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An FSCV study on the effects of targeted typical and atypical DAT inhibition on dopamine dynamics in the nucleus accumbens shell of male and female mice

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Abstract

Understanding the neurochemistry underlying sex differences in psychostimulant use disorders (PSUD) is essential for developing related therapeutics. Many psychostimulants, like cocaine, inhibit the dopamine transporter (DAT), which is largely thought to account for actions related to their misuse and dependence. Cocaine-like, typical DAT inhibitors, preferentially bind DAT in an outward-facing conformation while atypical DAT inhibitors, like modafinil, prefer a more inward-facing DAT conformation. Modafinil and *R*-modafinil have emerged as potential therapeutic options for selected populations of individuals affected by PSUD. In addition, analogs of modafinil (JJC8-088 and JJC8-091) with different pharmacological profiles have been explored as potential PSUD medications in preclinical models. In this work, we employ fast scan cyclic voltammetry (FSCV) to probe nucleus accumbens shell (NAS) dopamine (DA) dynamics in C57BL/6 male and female mice. We find that cocaine slowed DA clearance in both male and female mice but produced more robust increases in evoked NAS DA in female mice. *R*-modafinil produced mild increases in evoked NAS DA and slowed DA clearance across the sexes. The modafinil analog, JJC8-088, a typical DAT inhibitor, produced increases in evoked NAS DA in female and male mice. Finally, JJC8-091, an atypical DAT inhibitor, produced limited increases in evoked NAS DA and slowed DA clearance in both sexes. In this work we begin to tease out how sex differences may alter the effects of DAT targeting and highlight how this may help focus research toward effective treatment options for PSUD.

Graphical Abstract

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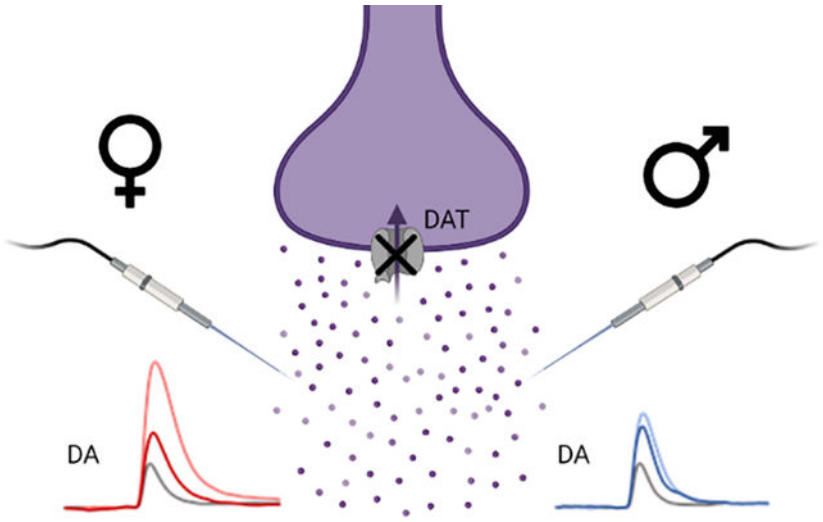
Author Contributions

The experiments were performed by MH, AYC, and MKB. The manuscript was written by MH and GT with helpful edits and contributions from all other authors.

Conflict of Interest

The authors declare no competing financial interest.



Keywords

Dopamine; FSCV; substance use disorder; sex differences; dopamine transporter; modafinil

Introduction

Psychostimulants like cocaine and methylphenidate are commonly misused across the globe. The number of people affected by psychostimulant use disorders (PSUD), identified by a dependence on illicit or misused stimulants, has increased in past years and has been exacerbated by the covid pandemic.[1] Overdose rates have also risen, in part, due to increases in polysubstance use of synthetic opioids like fentanyl merging the opioid crisis with PSUD.[2]

Psychostimulants act through indirectly modulating dopamine (DA) neurotransmission. For example, cocaine acts via blockade of the dopamine transporter (DAT) which reduces DA reuptake and clearance, and increases DA levels in the synaptic cleft.[3, 4] It is thought that these neurochemical actions producing elevated levels of DA in the mesolimbic system are largely linked to the euphoria, which would promote recreational use, possibly leading to dependence in many cocaine users.[3]

Evidence of differences in substance use and addiction between males and females has been reported.[5–7] Studies have shown that females more readily escalate or acquire self-administration, in rodents,[8–10] or drug use in humans,[11, 12] compared to males. For instance, female rodents exhibit higher motivation to access the drug.[13, 14] In humans, females reported higher craving in response to drug associated cues[15] and experience more debilitating withdrawal symptoms. [11, 16] Finally, in both rodents and humans, females have a higher risk of relapse/reinstatement to drug use (humans[17]; rodents[18, 19]) than males. Notably, in females, cycling levels of the hormone estradiol have been linked to more rapid acquisition of drug taking and other addictive-like behaviors and may account for some of the behavioral differences observed in substance use studies (reviewed in [5, 20]).

Sex differences in neurochemical responses related to substance use have also been reported (reviewed in[5, 7, 20]). Notably, cocaine administration produced an amplified effect on nucleus accumbens DA signaling in female rodents, which may be dependent on changing levels of cycling hormones during the female estrous cycle (i.e., high estradiol).[21–23]

At variance with cocaine and other addictive psychostimulants, another class of DA uptake inhibitors (DUIs), known as the atypical DAT blockers, produces different levels of behavioral and neurochemical effects.[24–27] Among these atypical DUI, modafinil (Provigil) and *R*-modafinil (Nuvigil; the long acting enantiomer of modafinil), drugs currently approved for the treatment of sleep disorders, shows limited, if any, addictive effects, in clinical and preclinical studies.[28–31] Several studies have shown that modafinil or its *R*-enantiomer preferentially bind to a conformation of DAT distinct from that preferred by addictive, cocaine-like psychostimulants.[32–34] DAT is a dynamic membrane transporter protein, and its conformation fluctuates based on its function as a transporter, and what substrate is bound. Typical DAT inhibitors, like cocaine, preferentially bind DAT in an outward-facing DAT conformation while atypical DAT inhibitors, like modafinil and its analogues, such as JJC8-091 prefer a more inward-facing DAT conformation.[35, 36] Importantly, *R*-modafinil elicits behavioral and neurochemical effects that are at variance with those shown by typical DAT inhibitors [37, 38](reviewed in [28]). The cocaine-like typical DAT inhibitor JJC8-088 has been shown to stimulate ambulatory activity and to produce cocaine-like changes in NAS DA dynamics.[35, 36] The atypical DAT inhibitor JJC8-091, in contrast, does not produce an increase in locomotion and produces little to no increases in NAS DA levels.[35, 39] However, both modafinil analogs inhibit DAT and slow NAS DA clearance rates.[35, 36] Thus far, most preclinical studies, including those with modafinil and modafinil analogs, are often conducted exclusively in male rodents.

