, Old Targets: Tweaking the Dopamine System to Treat Psychostimulant Use Disorders. *Annu Rev Pharmacol Toxicol* **61**:609–628.

Okunola-Bakare OM, Cao J, Kopajtic T, Katz JL, Loland CJ, Shi L, and Newman AH (2014) Elucidation of structural elements for selectivity across monoamine transporters: novel 2-[(diphenylmethyl)sulfinyl]acetamide (modafinil) analogues. *J Med Chem* **57**:1000–1013.

Rais R, Rojas C, Wozniak K, Wu Y, Zhao M, Tsukamoto T, Rudek MA, and Slusher BS (2014) Bioanalytical method for evaluating the pharmacokinetics of the GCP-II inhibitor 2-phosphonomethyl pentanedioic acid (2-PMPA). *J Pharm Biomed Anal* **88**:162–169.

Rais R, Wozniak K, Wu Y, Niwa M, Stathis M, Alt J, Giroux M, Sawa A, Rojas C, and Slusher BS (2015) Selective CNS Uptake of the GCP-II Inhibitor 2-PMPA following Intranasal Administration. *PLoS One* **10**:e0131861.

Reith MEA, Blough BE, Hong WC, Jones KT, Schmitt KC, Baumann MH, Partilla JS, Rothman RB, and Katz JL (2015) Behavioral, biological, and chemical perspectives on atypical agents targeting the dopamine transporter. *Drug Alcohol Depend* **147**:1–19.

Tanda G, Hersey M, Hempel B, Xi Z-X, and Newman AH (2021) Modafinil and its structural analogs as atypical dopamine uptake inhibitors and potential medications for psychostimulant use disorder. *Curr Opin Pharmacol* **56**:13–21.

Thomsen M, Barrett AC, Negus SS, and Caine SB (2013) Cocaine versus food choice procedure in rats: environmental manipulations and effects of amphetamine. *J Exp Anal Behav* **99**:211–233.

Thomsen M, Fink-Jensen A, Woldbye DPD, Wörtwein G, Sager TN, Holm R, Pepe LM, and Caine SB (2008) Effects of acute and chronic aripiprazole treatment on choice between

cocaine self-administration and food under a concurrent schedule of reinforcement in rats. *Psychopharmacology (Berl)* **201**:43–53.

Tunstall BJ, Ho CP, Cao J, Vendruscolo JCM, Schmeichel BE, Slack RD, Tanda G, Gadiano AJ, Rais R, Slusher BS, Koob GF, Newman AH, and Vendruscolo LF (2018) Atypical dopamine transporter inhibitors attenuate compulsive-like methamphetamine self-administration in rats. *Neuropharmacology* **131**:96–103.

Zhang H-Y, Bi G-H, Yang H-J, He Y, Xue G, Cao J, Tanda G, Gardner EL, Newman AH, and Xi Z-X (2017) The Novel Modafinil Analog, JJC8-016, as a Potential Cocaine Abuse Pharmacotherapeutic. *Neuropsychopharmacology* **42**:1871–1883.

Footnotes

This work was supported by the National Institute of Health Grants from the National Institute on Drug Abuse [P50 DA006634]; the National Institute of Neurological Disorders and Stroke [U44 NS116931]; and the NIDA Intramural Research Program [1ZIADA000389-26].

Conflict of Interest: Rahimi and Childers held executive roles at EncepHeal Therapeutics, Inc. while conducting these studies. EncepHeal Therapeutics held an exclusive license (NIH License: L-226-2017) for intellectual property tested in these studies. Newman and Cao are inventors on this NIH patent. Furthermore, NIH grant funds awarded to EncepHeal Therapeutics, Inc. (NIH Grant: U44 NS116931) supported these activities. The remaining authors declare no conflict of interest.

Figure Legends

Figure 1. Timeline for weekly chronic drug treatments.

