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Atypical dopamine transporter inhibitors R-modafinil and JHW 007 differentially affect D2 autoreceptor neurotransmission and the firing rate of midbrain dopamine neurons

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Abstract

Abuse of psychostimulants like cocaine that inhibit dopamine (DA) reuptake through the dopamine transporter (DAT) represents a major public health issue, however FDA-approved pharmacotherapies have yet to be developed. Recently a class of ligands termed “atypical DAT inhibitors” has gained attention due to their range of effectiveness in increasing extracellular dopamine (DA) levels without demonstrating significant abuse liability. These compounds not only hold promise as therapeutic agents to treat stimulant use disorders but also as experimental tools to improve our understanding of DAT function. Here we used patch clamp electrophysiology in mouse brain slices to explore the effects of two atypical DAT inhibitors (R-modafinil and JHW 007) on the physiology of single DA neurons in the substantia nigra and ventral tegmental area. Despite their commonalities of being DAT inhibitors that lack cocaine-like behavioral profiles, these compounds exhibited surprisingly divergent cellular effects. Similar to cocaine, R-modafinil slowed DA neuron firing in a D2 receptor-dependent manner and rapidly enhanced the amplitude and duration of D2 receptor-mediated currents in the midbrain. In contrast, JHW 007 exhibited little effect on firing, slow DAT blockade, and an unexpected inhibition of D2 receptor-mediated currents that may be due to direct D2 receptor antagonism. Furthermore, pretreatment with JHW 007 blunted the cellular effects of cocaine, suggesting that it may be valuable to investigate similar DAT inhibitors as potential therapeutic agents. Further exploration of these and other atypical DAT inhibitors may reveal important cellular effects of compounds that will have potential as pharmacotherapies for treating cocaine use disorders.

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Chemical compounds

Chemical compounds studied in this article: R-modafinil (PubChem CID: 9690109); JHW 007 (PubChem CID: 10091491); Cocaine (PubChem CID: 446220)

Keywords

Dopamine neuron; dopamine transporter; cocaine; D2 autoreceptor; mouse; substantia nigra; ventral tegmental area; neurotransmission; firing

1. INTRODUCTION

Cocaine abuse negatively impacts individuals and society, but despite considerable effort there is currently no FDA approved pharmacological treatment. In the brain, cocaine acutely produces a large, rapid increase in extracellular levels of the neurotransmitter dopamine (DA, Di Chiara & Imperato, 1988) by blocking reuptake by the plasmalemmal DA transporter (DAT) (Ritz et al., 1987; Schmitz et al., 2003). Effects on DAT are associated with the pleasurable and euphoric feelings associated with cocaine (Koob et al., 1998; Nestler, 2005) and mice that express cocaine-insensitive DATs will not self-administer cocaine (Thomsen et al., 2009). On a cellular level, the acute effects of cocaine can be observed as a decrease in DA neuron firing rate (Bunney et al., 2001) and a robust enhancement of the amplitude and time course of dendrodendritic neurotransmission between DA neurons (Beckstead et al., 2004; Branch and Beckstead, 2012; Courtney et al., 2012). An ideal pharmacological treatment for cocaine abuse might be expected to prevent the acute effects of cocaine on DA neuron excitability while also producing minimal effects on its own. However, drugs targeting DAT, such as methylphenidate, GBR12909, benztropine, and ($\pm$)-modafinil have shown limited clinical utility in preventing or treating cocaine abuse (Newman and Kulkarni, 2002; Czoty et al., 2016).

Recently a new class of ligands termed “atypical DAT inhibitors” has gained significant attention due to their ability to increase extracellular DA levels without exhibiting significant abuse liability (Reith et al., 2015). These atypical agents are structurally diverse DAT inhibitors that include the benztropine analogue JHW 007 (Agoston et al., 1997) and the benzhydryl compound R-modafinil, the latter of which is currently approved by the FDA as both the R-isomer and the racemate to treat narcolepsy, sleep apnea, and shift-work sleep disorders. These compounds have less propensity than cocaine to increase locomotion or produce reinforcement in rodents (Cao et al., 2011; Desai et al., 2005; Li et al., 2013) and JHW 007 has been shown to reduce cocaine and methamphetamine self-administration (Hiranita et al., 2009; Li et al., 2013; Hiranita et al., 2014) whereas R-modafinil is less effective (Zhang et al., 2017). In contrast to cocaine, R-modafinil and JHW 007 prefer binding to an occluded conformation of DAT (Loland et al., 2008; Loland et al., 2012). Neurochemically, this has been observed as a gradual increase of extracellular DA in the nucleus accumbens that lasts longer and reaches a much lower peak compared to cocaine (Kohut et al., 2014; Loland et al., 2012; Tanda et al., 2009). Together these observations indicate promise for atypical DAT inhibitors as treatments for psychostimulant abuse. However, no study to date has measured the effects of R-modafinil and JHW 007 on dendritic neurotransmission in single DA neurons, or tested their ability to block the effects of cocaine on the excitability of single DA neurons.