The present study aims to investigate how the actions of a stimulant, like cocaine, on brain DA may differ from those produced by some novel therapeutic options for PSUD: *R*-modafinil and the modafinil analogs JJC8-088 and JJC8-091, in male and female mice. Since atypical DAT inhibitors show limited efficacy in stimulating increases in DA levels and attenuate the behavioral effects of cocaine when administered in combination, we postulate that the actions of these drugs may be less dependent on sex than cocaine-like inhibitors of DAT. It is possible that dimorphisms in NAS DA dynamics following the administration of these potential therapeutic agents may exist, which may be useful to better understand the potential of these new drug agents as medications for PSUD.

Results and Discussion

Cocaine inhibits DAT and produces robust effects on evoked DA release in female mice

We show representative color plots (Figure 1A) of evoked NAS DA release at control/baseline as well as 10, 30, and 90 min post administration of cocaine (10 mg/kg). Additionally, we show the averaged evoked NAS DA release at control/baseline and in response to an acute dose of cocaine in male and female mice at doses of 3 mg/kg (Figure 1B) and 10 mg/kg (Figure 1C) at timepoints 10, 30, and 90-min post cocaine administration. Prior to cocaine or any other drug administration, there were no observed sex differences in control or baseline evoked NAS DA release amplitude (male (n=54): 0.25 ± 0.02 μM; female

(n=54): 0.24 ± 0.02 μM; unpaired t-test: t(106) = 0.45, p = 0.65) or $t_{1/2}$ (an indicator of DA clearance rate) (male (n=54): 1276.26 ± 46.33 ms; female (n=54): 1228.30 ± 38.68 ms; unpaired t-test: t(106) = 0.79, p = 0.43).

In Figure 2, we show a full 2-hour time course of cocaine-induced effect on normalized maximum evoked DA release (Figure 2A) and $t_{1/2}$ (Figure 2B) in male and female mice showing a dose-dependent increase in DA_{max} [repeated measures three-way ANOVA: main effect of time ($F_{27,540}$=31.53, p < 0.0001), main effect of dose ($F_{1,20}$=9.99, p = 0.005), main effect of sex ($F_{1,20}$=0.02, p = 0.88), interaction between time × dose ($F_{27,540}$=4.20, p < 0.0001), interaction between time × sex ($F_{27,540}$=4.15, p < 0.0001), interaction between dose × sex ($F_{1,20}$=1.49, p = 0.24), and 3-way interaction ($F_{27,540}$=1.95, p = 0.003)] and $t_{1/2}$ [repeated measures three-way ANOVA: main effect of time ($F_{27,540}$=22.80, p < 0.0001), main effect of dose ($F_{1,20}$=6.92, p = 0.02), main effect of sex ($F_{1,20}$=0.007, p = 0.94), interaction between time × dose ($F_{27,540}$=3.39, p < 0.0001), interaction between time × sex ($F_{27,540}$=1.24, p = 0.19), interaction between dose × sex ($F_{1,20}$=0.32, p = 0.58), and 3-way interaction ($F_{27,540}$=1.15, p = 0.27)]. Notably, we observed that the cocaine-induced increases in evoked NAS DA appear at a faster rate/at a larger extent in female mice than in male mice [repeated measures three-way ANOVA of DA_{max} 5–15 min post cocaine: main effect of time ($F_{2,40}$=17.45, p < 0.0001), main effect of dose ($F_{1,20}$=1.65, p = 0.21), main effect of sex ($F_{1,20}$=4.61, p = 0.04), interaction between time × dose ($F_{2,40}$=2.38, p = 0.11), interaction between time × sex ($F_{2,40}$=0.95, p = 0.39), interaction between dose × sex ($F_{1,20}$=0.04, p = 0.85), and 3-way interaction ($F_{2,40}$=2.51, p = 0.09)], but that sex-dependent effect leveled out by at later timepoints post cocaine.

Other researchers have shown similar effects of sex, where observed DA responses appear greater in females than in males following cocaine administration.[11, 21–23, 40, 41] This literature suggests that there are some mild differences in the neurochemistry of DA in the nucleus accumbens that may account for the differences in cocaine-induced increases in evoked NAS DA release that we observed. This is of particular interest because these differences in DA neurochemistry may underly the changes in cocaine-induced behavior effects by sex.[5–7] Based on the literature we postulate that there are four potential biological factors that account for the sex differences in NAS dopaminergic responses to cocaine.

First, it has long been speculated that due to natural differences in body size and mass that there may be sex differences in drug pharmacokinetics or metabolism. Studies have shown consistent concentrations of cocaine in plasma and brain tissue following cocaine administration in male and female rats, and plasma of men and women, however, sex differences in cocaine metabolite distribution were observed in rats.[42, 43] Therefore, we cannot exclude that sex differences in cocaine metabolism could, in part, contribute to the neurochemical differences in cocaine responses observed in the male and female mice.

Second, cycling levels of hormones, especially estradiol may account at least in part for sex differences in dopaminergic responses to cocaine. Calipari et al. 2017 showed very similar results in the nucleus accumbens and noted that the more robust cocaine-induced increase in evoked DA in female mice could be linked to the cycling hormone, estradiol.[21] In particular, they note that hormones could influence firing rate in the VTA. This effect is

likely muffled in our experiments because our sample size in females is not large enough to analyze variability throughout the cycling hormone levels of the estrous cycle. Instead, we present our data as a pooled average of female responses regardless of estrous cycle stage/hormone levels. Yoest et al. 2019 showed that estradiol benzoate acutely regulates DA dynamics in female mice in response to cocaine.[23] Similarly, Cummings et al. 2014 found that ovariectomized female rats treated with estradiol benzoate showed increased dopaminergic responses to cocaine.[22] The role of estradiol has been well reviewed and likely influences some of the factors to be discussed further.[7, 44]

Third, differences in transporter numbers or function may also account for the sex differences we observed following cocaine administration. While previous studies have shown estradiol-dependent changes in DA reuptake in the nucleus accumbens,[45] we do not observe any sex-dependent effects, see Figure 2B. It is notable that Calipari et al. 2017 showed estrus-dependent increases in phosphorylation of DAT that may transiently increase the efficiency of DAT in the nucleus accumbens of female mice.[21] We did not observe any sex differences in $t_{1/2}$ following cocaine administration in our experiments which indicates that there were no overall changes in DAT function or efficacy but we did not directly measure levels of DAT phosphorylation.