Figure 2. Pharmacokinetic analyses of JJC8-088 and JJC8-091 in nonhuman primates following IV administration. (A) Plasma concentration (pmol/mL) time profile of JJC-088 (IV; 0.7 mg/kg) and (B) JJC8-091(IV; 1.9 mg/kg) (C) Pharmacokinetic parameters of JJC8-088 and -091. $T_{1/2}$ half-life; C_0 initial concentration; AUC_∞ area under curve extrapolated to infinity; V_d volume of distribution.

Figure 3. Effects of acute JJC8-088 on cocaine vs. food choice in three rhesus monkeys. Ordinate: percent of total trials in which cocaine was chosen; Abscissa: cocaine dose (mg/kg per injection). Each point represents the mean (± SD) of two determinations.

Figure 4. Effects of acute JJC8-091 on cocaine vs. food choice in three rhesus monkeys. Ordinate: percent of total trials in which cocaine was chosen; Abscissa: cocaine dose (mg/kg per injection). Each point represents the mean (± SD) of two determinations.

Figure 5. Effect of chronic JJC8-088 as a function of weeks of treatment in three rhesus monkeys responding under a concurrent cocaine vs. food choice schedule of reinforcement. Ordinates: (**A**) percent of total trials in which cocaine was chosen; Abscissae: unit dose of cocaine available as an alternative to one food pellet. Data points are single-point determinations.

Figure 6. Effect of chronic JJC8-091 as a function of weeks of treatment in three rhesus monkeys responding under a concurrent cocaine vs. food choice schedule of reinforcement. Ordinates: percent of total trials in which cocaine was chosen; Abscissae: unit dose of cocaine available as an alternative to one food pellet. Data points are single-point determinations.

JPET Fast Forward. Published on December 6, 2022 as DOI: 10.1124/jpet.122.001363
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1. Chronic JJC8-088 (top panel) and JJC8-091 (bottom panel) on cocaine-food choice ED50 each week (day 5) over the treatment time period for three monkeys.

Chronic JJC8-088 Treatment on Cocaine - Food Choice ED50											
R-1690	Week	Baseline	1	2	3	4	5	6	7	8	9
	Dose (mg/kg)	N/A	0.7	0.7	0.7	1	1	1	1	0.7	0
	ED50	0.017	0.008	0.004	0.005	0.005	0.017	0.017	0.017	0.017	0.017
R-1710	Week	Baseline	1	2	3	4	5	6	7	8	9
	Dose (mg/kg)	N/A	0.7	0.7	0.7	0.7	1	1	1.7	1.7	1
	ED50	0.007	0.005	0.014 *	0.014 *	0.013 *	0.1 **	0.063 *	0.003	0.003	0.047 *
R-1688	Week	Baseline	1	2	3	4	5	6	7	8	9
	Dose (mg/kg)	N/A	0.7	0.7	0.7	1	1	1.7	3	3	1.7
	ED50	0.007	0.003	0.003	0.004	0.003	0.003	0.007	0.11 *	0.006	0.003
Chronic JJC8-091 Treatment on Cocaine - Food Choice ED50											
R-1691	Week	Baseline	1	2	3	4	5	6	7	8	
	Dose (mg/kg)	N/A	3.6	3.6	3.6	5.6	5.6	10	17	10	
	ED50 (mg/kg)	0.017	0.015	0.018 *	0.003	0.015	0.011	0.017	0.003	0.012	
R-1710	Week	Baseline	1	2	3	4	5	6	7		
	Dose (mg/kg)	N/A	3.6	3.6	5.6	5.6	5.6	10	10		
	ED50	0.016	0.004	0.003	0.007	0.017 *	0.047 *	0.015	0.012		
R-1688	Week	Baseline	1	2	3						
	Dose (mg/kg)	N/A	17	17	17						
	ED50	0.017	0.005	0.015	0.017						
* Indicates right shift; ** slope flat											

* Indicates right shift in ED50; ** slope precludes calculation of ED50 (i.e., Flat).

JPET Fast Forward. Published on December 6, 2022 as DOI: 10.1124/jpet.122.001363
This article has not been copyedited and formatted. The final version may differ from this version.