To address this, we employed patch clamp electrophysiology in midbrain slices from adult mice. We then compared the effects of the prototypical DAT inhibitor cocaine with atypical compounds R-modafinil and JHW 007 on DA neuron firing and D2 autoreceptor-mediated inhibitory postsynaptic currents (D2-IPSCs). Our results show that R-modafinil, like cocaine, rapidly enhances the amplitude and time of activation of D2-IPSCs and slows DA neuron firing in a D2 receptor-dependent manner. In contrast, JHW 007 modestly slowed DA neuron firing and actually decreased the amplitude of D2-IPSCs at high concentrations. JHW 007 also strongly inhibited the effects of cocaine on DA neuron firing, and blunted the cocaine-induced increase in amplitude of D2-IPSCs. Our results are the first direct observations of the cellular actions of atypical DAT inhibitors on DA mediated neurotransmission, and could suggest that combining weak inhibition of DAT and D2 receptor antagonism could counteract some of the cellular effects of cocaine.

2. MATERIALS AND METHODS

2.1 Animals

Male DBA/2J mice aged 7–12 weeks were used for all experiments. Mice were housed on a 12/12 hour reverse light cycle. Mice were sacrificed in accordance with accepted practices using procedures approved by the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee.

2.2 Brain slice preparation and dopamine neuron identification

Mice were anesthetized using isoflurane, decapitated, and their brains removed. The midbrain was blocked with a razor blade, placed in an ice cold bath of carboxygenated (95% O₂/5% CO₂) artificial cerebral spinal fluid (aCSF) (in mM: 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 2.4 CaCl₂, 1.2 NaH₂PO₄, 21.4 NaHCO₃, and 11.1 D-glucose) with kynurenic acid (1.3 mM) added, and cut into horizontal slices (200 µm thick) using a vibrating microtome. Slices containing the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) were collected, stored in a scintillation vial containing aCSF and the NMDA receptor antagonist MK-801 (30 µM), and incubated at 35°C for 30 minutes before being placed in a perfusion bath for recording. DA neurons of the SNc and VTA were visualized with an infrared light-emitting diode and located in the brain slice relative to the medial terminal nucleus of the accessory optic track and the interpeduncular fossa. Firing rate, spike width, and I_h were recorded. All whole cell experiments quantified DA-mediated outward currents that have only been observed in midbrain DA neurons but not in other nearby cell types (Branch et al., 2016).

2.3 Experimental design

We performed recordings of DA neurons in the SNc and VTA to determine the effects of two atypical DAT inhibitors, R-modafinil and JHW 007, on cell excitability and DA D2 autoreceptor-mediated currents. VTA cells were a minimum of 100 µm medial of the medial terminal nucleus of the accessory optic tract. In most cases there were no strong indications of differences in cellular responses between brain regions, so data were pooled (except where noted).

DA neuron firing rates were recorded using loose cell attached recordings and high resistance pipettes (~8 MΩ) filled with a Na HEPES-based internal solution (plus 20 mM NaCl, Branch and Beckstead, 2012). Baseline firing rates were recorded for 5 minutes followed by bath perfusion of drugs.

D2 autoreceptor currents from midbrain DA neurons were measured using whole cell voltage-clamp recordings (holding potential −55 mV) using low resistance pipettes (~2.5 MΩ) and an intracellular solution that contained 115 mM potassium methyl sulfate, 20 mM NaCl, 1.5 mM MgCl₂, 10 mM HEPES, 10 mM BAPTA, 2 mM ATP, and 0.4 mM GTP, pH 7.35–7.40, 270 mOsm/l (Branch & Beckstead, 2012). For all experiments, baseline currents were measured for 5 minutes followed by bath perfusion of drugs. To evoke D2 autoreceptor-mediated IPSCs we used a bipolar platinum electrode (FHC Inc. model MX212EP[MB2], Bowdoin, ME) to stimulate episodic release of endogenous DA (a train of five stimulations at 40 Hz every 60 seconds). D2-IPSCs were experimentally isolated by adding antagonists for GABA_A (100 μM picrotoxin), GABA_B (100 nM CGP55845), AMPA (10 μM DNQX), and nACh (100 μM hexamethonium) receptors to the aCSF. In separate experiments, D2 receptor-mediated currents were evoked through iontophoresis of exogenous DA. The tip of a thin tapered glass pipette was placed near the soma of the cell being recorded and DA (1 M in the pipette) was ejected (+200 nA pulse for 25–200 ms) every 60 seconds (Branch & Beckstead, 2012).