Fourth, sex differences in DA receptor activation, especially autoreceptors may play a role. In particular, researchers have noted that D2 autoreceptor feedback mechanisms appear to be blunted in females and estradiol is linked to higher DA firing rates and this could largely explain the increased cocaine-induced DA release in female mice.[21, 41] Estradiol has also been shown to modify D2R affinity states.[46] It is also possible that the neurochemical sex differences observed by us and others in response to cocaine may be further linked to differences in autoreceptor control of DAT activity.[41]

Overall, we find that cocaine administration slows DA clearance and increases maximum evoked DA release in the NAS in both male and female mice. This effect on evoked DA release is observed consistently in both sexes however, quantitatively it is on average larger in female mice in what may be an estradiol-dependent manner as suggested by the literature.[21]

R-Modafinil inhibits DAT in male and female mice

Next, we aimed to test the effect of *R*-modafinil administration on NAS DA dynamics. We show representative color plots (Figure 3A) of evoked NAS DA release at control/baseline as well as 30 and 90-min post administration of *R*-modafinil (10 mg/kg). Additionally, we show the averaged evoked NAS DA release response to an acute administration of *R*-modafinil in male and female mice at doses of 10 mg/kg (Figure 3B) and 32 mg/kg (Figure 3C) at timepoints 30 and 90-min post *R*-modafinil administration.

In Figure 4, we show a full 2-hour time course of *R*-modafinil-induced effect on normalized maximum evoked DA release (Figure 4A) and $t_{1/2}$ (Figure 4B) in male and female mice. *R*-modafinil administration produced dose-dependent increases in NAS evoked DA release and increases in $t_{1/2}$ [DA release: (DA_{max}): repeated measures three-way ANOVA: main effect of time ($F_{27,648}$=24.93; $p < 0.0001$), main effect of dose ($F_{1,24}$=14.24; $p = 0.0009$),

main effect of sex ($F_{1,24}$=0.00003; p = 0.99), interaction between time × dose ($F_{27,648}$=3.84; p < 0.0001), interaction between time × sex ($F_{27,648}$=0.24; p > 0.9999), interaction between dose × sex ($F_{1,24}$=0.06; p = 0.81), and 3-way interaction ($F_{27,648}$=0.17, p > 0.9999); DA clearance ($t_{1/2}$): repeated measures three-way ANOVA: main effect of time ($F_{27,648}$=57.39; p < 0.0001), main effect of dose ($F_{1,24}$=14.26; p = 0.0009), main effect of sex ($F_{1,24}$=0.002; p = 0.97), interaction between time × dose ($F_{27,648}$=7.39; p < 0.0001), interaction between time × sex ($F_{27,648}$=0.77; p = 0.80), interaction between dose × sex ($F_{1,24}$=0.35; p = 0.56), and 3-way interaction ($F_{27,648}$=0.94, p = 0.55)].

The observed effect of *R*-modafinil on NAS DA is consistent with what was observed previously in male Swiss Webster mice.[38] Thus, *R*-modafinil effects on evoked NAS DA appear to be consistent in male and female mice. In agreement with previously published data, we see that the actions of cocaine on stimulation of evoked DA are more robust and shorter-lasting than the effects of *R*-modafinil which appear more prolonged/longer lasting.[34] Interestingly, *R*-modafinil has a lower affinity for DAT than cocaine (3050 nM vs 72 nM)[36, 47] and it appears to produce a smaller increase in $t_{1/2}$ or less inhibition of DA clearance than that observed following cocaine administration. Differences in DA dynamics elicited by cocaine or *R*-modafinil may result in their different behavioral effects that could also influence their reinforcing effects and, especially for cocaine, the occurrence of misuse and dependence.[28, 29]

We postulate that the lack of sex-dependent differences following the administration of atypical DAT inhibitors like R-modafinil could support the differences between typical and atypical DAT inhibitors in altering DAT number or function in the neuronal membrane. Novel super-resolution imaging has shown that DAT is sequestered into nanodomains in the plasma membrane to allow for highly regulated control of dopamine dynamics via DAT distribution.[48, 49] Further localization to these nanoclusters may denote DAT conformations, as nanoclustered DAT proteins were found to be in the inward-facing conformation.[49] Interestingly, the D2R antagonism decreased localization of DAT to nanodomains thereby promoting the unclustering of DAT proteins which are, in contrast, in an outward-facing conformation.[49] Thus the blunting of D2R feedback, like that seen at times of high estradiol levels in females,[21, 41] may act synergistically with the cocaine/typical DAT inhibitors to increase unclustering of DAT proteins. In contrast, these forces may act in opposition with the atypical DAT inhibitors. This may support our findings of sex differences only in response to typical DAT inhibition (like that seen with cocaine administration) and warrants further investigation.

Unfortunately, modafinil has shown limited clinical efficacy for PSUD, with best results obtained in selected populations without co-occurring alcohol use disorder (reviewed in [28]). Thus, novel analogs of the parent drug with different pharmacological profiles and potential for a broader efficacy for PSUD have been synthesized and are currently being tested.[28, 35, 36, 50, 51]

Modafinil analogs inhibit DAT in male and female mice

Next, we analyzed the effect of administration of the two modafinil analogs, JJC8-088 and JJC8-091 on NAS DA dynamics.

We show representative color plots (Figure 5A) of evoked NAS DA release at control/baseline as well as 30 and 90-min post administration of JJC8-88 (10 mg/kg). Additionally, we show the averaged evoked NAS DA release response to an acute administration of JJC8-088 in male and female mice at doses of 10 mg/kg (Figure 5B) and 32 mg/kg (Figure 5C) at timepoints 30 and 90-min post JJC8-088 administration.

In Figure 6, we show a full 120 min time course of JJC8-088-induced effect on normalized maximum evoked DA release (Figure 6A) and $t_{1/2}$ (Figure 6B) in male and female mice. JJC8-088 produced increases in evoked NAS DA release and slowed DA clearance in a dose-dependent but not sex-dependent manner [DA release: (DA_{max}): repeated measures three-way ANOVA: main effect of time ($F_{27,648}$=58.66; $p < 0.0001$), main effect of dose ($F_{1,24}$=9.76; $p = 0.005$), main effect of sex ($F_{1,24}$=2.69; $p = 0.11$), interaction between time × dose ($F_{27,648}$=10.22; $p < 0.0001$), interaction between time × sex ($F_{27,648}$=1.46; $p = 0.06$), interaction between dose × sex ($F_{1,24}$=0.18; $p = 0.67$), and 3-way interaction ($F_{27,648}$=0.32, $p > 0.9996$); DA clearance ($t_{1/2}$): repeated measures three-way ANOVA: main effect of time ($F_{27,648}$=51.96; $p < 0.0001$), main effect of dose ($F_{1,24}$=7.48; $p = 0.01$), main effect of sex ($F_{1,24}$=0.16; $p = 0.70$), interaction between time × dose ($F_{27,648}$=7.81; $p < 0.0001$), interaction between time × sex ($F_{27,648}$=1.31; $p = 0.14$), interaction between dose × sex ($F_{1,24}$=0.01; $p = 0.92$), and 3-way interaction ($F_{27,648}$=0.31, p=0.999)].