Table 2. Effects of Chronic Treatment of JJC Compounds on Food-Only (Component 1) Responding

Chronic JJC8-088 Treatment

	Monkey	BL	1	2	3	4	5	6	7	8	9
	Dose (mg/kg)	-	0.7	0.7	0.7	1.0	1.0	1.0	1.0	0.7	0.0
R-1690	Mean	10	9.25	10	9.5	5.0	0.0	0.0	0.0	0.25	10
	SD	0.0	1.5	0.0	1.0	3.6	0.0	0.0	0.0	0.5	0.0
	Dose (mg/kg)	-	0.7	0.7	0.7	0.7	1.0	1.0	1.7	1.7	1.0
R-1710	Mean	10	0.0	7.5	10	10	7.75	10	0.0	0.0	2.75
	SD	0.0	0.0	5.0	0.0	0.0	1.7	0.0	0.0	0.0	1.9
	Dose (mg/kg)	-	0.7	0.7	0.7	1.0	1.0	1.7	3.0	3.0	1.7
R-1688	Mean	10	10	10	10	10	10	10	8.75	4.25	7.25
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	2.6	1.9

Chronic JJC8-091 Treatment

	Monkey	BL	1	2	3	4	5	6	7	8
	Dose (mg/kg)	-	3.6	3.6	3.6	5.6	5.6	10	17	10
R-1691	Mean	10	10	10	10	10	10	10	6.0	10
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.9	0.0
	Dose (mg/kg)	-	3.6	3.6	5.6	5.6	5.6	10	10	
R-1710	Mean	10	10	10	10	7.25	8.25	10	9.5	
	SD	0.0	0.0	0.0	0.0	3.4	3.5	0.0	1.0	
	Dose (mg/kg)	-	17	17	17					
R-1688	Mean	10	0	0.5	7.5					
	SD	0.0	0.0	1.0	5.0					

For Weeks 1-9, each point represents mean data from four consecutive Food-only (component) number of reinforcers (Max = 10)