2.4 Drugs

R-modafinil and JHW 007 were synthesized in the Newman lab (NIDA-IRP) according to literature procedures (Agoston et al., 1997, Prisinzano et al., 2004, Cao et al., 2011), and cocaine hydrochloride was obtained from the NIDA drug supply program (Bethesda, MD).

2.5 Data and statistical analysis

Data were recorded using Labchart (AD Instruments, Colorado Springs, CO) and Axograph software (www.axograph.com). During episodic stimulation, data were sampled at 50 kHz and filtered at 6 kHz. Maximum drug effects were calculated as the largest change from baseline observed for rolling three-sweep averages. Statistical analyses were performed with Graphpad Prism 6.0 and 7.0 (www.graphpad.com). Two-way repeated measures ANOVAs were used to test for differences between groups over time or across brain regions. One-way ANOVAs or t-tests were used to test for differences of maximal effects between treatment groups. Sidak's, Tukey's, or Dunnett's multiple comparisons tests were used for simple comparisons following significant ANOVAs. Asterisks were used to represent *P* values when comparisons were statistically significant: * *P* 0.05, ** *P* 0.01, *** *P* 0.001, **** *P* 0.0001. Summary data are presented as mean ± SEM.

3. RESULTS

3.1 Atypical DAT inhibitors differentially affect midbrain DA neuron cell excitability

We first used loose cell-attached recordings to measure firing rate of midbrain DA neurons in brain slices from young adult mice. In midbrain slice preparations from rodents DA neurons fire in a rhythmic, pacemaker manner (Grace and Onn, 1989; Fig 1A, top). As

expected, bath perfusion of the prototypical DAT inhibitor cocaine substantially reduced DA neuron firing rate (Figure 1A, bottom) and in some cells halted firing altogether. This effect was blocked by the D2-type receptor antagonist sulpiride (200 nM, Figure 1B,C, $t_{14} = 2.965$, $P = 0.0102$) and was produced presumably by a rise in extracellular DA concentration. We observed a similar result with the benzhydryl-based atypical DAT inhibitor R-modafinil, which decreased DA neuron firing rate in a concentration- and D2 receptor-dependent manner (Figure 1D,E, one-way ANOVA $F_{2,27} = 8.467$, $P = 0.0014$ and Tukeys' multiple comparisons test). In contrast, a large concentration of the benztropine-analogue and atypical DAT inhibitor JHW 007 (10 μM) did not substantially alter DA neuron firing rate during a standard ten-minute application, either in the presence or absence of sulpiride (Figure 1F, two-way ANOVA, main effect of treatment, $F_{1,7} = 0.2431$, $P = 0.1908$, $n = 3-6$). The results seemed to indicate a possible effect during the washout of JHW 007, so we next tested a longer, twenty-minute application. This longer perfusion was able to slightly decrease firing but remained unaffected by the presence of sulpiride (Figure 1G, two-way ANOVA, main effect of group, $F_{1,8} = 0.2431$, $P = 0.6353$, $n = 5$), suggesting that this decrease was not due to D2 receptor activation. The slow effect of JHW 007 on dopamine neuron firing rate is consistent with previous reports of a slow action when compared to cocaine (Desai et al., 2005), and further experiments did indicate some washout of JHW 007 after 25–30 minutes (not shown). This initial characterization of the cellular effects of atypical DAT inhibitors suggested that while R-modafinil may act on DA neuron excitability in a similar manner to cocaine, JHW 007 appears to differ mechanistically.