We observed that the typical DAT inhibitor and modafinil analog JJC8-088 does produce cocaine-like increases in NAS DA, although interestingly these effects appear to be largely sex-independent. Previous studies in rodents have shown that JJC8-088 exhibits behavioral and neurochemical effects consistent with typical DAT inhibitors,[35, 38] For instance, JJC8-088 produced acquisition and maintenance of self-administration behavior in naïve and cocaine-trained rats, suggesting it has indeed cocaine-like, typical reinforcing effects. Also, other than eliciting cocaine-like increases in extracellular DA levels and in evoked DA release and clearance, JJC8-088 was shown to produce cocaine-like stimulation of ambulatory activity in mice. Altogether, the effects of JJC8-088 might suggest that the sex-dependent increases in NAS DA observed following cocaine administration may not necessarily be a hallmark of all typical DAT inhibitors or, alternatively may indicate that there is a spectrum in ‘typicality’ of DAT inhibitors.

Figure 7A shows representative color plots of evoked NAS DA release at control/baseline as well as 30 and 90-min post JJC8-091 administration (10 mg/kg). Additionally, we show the averaged evoked NAS DA release response to an acute administration of JJC8-091 in male and female mice at doses of 10 mg/kg (Figure 7B) and 32 mg/kg (Figure 7C) at timepoints 30 and 90-min post JJC8-088 administration.

In Figure 8, we show a full 2-hour time course of JJC8-091-induced effect on normalized maximum evoked DA release (Figure 8A) and $t_{1/2}$ (Figure 8B) in male and female mice. JJC8-091 increased evoked NAS DA release and slowed DA clearance in dose and sex-independent manners [DA release: (DA_{max}): repeated measures three-way ANOVA : main effect of time ($F_{27,648}$=5.52; $p < 0.0001$), main effect of dose ($F_{1,24}$= 0.02; $p = 0.90$), main effect of sex ($F_{1,24}$=0.003; $p = 0.96$), interaction between time × dose ($F_{27,648}$=0.37; $p = 0.999$), interaction between time × sex ($F_{27,648}$=0.58; $p = 0.96$), interaction between dose

× sex ($F_{1,24}$=0.07; p = 0.80), and 3-way interaction ($F_{27,647}$=0.21, p > 0.9999); post hoc Dunnett analysis of last control vs 30 min post JJC8-091 administration (p = 0.003); DA clearance ($t_{1/2}$): repeated measures three-way ANOVA: main effect of time ($F_{27,648}$=13.30; p < 0.0001), main effect of dose ($F_{1,24}$=0.00006; p = 0.999), main effect of sex ($F_{1,24}$=0.14; p = 0.72), interaction between time × dose ($F_{27,648}$=0.66; p = 0.91), interaction between time × sex ($F_{27,648}$=1.09; p = 0.35), interaction between dose × sex ($F_{1,24}$=0.25; p = 0.62), and 3-way interaction ($F_{27,648}$=1.19, p = 0.23); post hoc Dunnett analysis of last control vs 30 min post JJC8-091 administration (p = 0.0005)].

Notably, in these experiments we did not observe a dose-dependent increase in $t_{1/2}$ (and thus DAT inhibition) following JJC8-091 administration. This appears at contrast with previously reported neurochemical data in Swiss webster mice[38] and in Sprague Dawley rats following the administration of JJC8-091.[35] We postulate that C57BL/6 male mice may be less sensitive to JJC8-091 administration, a phenomenon we plan to pursue more in future studies. However, we cannot rule out the potential off-target pharmacological effects of large doses of JJC8-091, [36] which might play a role in the results obtained under the current experimental conditions.

In conclusion, we found that the atypical DAT inhibitors *R*-modafinil and JJC8-091 and the typical DAT inhibitor JJC8-088 appear to have less sex-based differences in their effects on NAS DA dynamics than those observed by cocaine administration. The sex-based discrepancies in cocaine-induced DA response have largely been attributed to estradiol dependent changes in D2 autoreceptor activation and DAT number and efficiency.[21, 41] It now seems possible that these sex differences do not significantly influence DA dynamics produced by *R*-modafinil and modafinil analogs and possibly their effects as PSUD medications. We highlight that the consistency in dopaminergic response to atypical DAT inhibitors like *R*-modafinil and JJC8-091 supports their potential efficacy in the treatment of PSUD across the sexes.

Methods

Chemicals & Reagents

Electrodes were calibrated using solutions of 0, 0.5, 1, 2, and 3 ug/mL dopamine hydrochloride (Sigma-Aldrich, Burlington, MA) in 0.1 M Phosphate buffered saline (Sigma-Aldrich, Burlington, MA). Cocaine hydrochloride (NIDA Drug Supply Program) was dissolved in sterile saline (Hospira, Lake Forest, IL). Modafinil analogs, JJC8-088 fumarate salt and JJC8-091 fumarate salt (synthesized by J. Cao in the Medicinal Chemistry Section, NIDA-IRP as previously described [36]) were dissolved into a solution made up of 10% DMSO (Sigma-Aldrich, Burlington, MA), 15% Tween-80 (Sigma-Aldrich, Burlington, MA), and 75% sterile water (Hospira, Lake Forest, IL) and sonicated until fully dissolved. Drug doses were selected based on previously published behavioral and neurochemical studies [35, 38] and injected at a volume of 10 mL/kg.

Animals

Male and female C57BL/6 mice (Charles River, Wilmington, MA), aged 8–12 weeks, were housed in groups with ad libitum access to food and water. Animal facilities were temperature and humidity controlled and maintained on a 12 h light/dark schedule (lights on at 7AM and lights off at 7PM). All experiments were performed in experimentally naïve animals during the light hours of the day. Following neurochemical studies vaginal smears were performed and cytology analyzed under a microscope in order to identify estrous cycle stage (data not reported) as described in Caligioni 2009.[52] Animal procedures included in this work were approved and performed in accordance with guidelines from the Animal Care and Use Committee of the National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD, USA which is fully accredited by the AAALAC International.

Fast Scan Cyclic Voltammetry

Surgery.—C57BL/6 mice were anesthetized with an i.p. injection of 33% w/v urethane (Sigma-Aldrich, Burlington, MA) dissolved in sterile saline (Hospira). As previously described, stereotaxic surgery was performed to implant a carbon fiber microelectrode into the NAS (AP= +1.5 mm; ML= −1.3 mm; DV= −4.2 to 5.0 mm) and a stimulating electrode made of a bipolar tungsten electrode (Plastics One Inc., Roanoke, VA) was implanted into the MFB (AP= −1.5 mm; ML= −1.0 mm; DV= −4.5 mm).[38] A reference electrode (Ag/AgCl) was implanted in the contralateral side of the brain.