Figure 1

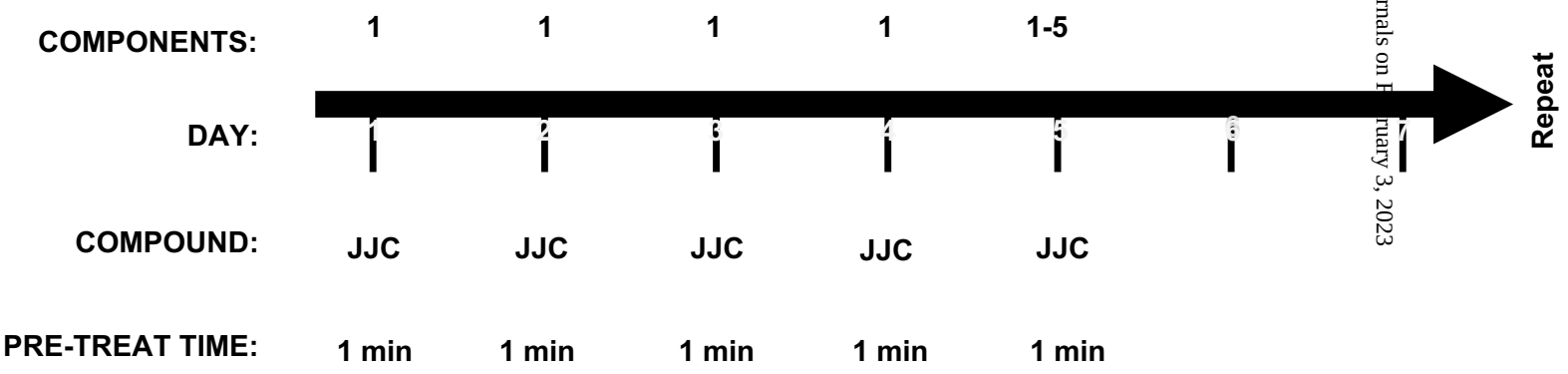
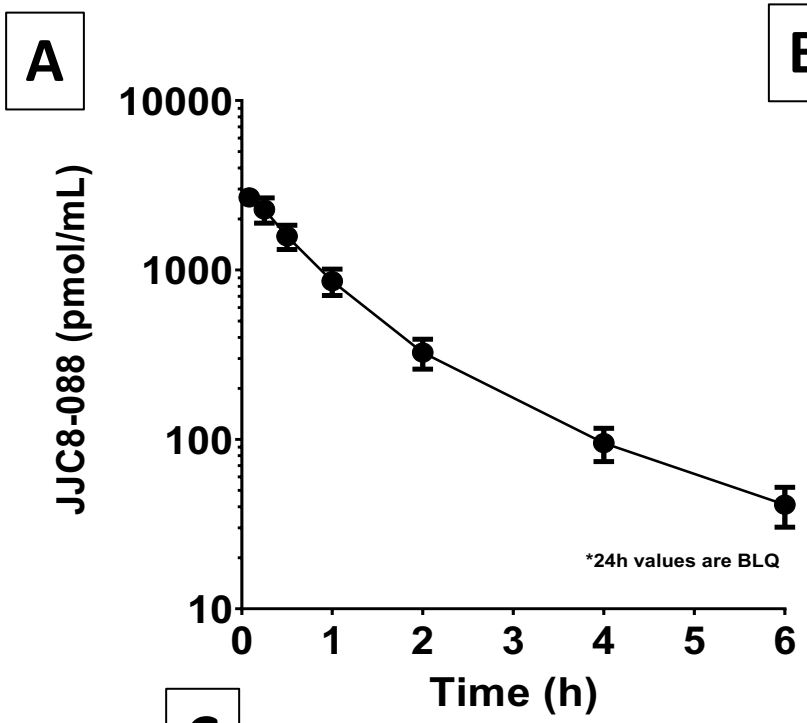
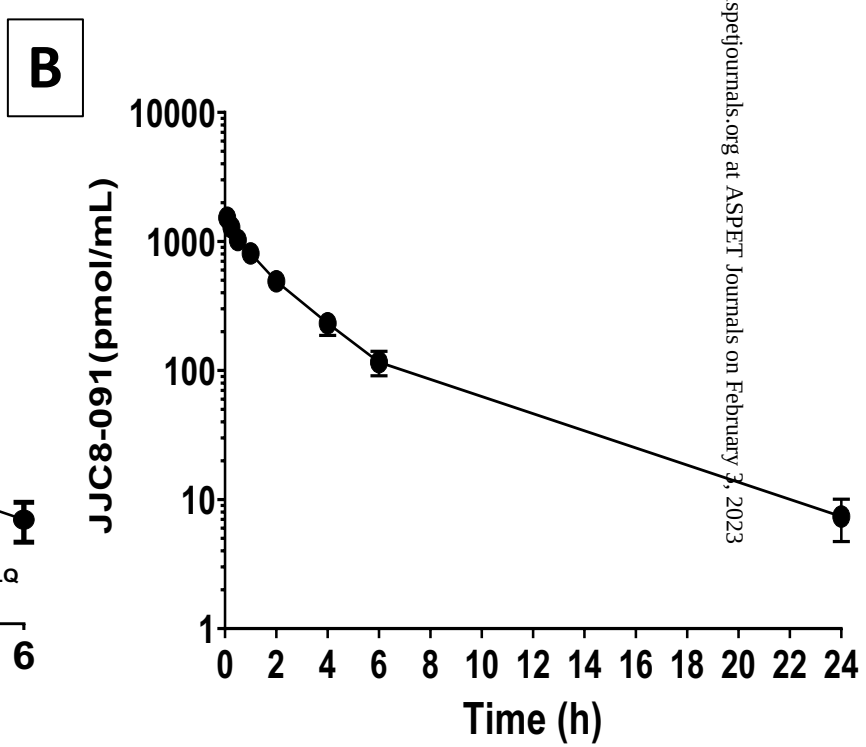