3.2 Atypical DAT inhibitors differentially affect D2 autoreceptor IPSC amplitude and width

We next sought to determine the effects of these atypical DAT inhibitors on local dendritic dopamine transmission. To accomplish this, we used whole cell patch clamp electrophysiology of midbrain DA neurons to measure D2 autoreceptor-mediated neurotransmission (Beckstead et al., 2004). Cells were voltage clamped at -55 mV to ground and D2-IPSCs were electrically evoked (Figure 2A). As previously reported (Beckstead et al., 2004; Branch and Beckstead, 2012), bath perfusion of cocaine (10 μM) increased the amplitude of D2-IPSCs compared to baseline (Figure 2B,C), greatly increased the width at half-maximal amplitude (which hereafter will be simply referred to as 'width', Figure 2B,D), and consistently produced an outward shift in the holding current (44.9 ± 6.78 pA, $n = 8$, data not shown). Bath perfusion of R-modafinil (10–100 μM) again produced similar effects to cocaine by increasing the amplitude of D2-IPSCs in a concentration-dependent manner (Figure 2E,F,G) and augmenting the width preferentially at the higher concentration (Figure 2E,F,H). R-modafinil also produced an outward shift in the holding current (10 μM : 21.6 ± 5.02 pA, $n = 5$; 100 μM : 39.1 ± 3.28 pA, $n = 14$, data not shown). Like the results presented in Figure 1 this again suggests that cocaine and R-modafinil act similarly on DAT, DA neurotransmission, and DA cell firing.

We next tested the effects of JHW 007 on D2-IPSCs, and opted to test lower concentrations based on published differences in DAT affinity (12.0 nM for JHW 007 versus 3,260 nM for R-modafinil; Cao et al., 2010; Kopajtic et al., 2010). Bath perfusion of JHW 007 again produced divergent results from those obtained with cocaine and R-modafinil. A ten-minute perfusion of JHW 007 (0.1–1 μM) had little effect on the amplitude and width of D2-IPSCs

(Figure 3A,C,D). Surprisingly, a high concentration (10 μ M) of JHW 007 actually *decreased* the amplitude of D2-IPSCs (Figure 3B,C). In a separate experiment, 10 μ M JHW 007 had no effect on GABA_B receptor/GIRK channel-mediated IPSC amplitudes ($-4.04 \pm 10.4\%$) or widths ($+3.74 \pm 6.91\%$, $n = 9$, data not shown). This suggests that the effects on DA currents were not due to direct blockade of GIRK channels but we probably due to antagonism of D2 receptors. The fact that D2-IPSC inhibition occurred much more rapidly than the effects on firing rate (that were not sulpiride-sensitive, Figure 1F) also suggests that distinct targets are involved. Figure 4 summarizes the maximal effects of DAT inhibitors on D2-IPSC amplitude and width from Figures 2 and 3 (one-way ANOVA followed by Dunnett's multiple comparisons test versus 10 μ M cocaine). Current width was not analyzed subsequent to perfusion of 10 μ M JHW 007 because the current amplitudes were reduced to the point that width could not be accurately measured. Taken together these data provide evidence of a divergence between the effects of JHW 007 on DA neuron excitability and neurotransmission when compared to other DAT inhibitors.

3.3 Effects of atypical DAT inhibitors on iontophoretically-induced DA currents

Although bath perfusion of atypical DAT inhibitors affected D2-IPSCs, it was unclear if the effects were pre- or post-synaptic in nature. To determine the locus of effects on neurotransmission we next induced DA-mediated currents episodically through iontophoresis of exogenous DA (1 M in the pipette, Beckstead et al., 2004). Similar to the D2-IPSC experiments, R-modafinil (10–100 μ M) enhanced currents induced by DA iontophoresis (Figure 5A,B). Summary data (Figure 5C,D) suggest a similar time course for the effects of R-modafinil on the amplitude and width of D2 receptor-mediated currents when compared to the data on D2-IPSCs shown in Figure 2. Consistent with D2 receptor antagonism, JHW 007 (1–10 μ M) also showed a similar reduction in the amplitude of DA-mediated currents (Figure 5E,F,G) when compared to effects on D2-IPSCs presented in Figure 3. However, JHW 007 here produced an enhancement of current width (Figure 5E,F,H) that was not observed with D2-IPSCs. This result is consistent with modest inhibition of DAT function, which would preferentially affect DA traveling extrasynaptically between the tip of the iontophoretic pipette and the target D2 receptors on the neuron.

We also explored whether R-modafinil and JHW 007 exhibit differential effects on DA currents in the SNc versus the VTA. Throughout this study data for both atypical DAT inhibitors were obtained in both the SNc and VTA, and most comparisons showed no apparent difference between the two regions and, therefore, most SNc and VTA data were pooled and not shown separately. However, R-modafinil did produce a larger enhancement of iontophoretically-induced current amplitudes in the SNc versus the VTA (Figure 6A; Two-way ANOVA, main effect of brain region $F_{1,26} = 11.04$, $P = 0.0027$; Sidak's multiple comparisons test for 100 μ M R-modafinil $P = 0.0030$). Similarly, R-modafinil also produced a greater enhancement of current widths in the SNc versus the VTA (Figure 6B; Two-way ANOVA, main effect of brain region $F_{1,26} = 9.748$, $P = 0.0044$; main effect of concentration $F_{1,26} = 10.15$, $P = 0.0037$, Sidak's multiple comparisons test for 100 μ M R-modafinil $P = 0.0041$). This is consistent with a recent voltammetry study that described larger effects of ($\pm$)-modafinil in the dorsal versus ventral striatum (Bobak et al., 2016). By contrast, there was no indication of a region-specific effect of JHW 007 on the amplitude or width of

iontophoretically-induced DA currents either during drug perfusion or during drug washout (data not shown).