Data Collection & Analysis.—Carbon fiber microelectrodes were fabricated as previously described.[38] A dopamine-specific waveform was applied by scanning −0.4 V to 1.3 V to −0.4 V at a rate of 400 V/s. Dopamine release was evoked using an electrical stimulation (24 pulses, 180 μA, 60 Hz, 4 ms duration). FSCV was performed using a UEI potentiostat, breakout box, and Tarheel-CV software (UNC electrical shop, Chapel Hill, NC). Stimulation was controlled with digitimer neurologs NL800A (Ft. Lauderdale, FL). Dopamine was identified by a sharp release and clearance event immediately following stimulation with an oxidation at 0.6 V. Data was analyzed using HDCV (UNC, Chapel Hill, NC) and Igor Pro (Wavemetrics, Portland, OR; as described in Keighron et al. 2019[38]) for identification of maximum evoked dopamine release (DA_{max}) and $t_{1/2}$ as an indicator of the rate of dopamine clearance ($t_{1/2}$= time it takes for half of the dopamine to be cleared; described further in Park et al. 2011[53]).

Histology.—Immediately after the end of neurochemical studies, a high voltage was applied to the carbon fiber microelectrode in order to lesion the brain tissue for histological identification of electrode placement. Animals were humanely sacrificed and brain tissue was collected and stored in formalin (4% formalde-fresh solution, Fisher Scientific, Waltham, MA) for at least 7 days. Three days prior to tissue slicing, brains were switched to a 30% sucrose solution (Sigma-Aldrich, Saint Louis, MO). Tissue was sectioned via cryostat at a width of 30 μm (Leica Biosystems, Deer Park, IL) and analyzed under a light microscope for correct electrode placement.

Experimental Design & Statistical Analyses

Experimentally naïve mice were arbitrarily assigned to a treatment and only one experiment was performed on each animal. A standard sample size of 5 was selected based on a previously published post-hoc power analysis.[54] Experimental data was collected every 5 min as follows: 4 control evoked DA files (stable with less than 15% variability in signal), pharmacological agent was administered, and data collection continued for 2 hour post drug. Drug treatments (cocaine, *R*-modafinil, JJC8-088, JJC8-091) at doses of 3, 10, or 32 mg/kg were arbitrarily assigned and only one treatment was administered to each animal for the purpose of these studies. Herein, data are presented as group means ± SEM. For DA_{max} and $t_{1/2}$ analyses, data is normalized and expressed as a percentage of control DA values. Data was excluded if the signal was not stable for the duration of the experiment, if the animal did not survive, or if histology could not confirm proper placement in the NAS. Of the data included 4 individual data points were removed as outliers from the dataset and replaced with an averaged normalized response of other animals in the treatment group.

Statistical analysis was completed using Graphpad Prism (San Diego, CA) using a paired t-test or ANOVA for repeated measures, over time, and dose applied to the data obtained from analysis of successive evoked DA release of each group. The Grubb's test for outliers was used as needed to confirm that data was not statistically different than the mean.

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Abbreviations

DA	Dopamine
DAT	Dopamine transporter
DA_{max}	Maximum evoked dopamine release
MFB	Medial forebrain bundle
NAS	Nucleus accumbens shell
PSUD	Psychostimulant use disorder
$t_{1/2}$	Measure of dopamine clearance

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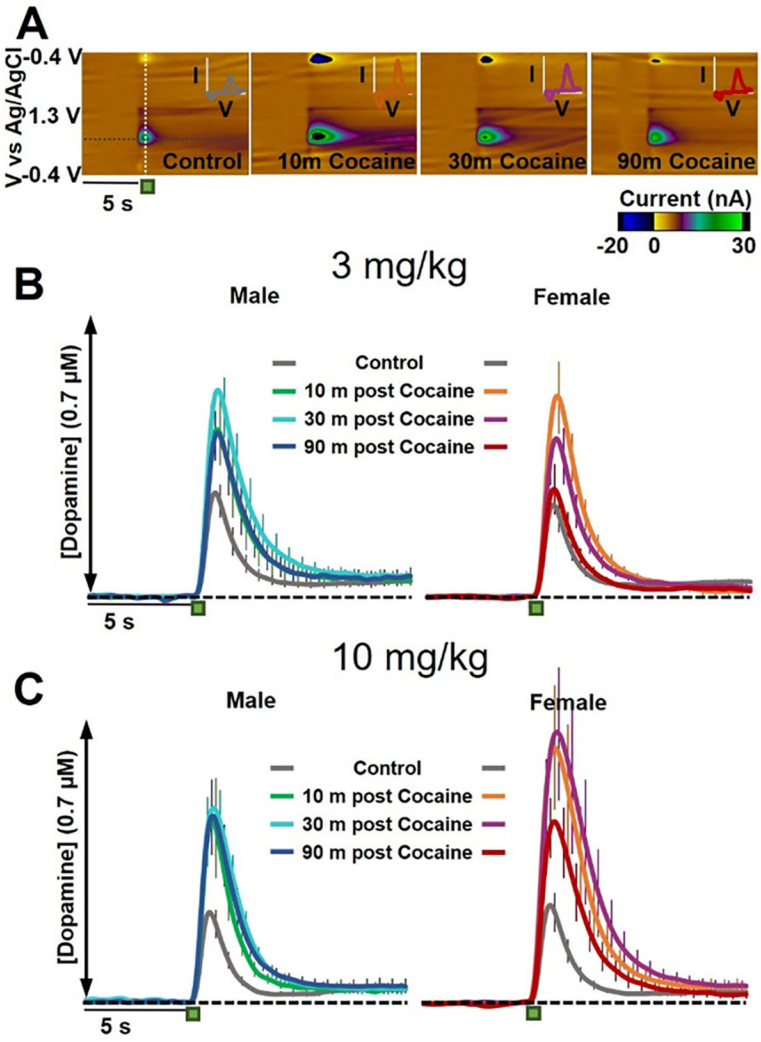

Figure 1: Cocaine administration increases evoked DA release and slows DA clearance in the NAS.

(**A**) Representative color plots of evoked NAS DA for a female mouse treated with 10 mg/kg Cocaine. (**B**) The averaged evoked NAS DA response in male and female mice at control levels (shown in grey), 10 min (male: green, female: orange), 30 min (male: teal, female: purple), and 90 min (male: blue, female: red) post cocaine administration of 3 mg/kg; i.p. and (**C**) 10 mg/kg; i.p. Stimulation is marked by green box below the plots and traces. Data is shown as the average with SEM in correspondingly colored error bars (n=6 for each treatment).