Figure 2

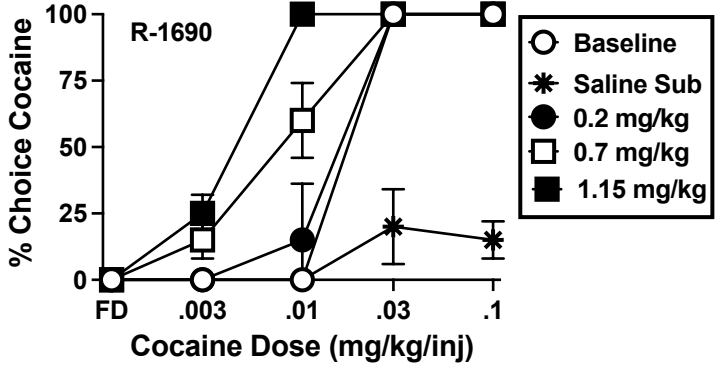




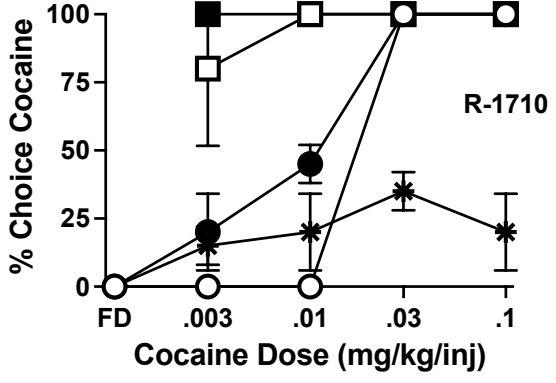
C

Compound	Dose i.v. (mg/kg)	$T_{1/2}$ (h)	C_0 (pmol/ml)	AUC_∞ (h*pmol/mL)	V_d (L/kg)
JJC8-088	0.7	1.14 ± 0.07	2921.9 ± 270.3	2912.4 ± 476.7	0.96 ± 0.15
JJC8-091	1.9	3.35 ± 0.67	1678.9 ± 249.4	3509.2 ± 592.8	7.86 ± 0.51