3.4 Effects of pretreatment with atypical DAT inhibitors on cocaine action

As atypical DAT inhibitors have been touted as potential therapeutics to treat cocaine abuse, we next sought to determine the interaction between these compounds and cocaine on a cellular level. As data up to this point indicated that cocaine and R-modafinil act similarly on single DA neurons, JHW 007 represented the better candidate to act as an “antagonist” of cocaine’s actions. Bath perfusion of 1 μ M JHW 007 had no effect on its own on DA neuron firing rate (Figure 7A). However, after a 10-minute pretreatment the actions of cocaine were dramatically reduced (Figure 7B, $t_{16} = 3.449$, $P = 0.0033$), indicating that JHW 007 can act on a cellular level to limit the action of cocaine.

To explore this further, we next tested the effects of pretreatment with atypical DAT inhibitors on the cocaine-induced enhancement of D2-IPSCs. Figure 8A illustrates the enhancement of D2-IPSC amplitude and width produced by 10 μ M cocaine alone. Figure 8B indicates the effects of applying 1 μ M JHW 007 followed by a co-application of 10 μ M cocaine. Consistent with the data from Figure 3C, 1 μ M JHW 007 alone either had no effect on IPSC amplitudes or slightly reduced them (mean -15.1% , range -37.0% to $+13.7\%$). Pretreatment with JHW 007 (1 μ M for ten minutes or 100 nM for 20 minutes) appeared to blunt the increase in amplitude produced by cocaine (Figure 8C,D,E) but only produced a non-significant reduction on IPSC width (Figure 8F). Consistent with our previous findings, 10 μ M R-modafinil had very similar effects to cocaine on D2-IPSCs (Figure 9A). 10 μ M R-modafinil pretreatment enhanced IPSC amplitudes (Figure 9B) and to a lesser extent width (Figure 9D). Overall R-modafinil seemed to occlude the effects of cocaine rather than inhibit them (Figure 9C,E), indicating a mechanism of action distinct from JHW 007.

4. DISCUSSION

4.1 Physiological actions of DAT inhibitors on DA neurons

DAT is an integral membrane protein that is highly expressed on the dendrites and axon terminals of dopaminergic neurons in the ventral midbrain (Blanchard et al., 1994; Apuschkin et al., 2015). Its primary physiological role is to translocate DA from the extracellular space to the cytoplasm, thus limiting DA signaling (Beckstead et al., 2004; Marcott et al., 2014). Most rapidly acting DAT inhibitors like cocaine are psychomotor stimulants that are strongly reinforcing, as evidenced by their rampant abuse in humans and their ability to maintain self-administration behavior in animal models (Lile, 2006). However, despite decades of studies investigating the actions of DAT inhibitors, pharmacotherapeutics to effectively treat cocaine abuse or prevent relapse remain elusive (Reith et al., 2015).

Atypical DAT inhibitors have recently drawn attention in this regard due to their ability to increase extracellular DA while exhibiting no or limited abuse liability. R-modafinil and JHW 007 both bind DAT (Agoston et al., 1997; Katz et al., 2004; Kopajtic et al., 2010; Loland et al., 2012) and increase extracellular DA concentrations, albeit to a lesser extent

than cocaine (Tanda et al., 2009; Loland et al., 2012). The present work sought to describe the cellular effects of these compounds on DA neuron firing and dendritic DA neurotransmission, as well as their ability to disrupt cocaine action on a cellular level. Our results suggest that R-modafinil acts on DA neuron firing and D2-IPSCs similarly to cocaine. Cocaine has previously been reported to slow DA neuron firing in rodent brain slices in a D2 receptor-dependent manner (Brodie and Dunwiddie, 1990) and enhance the amplitude and duration of D2-IPSCs (Beckstead et al., 2004; Branch and Beckstead, 2012). Our findings suggest that R-modafinil also reduces firing in a concentration- and D2 receptor-dependent manner consistent with previous work on the racemate (Korotkova et al., 2007; Federici et al., 2013). Like cocaine, R-modafinil also enhanced the amplitude and width of D2-IPSCs in a concentration-dependent manner, at concentrations consistent with its reported 17-fold lower affinity for DAT than cocaine (Loland et al., 2012). The fact that the amplitude and time course of R-modafinil effects varied little from that of cocaine suggests that any difference in behavior induced by these compounds is not a result of effects on excitability observable in single DA neurons. Like cocaine, ($\pm$)-modafinil is a stimulant (Young et al., 2011) and has been reported to have reinforcing properties in rhesus monkeys (Gold and Balster, 1996). Nevertheless, neither the racemate nor R-modafinil is self-administered in rodents and they do not exhibit abuse liability in humans, despite a mechanism of action that might predict this (Mereu et al. 2013 and 2017, Volkow et al. 2009; Zhang et al., 2017).