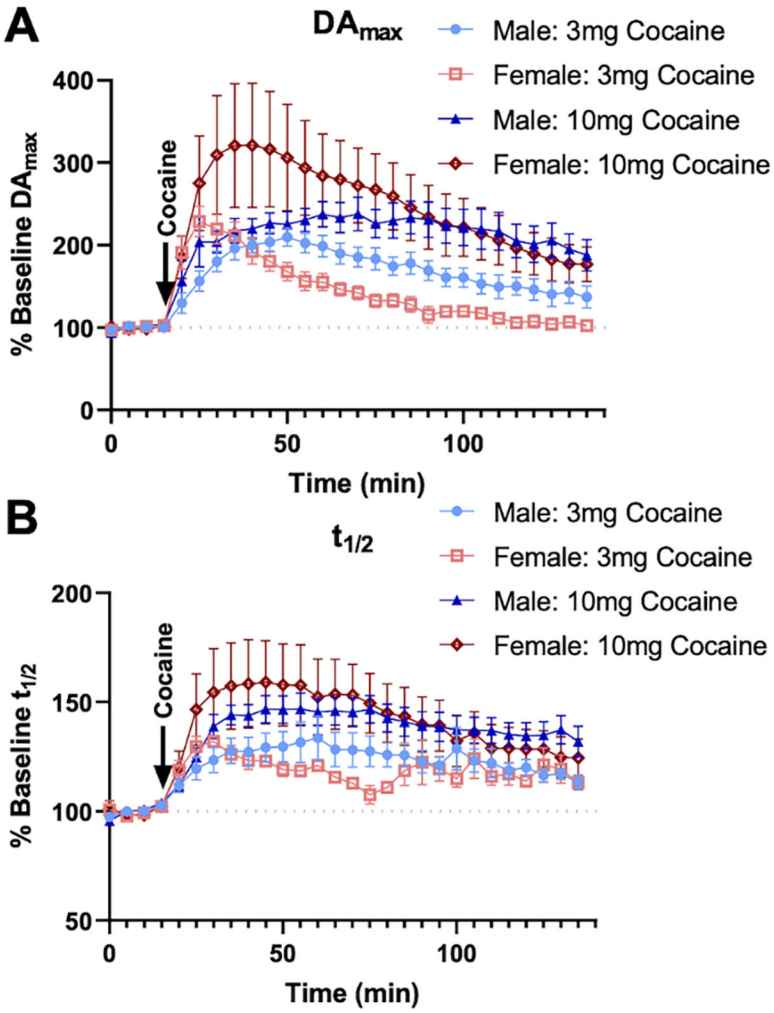

Figure 2: Cocaine administration increases evoked DA release and slows DA clearance in the NAS.

(**A**) Averaged normalized change in NAS DA_{max} and (**B**) DA clearance ($t_{1/2}$) in male and female mice. DA was analyzed every five min and after 4 consistent control files, cocaine (3 or 10 mg/kg; i.p.) was administered. DA was analyzed for 120 min after drug administration. (n=6 for each treatment group).

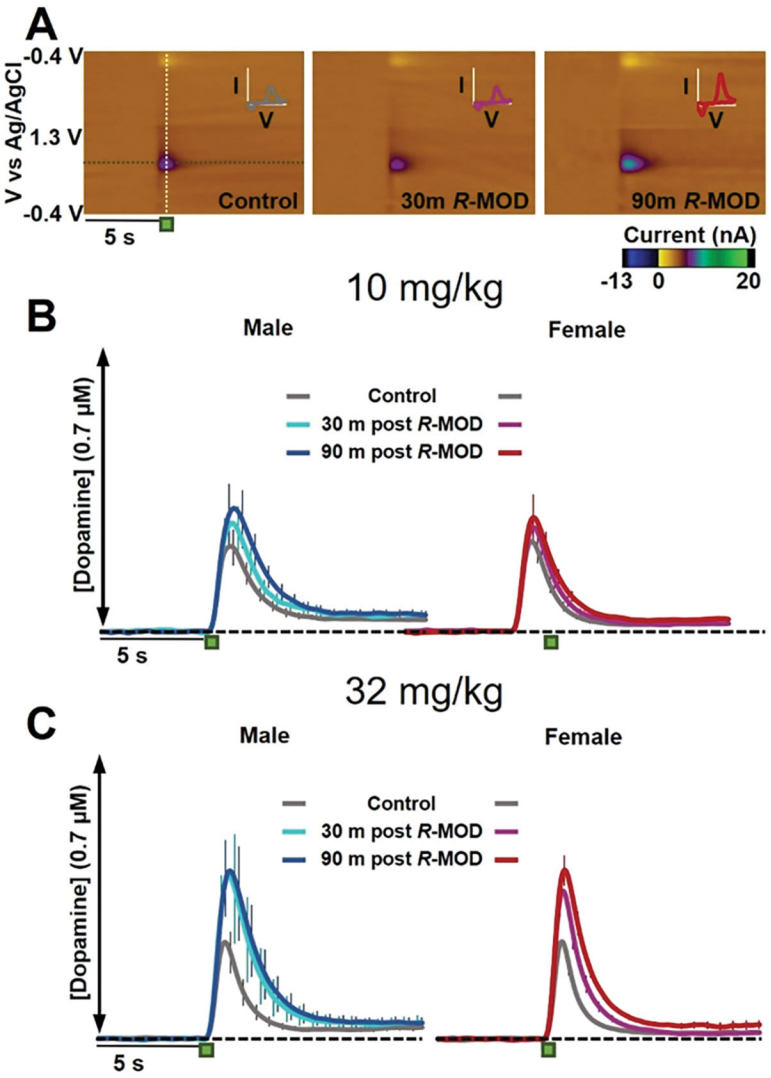

Figure 3: *R*-modafinil (*R*-MOD) administration increases evoked DA release and slows DA clearance in the NAS.

(**A**) Representative color plots of evoked NAS DA for a female mouse treated with 10 mg/kg *R*-modafinil. (**B**) The averaged evoked NAS DA response in male and female mice at control levels (shown in grey), 30 min (male: teal, female: purple), and 90 min (male: blue, female: red) post *R*-modafinil administration of 10 mg/kg; i.p. and (**C**) 32 mg/kg; i.p. Stimulation is marked by green box below the plots and traces. Data is shown as the average with SEM in correspondingly colored error bars (n=7 for each treatment group).