Figure 3



Acute JJC8-088



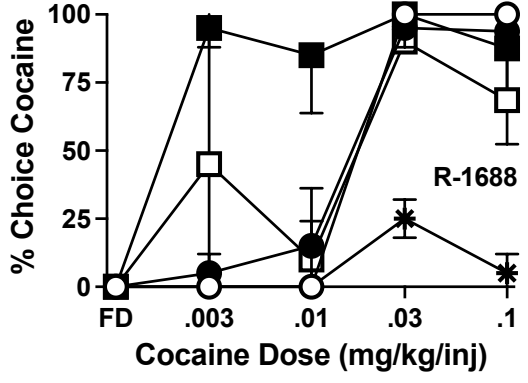


Figure 4

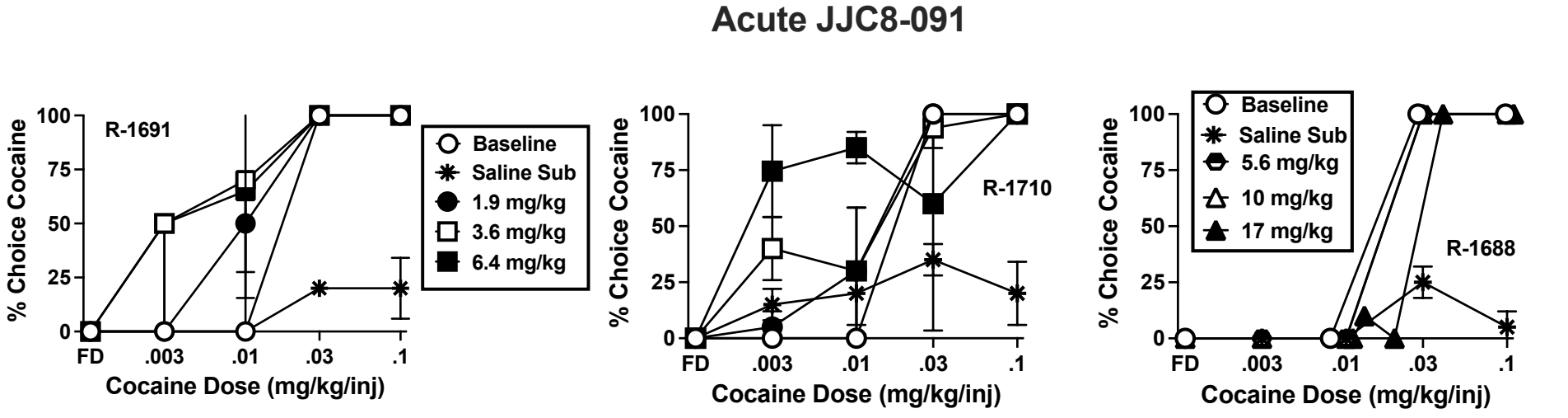
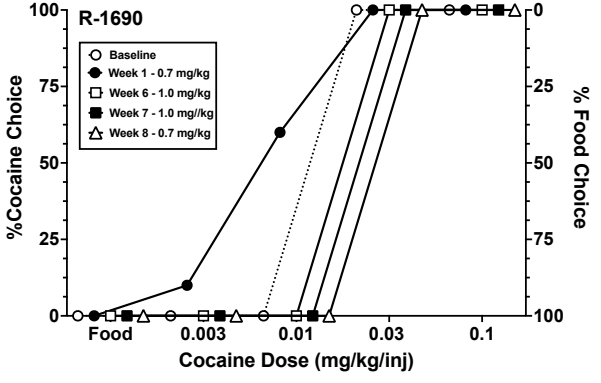
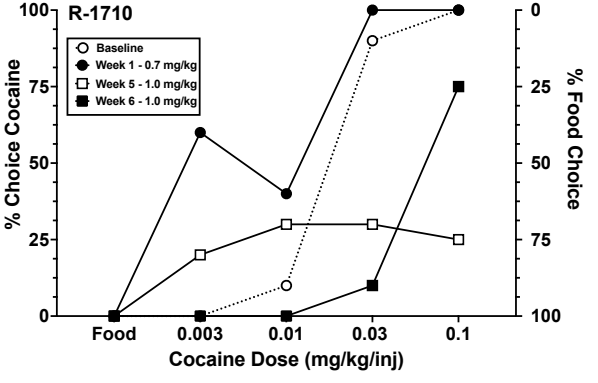

Acute JJC8-091
R-1691
% Choice Cocaine
100
75
50
25
0
FD
.003
.01
.03
.1
Cocaine Dose (mg/kg/inj)
Baseline
Saline Sub
1.9 mg/kg
3.6 mg/kg
6.4 mg/kg
R-1710
% Choice Cocaine
100
75
50
25
0
FD
.003
.01
.03
.1
Cocaine Dose (mg/kg/inj)
R-1688
% Choice Cocaine
100
75
50
25
0
FD
.003
.01
.03
.1
Cocaine Dose (mg/kg/inj)
Baseline
Saline Sub
5.6 mg/kg
10 mg/kg
17 mg/kg

Figure 5

Chronic JJC8-088





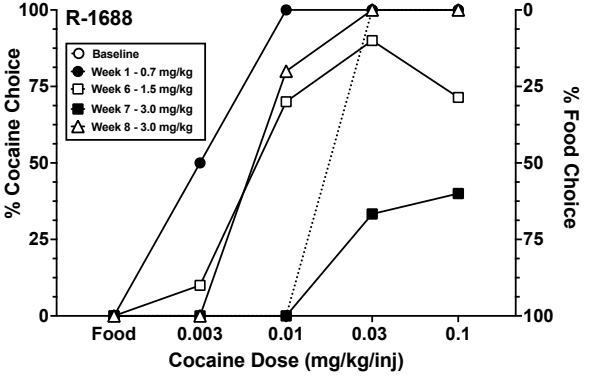


Figure 6

Chronic JJC8-091