The profile of cellular effects produced by JHW 007 were divergent from both cocaine and R-modafinil. Extended exposure to JHW 007 slightly slowed DA neuron firing, and was unaffected by pretreatment with the D2 receptor antagonist sulpiride. When JHW 007 was applied to D2-IPSCs it did not produce the rapid increase in amplitude followed by the sustained increase in width observed with cocaine both here and previously (Branch and Beckstead, 2012). DAT inhibition itself was difficult to observe during the 10-minute bath perfusion of JHW 007, and was only clearly evident when exogenous DA was applied by iontophoresis. The weak DAT inhibition of JHW 007 when compared to cocaine could be responsible for its lower abuse liability, as it does not appear to support self-administration and does not substitute in rats trained to self-administer cocaine (Hiranita et al., 2009).

4.2 Actions of DAT inhibitors on D2 autoreceptors

DAT inhibitors are typically classified as “indirect” agonists on DA receptors because they lack direct binding. Rather, binding to DAT increases the extracellular concentration of DA which in turn activates both synaptic and extrasynaptic receptors. This effect was clearly observed with cocaine and R-modafinil as an enhanced D2-IPSC amplitude and width. In contrast however, JHW 007 decreased IPSC amplitude in a manner consistent with D2 receptor antagonism. Unlike cocaine and R-modafinil (Zhang et al., 2017), JHW 007 has affinity for the D2L receptor (47.1 nM, Katz et al., 2004) and is a weak D2 receptor antagonist ($IC_{50} = 2 \mu M$, Cao et al., 2016) in addition to exhibiting 12 nM binding affinity for DAT (Kopajtic et al., 2010). This could explain how mid-micromolar concentrations of JHW 007 reduced DA-mediated current amplitudes. Nevertheless, JHW 007 has been reported to attenuate cocaine-induced locomotor stimulation in D2 receptor knockout mice,

possibly suggesting a complex mechanism of action that may involve sigma 1 receptors or other yet unidentified targets (Desai et al., 2014).

Increased D2 receptor current width can be interpreted as indirect evidence of DAT blockade, and all three compounds tested did increase this parameter to some extent. Cocaine and R-modafinil inhibited DAT rapidly and efficaciously, while DAT inhibition by JHW 007 was slow and incomplete. Increased D2 receptor current width by JHW 007 was not observed during IPSC experiments with ten-minute perfusion (Figure 3D), but did slowly develop during drug washout (not shown). The slow effect of JHW 007 on IPSC width is likely due to its reported slow association rate with DAT (Desai et al., 2005). Indirect evidence of uptake inhibition was more readily observed in experiments measuring currents induced by DA iontophoresis (Figure 5H). The reason for this divergence is that DA applied by iontophoresis relies on diffusion to reach target D2 receptors on the neuron being monitored, making the duration of action susceptible to uptake inhibition. By contrast, dendritic release of endogenous DA in the mouse midbrain exhibits a more local effect that is less sensitive to uptake inhibition (Beckstead et al., 2004; Ford et al., 2009; Ford et al., 2010; Courtney et al., 2012). The slow action of JHW 007 is consistent with previously published studies conducted in vivo. JHW 007 exhibits an association rate for DAT that is $\sim 10\times$ slower than that of cocaine (Desai et al., 2005), and can take hours to produce maximal enhancement of nucleus accumbens DA following an i.p. injection (Tanda et al., 2009). Consistent with the behavioral effects of these DAT inhibitors, slow onset of action can generally be associated with reduced abuse liability (Volkow et al., 2005). Additionally, R-modafinil and JHW 007 have sustained effects on extracellular DA levels (Loland et al., 2012; Tanda et al., 2009), in contrast with cocaine which produces a rapid increase in DA followed by a rapid decrease back to baseline (Tanda et al., 2009). The rapid increase in DA is associated with euphoria while the rapid decrease in DA levels is associated with dysphoria that can result in repeated use of cocaine to avoid aversive side effects (Koob & Le Moal, 1997; Koob et al., 1998). The relatively sustained enhancement of extracellular DA produced by R-modafinil and JHW 007 suggests that they may be useful as replacement therapies for cocaine abuse by stabilizing DA levels and reducing the sudden decrease, perhaps attenuating withdrawal and craving for the drug (Velazquez-Sanchez et al., 2013).