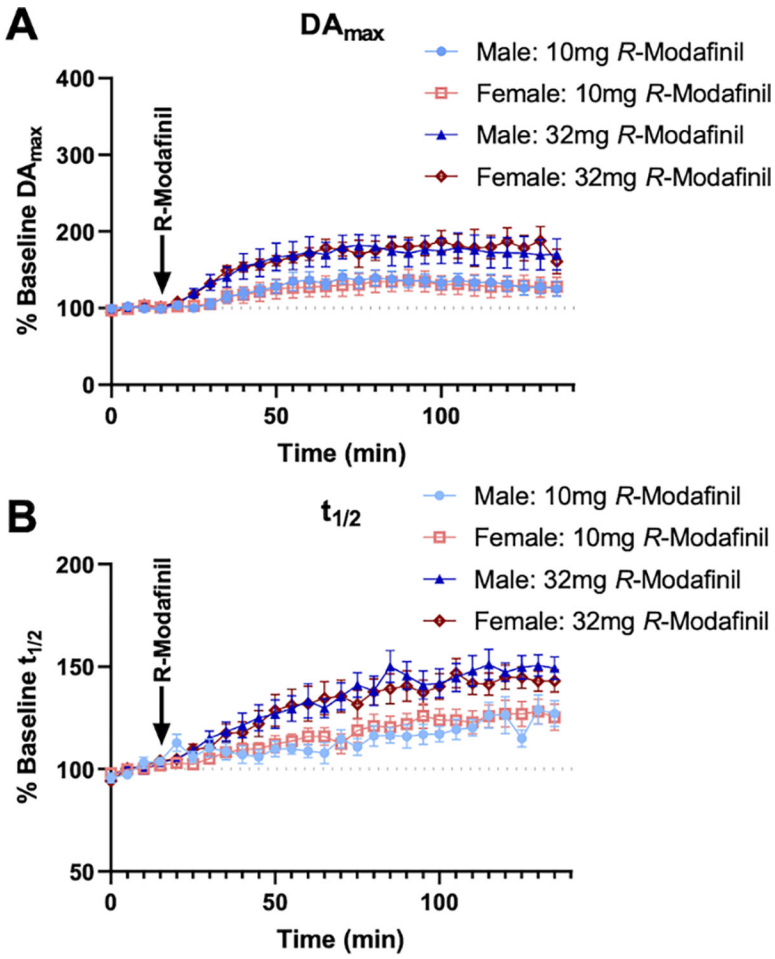

Figure 4: *R*-modafinil administration increases evoked DA release and slows DA clearance in the NAS.

(**A**) Averaged normalized change in NAS DA_{max} and (**B**) DA clearance ($t_{1/2}$) in male and female mice. DA was analyzed every five min and after 4 consistent control files, *R*-modafinil (10 or 32 mg/kg; i.p.) was administered. DA was analyzed for 120 min after drug administration. (n=7 for each treatment group).

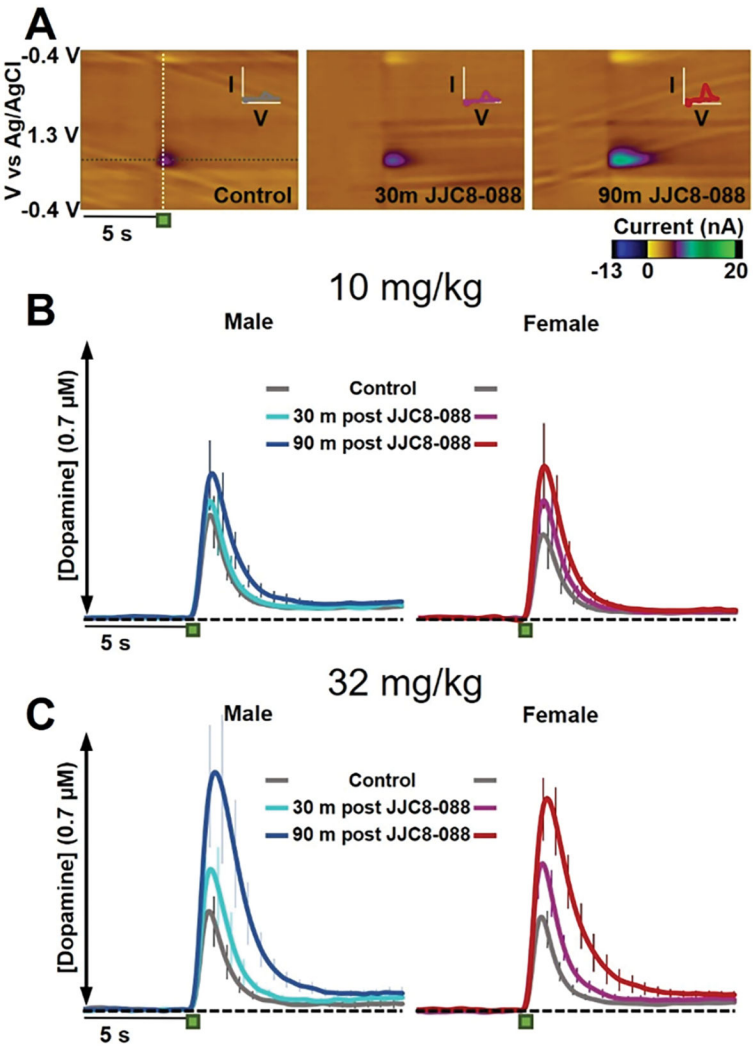

Figure 5: JJC8-088 administration increases evoked DA release and slows DA clearance in the NAS.

(**A**) Representative color plots of evoked NAS DA for a female mouse treated with 10 mg/kg JJC8-088. (**B,C**) The averaged evoked NAS DA response in male and female mice at control levels (shown in grey), 30 min (male: teal, female: purple), and 90 min (male: blue, female: red) post JJC8-088 administration (10 and 32 mg/kg; i.p.). Stimulation is marked by green box below the plots and traces. Data is shown as the average with SEM in correspondingly colored error bars (n=7 for each treatment group).

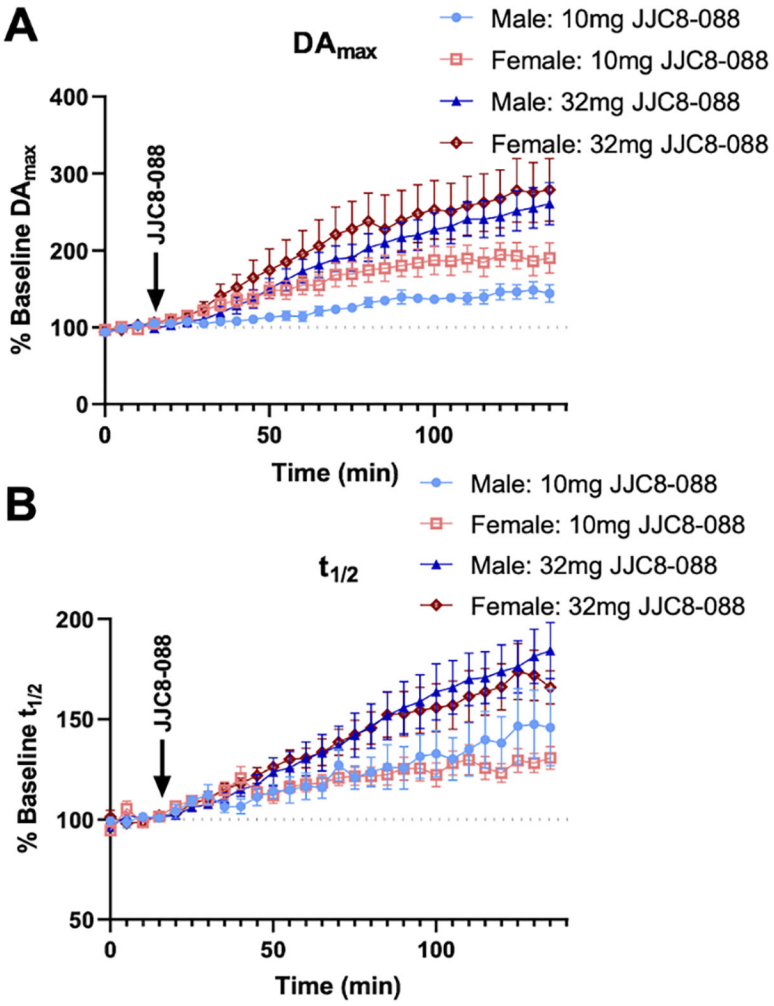

Figure 6: JJC8-088 administration increases evoked DA release and slows DA clearance in the NAS.