4.3 Interactions of JHW 007 and R-modafinil with cocaine

In humans, modafinil blunts cocaine-induced behavioral effects and could be useful in some subpopulations diagnosed with cocaine use disorder (Hart et al., 2008; Verrico et al., 2014; Kampman et al., 2015). JHW 007 has not been studied in humans, but preclinically is known to readily cross the blood brain barrier (Raje et al., 2003), antagonize cocaine-mediated conditioned place preference (Velazquez-Sanchez et al., 2010), block cocaine-induced locomotion, and attenuate both cocaine and methamphetamine self-administration in a dose-dependent manner (Desai et al., 2005; Desai et al., 2014; Li et al., 2013; Hiranita et al., 2014). Our goal was to determine if one or both of these compounds was able to functionally inhibit the physiological consequences of cocaine perfusion in single DA neurons. R-modafinil, consistent with observations obtained when it was applied alone, appeared to be largely cocaine-like in its cellular effects. These effects are probably best described as occlusion and are what might be expected if cocaine itself was applied sequentially in two

increasing concentrations. Indeed, although R-modafinil is not self-administered (Zhang et al., 2017) it does share other behavioral actions with cocaine in rodents (Loland et al., 2012). Notably, R-modafinil is not as effective in attenuating the self-administration of cocaine as JHW 007 or a recently reported modafinil analogue, JJC8-016 (Okunola-Bakare et al., 2014; Cao et al., 2016; Zhang et al., 2017). In contrast, JHW 007 appeared to blunt the cellular effects of cocaine in a concentration-dependent manner that did not resemble occlusion.

JHW 007 exhibits several features consistent with therapeutics that might be useful for treating cocaine abuse. An ideal treatment would not be reinforcing and would have little effect on normal cellular physiology, all while blunting the behavioral and cellular effects of cocaine. An additional consideration is that JHW 007, in addition to binding to DAT and D2 receptors, also exhibits high affinity for other potential targets (Katz et al., 2004; Cao et al., 2016). Future work will explore the role of these other targets in the cellular effects of atypical DAT inhibitors, and could reveal ideal cellular actions for the design of potential medications for the treatment of cocaine abuse.

Conclusions

Overall, this study demonstrates that the atypical DAT inhibitors R-modafinil and JHW 007 act distinctly on DA neuron firing and D2 receptor-mediated neurotransmission. Additionally, JHW 007 is able to reduce the cellular actions of cocaine which supports the notion that similar compounds have potential as therapeutics for treating human cocaine abuse.

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Abbreviations

DAT	dopamine transporter
DA	dopamine
SNC	substantia nigra pars compacta
VTA	ventral tegmental area
IPSC	inhibitory post-synaptic current

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Highlights

The effects of R-modafinil on single dopaminergic neurons are similar to cocaine

JHW 007 exhibits divergent electrophysiological effects from other DAT inhibitors

JHW 007, but not R-modafinil, partially inhibits the effects of cocaine on DA neurons

Investigating compounds like JHW 007 could lead to therapies for cocaine addiction

Figure 1. R-modafinil and cocaine, but not JHW 007, decrease DA neuron firing rates
 (A) Sample tracings and (B) summary data indicate that bath perfusion of the prototypical DAT inhibitor cocaine (10 μ M) causes a decrease in DA neuron firing rate that was blocked by pretreatment with the D2 receptor antagonist sulpiride (200 nM). (C) Maximal effects of data represented in panel A indicate a significant effect of sulpiride. (D) The atypical DAT inhibitor R-modafinil (10–100 μ M) caused a concentration-dependent decrease in DA neuron firing rate that was also blocked by pretreatment with sulpiride. (E) Maximal effects of data represented in panel D. (F) In contrast, the atypical DAT inhibitor JHW 007 (10 μ M)

produced minimal effects on dopamine neuron firing rate during a standard 10-minute perfusion and was not affected by sulpiride pretreatment. (G) A longer perfusion of JHW 007 (10 μ M) revealed a slowly-developing, modest decrease in firing rate that did not quickly wash out and was not affected by sulpiride pretreatment. * $P < 0.05$, ** $P < 0.01$.