(**A**) Averaged normalized change in NAS DA_{max} and (**B**) DA clearance ($t_{1/2}$) in male and female mice. DA was analyzed every five min and after 4 consistent control files, JJC8-088 (10 and 32 mg/kg; i.p.) was administered. DA was analyzed for 120 min after drug administration. (n=7 for each treatment group).

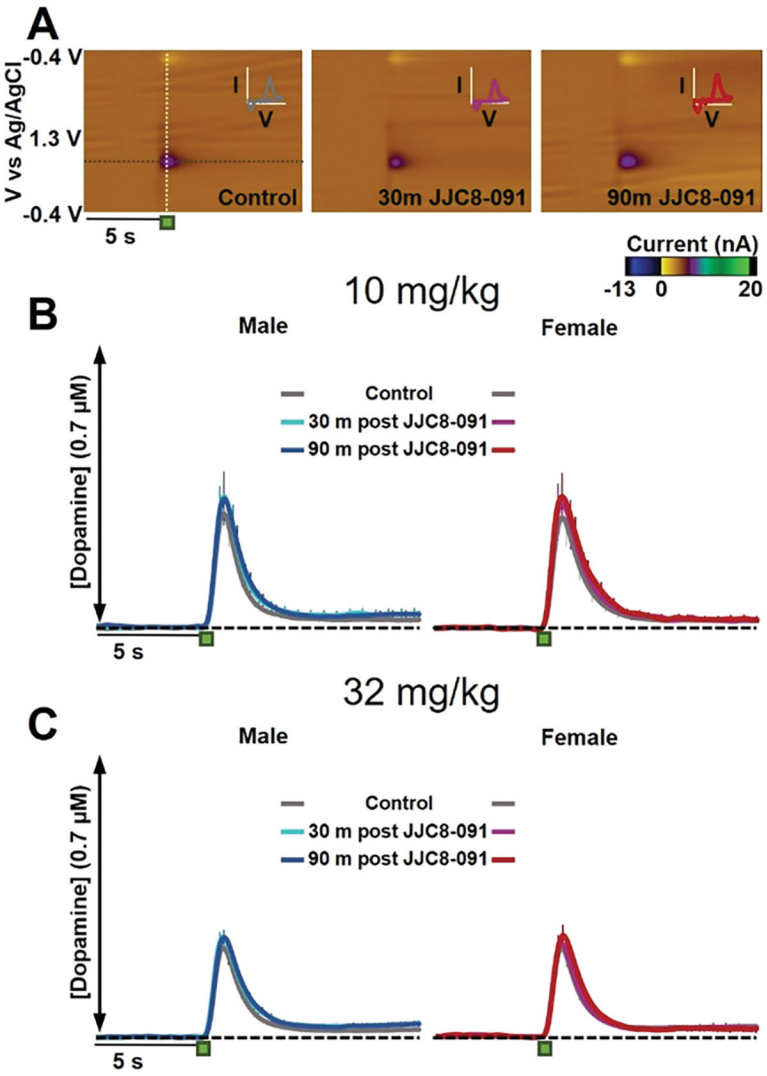

Figure 7: JJC8-091 administration increases evoked DA release and slows DA clearance in the NAS.

(**A**) Representative color plots of evoked NAS DA for a female mouse treated with 10 mg/kg JJC8-091. (**B,C**) The averaged evoked NAS DA response in male and female mice at control levels (shown in grey), 30 min (male: teal, female: purple), and 90 min (male: blue, female: red) post JJC8-091 administration (10 and 32 mg/kg; i.p.). Stimulation is marked by green box below the plots and traces. Data is shown as the average with SEM in correspondingly colored error bars (n=7 for each treatment group).

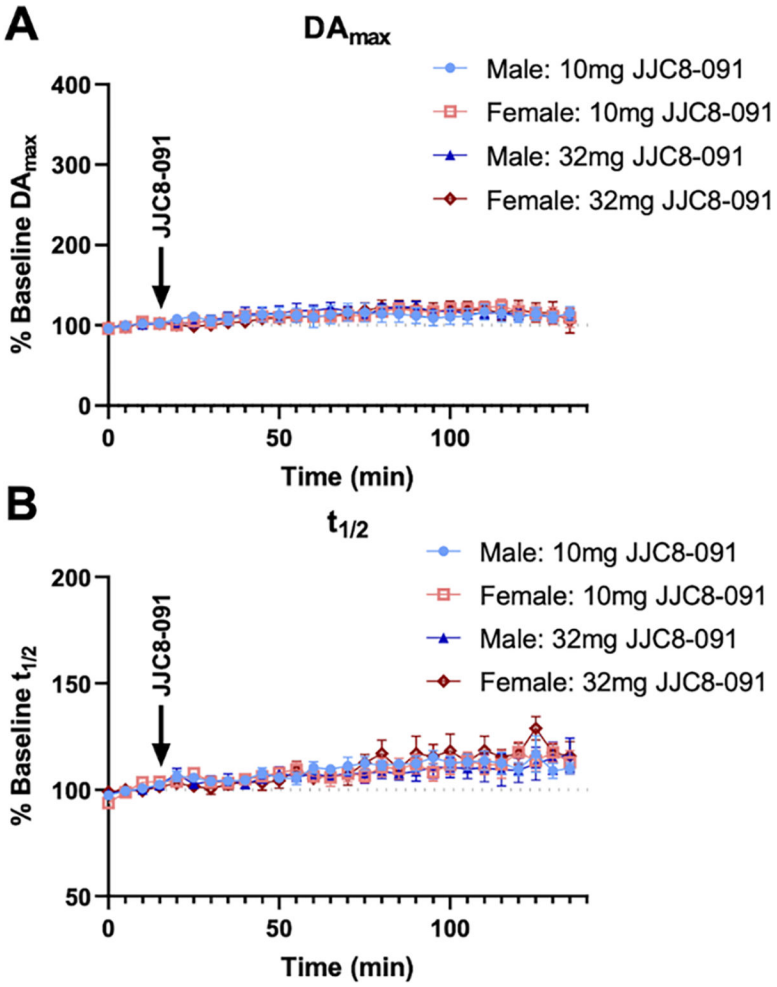

Figure 8: JJC8-091 administration increases evoked DA release and slows DA clearance in the NAS.

(**A**) Averaged normalized change in NAS DA_{max} and (**B**) DA clearance ($t_{1/2}$) in male and female mice. DA was analyzed every five min and after 4 consistent control files, JJC8-091 (10 and 32 mg/kg; i.p.) was administered. DA was analyzed for 120 min after drug administration. (n=7 for each treatment group).