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Figure 2. Cocaine and R-modafinil produce similar effects on the amplitude and width of electrically evoked D2 inhibitory postsynaptic currents

Representative D2-IPSCs from single cell recordings show baseline versus the effects of (A) the D2 receptor antagonist sulpiride (200 nM) and (B) cocaine (10 μ M). Summary data indicate that cocaine (10 μ M) increases the amplitude (C) and width (D) of D2-IPSCs. (E,F) Representative traces of the effects of R-modafinil on D2-IPSCs. Summary data indicate that, similar to cocaine, R-modafinil (10–100 μ M) increases the amplitude (G) and width (H) of D2-IPSCs in a concentration-dependent manner.

Figure 3. Unlike cocaine and R-modafinil, JHW 007 decreases D2-IPSC amplitudes and does not increase current width

Representative traces from DA neuron recordings indicate that (A) 100 nM JHW 007 had minimal effect on the amplitude of D2-IPSCs, while (B) 10 μ M JHW 007 greatly reduced their amplitude. (C) Summary data indicate that JHW 007 (100 nM - 1 μ M) decreased D2-IPSC amplitude in a concentration-dependent manner and (D) had little effect on width during the perfusion.

Figure 4. Cocaine and R-modafinil increase the amplitude and width of D2-IPSCs, but JHW 007 does not

Combined summary data from Figures 2 and 3 indicate that while (A) cocaine and R-modafinil increase D2-IPSC amplitudes, JHW 007 has the opposite effect. (B) Cocaine and R-modafinil increase D2-IPSC width, while JHW 007 has little effect. **** P = 0.0001

Figure 5. R-modafinil and JHW 007 differentially affect D2 receptor currents induced by iontophoresis of exogenous DA

(A,B) Representative traces from experiments where DA was iontophoretically applied onto a DA neuron show the effects of perfusing R-modafinil (10–100 μM). Similar to the experiments with D2-IPSCs, R-modafinil increased (C) the amplitude and (D) the width of currents elicited by iontophoresis in a concentration-dependent manner. (E,F) Representative traces show the effects of JHW 007 perfusion on currents elicited by iontophoresis of DA. (G) JHW 007 decreased D2 current amplitude while (H) increasing current width in a concentration-dependent manner.

Figure 6. R-modafinil has larger effects on DA iontophoresis-induced D2 receptor-mediated currents in the SNc versus the VTA

Summary data indicate that R-modafinil (10–100 μ M) had larger effects in the SNc versus the VTA on D2 receptor currents induced by DA iontophoresis. The effect was observed for both current amplitude (A) and width (B). ** $P < 0.01$

Figure 7. Pretreatment with JHW 007 reduces the effects of cocaine on DA neuron firing rate (A) While cocaine alone decreased firing rate, pretreatment with the atypical DAT inhibitor JHW 007 (1 μ M) attenuated the effect of co-applied cocaine. (B) Maximal effects from the data shown in panel A. ** $P < 0.01$.

Figure 8. Pretreatment with JHW 007 blunts the effect of cocaine on the amplitude of D2-IPSCs
 Representative D2-IPSCs indicate the effects of perfusing (A) 10 μ M cocaine alone, and (B) 1 μ M JHW 007 followed by JHW 007 + 10 μ M cocaine. (C1) JHW 007 pretreatment reduced the effect of cocaine on D2-IPSC amplitude. However, 1 μ M JHW 007 produced a slight decrease in IPSC amplitude on its own, so baselines were re-calculated as the average of the five sweeps immediately preceding cocaine perfusion (C2). Using this adjusted baseline, analyzing the maximum effect of cocaine (D) as well as the average of the last three sweeps of perfusion (E) indicated a significant blunting produced by JHW 007 pretreatment. Cocaine-induced enhancement of width was not significantly affected by JHW 007 pretreatment (F). * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure 9. Pretreatment with R-modafinil occludes the amplitude-enhancing effect of cocaine on D2-IPSCs

Representative D2-IPSCs indicate the effects of perfusing 10 μ M cocaine alone (A, top), and 10 μ M R-modafinil followed by R-modafinil + cocaine (A, bottom). (B) R-modafinil (10 μ M) treatment enhanced the amplitude of D2-IPSCs and occluded the effects of a subsequent perfusion of cocaine (10 μ M). (C) The maximal enhancement of amplitude after cocaine perfusion was unaffected by R-modafinil pretreatment. (D) R-modafinil treatment mildly enhanced the width of D2-IPSCs and did not blunt the substantial increase in width produced by cocaine. (E) The maximal enhancement of width after cocaine perfusion was unaffected by R-modafinil pretreatment.