The 5-HT$_{2A}$ Receptor (5-HT$_{2A}$R) Regulates Impulsive Action and Cocaine Cue Reactivity in Male Sprague-Dawley Rats

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ABSTRACT

Impulsivity and the attentional orienting response to cocaine-associated cues (*cue reactivity*) promote relapse in cocaine-use disorder (CUD). A time-dependent escalation of cue reactivity (*incubation*) occurs during extended, forced abstinence from cocaine self-administration in rats. The investigational serotonin (5-HT) 5-HT$_{2A}$ receptor (5-HT$_{2A}$R) antagonist/inverse agonist M100907 suppresses *impulsive action*, or the inability to withhold premature responses, and cocaine-seeking behaviors. The present preclinical study was designed to establish the potential for repurposing the Food and Drug Administration–approved selective 5-HT$_{2A}$R antagonist/inverse agonist pimavanserin as a therapeutic agent to forestall relapse vulnerability in CUD. In male Sprague-Dawley rats, pimavanserin suppressed impulsive action (*premature responses*) measured in the 1-choice serial reaction time (1-CSRT) task, similarly to M100907. We also used the 1-CSRT task to establish baseline levels of impulsive action before cocaine self-administration and evaluation of cue reactivity (lever presses reinforced by the discrete cue complex previously paired with cocaine delivery). We observed an incubation of cocaine cue reactivity between day 1 and day 30 of forced abstinence from cocaine self-administration. Baseline levels of impulsive action predicted incubated levels of cocaine cue reactivity in late abstinence. We also found that baseline impulsive action predicted the effectiveness of pimavanserin to suppress incubated cue reactivity in late abstinence from cocaine self-administration at doses that were ineffective in early abstinence. These data suggest that integration of clinical measures of impulsive action may inform refined, personalized pharmacotherapeutic intervention for the treatment of relapse vulnerability in CUD.

Introduction

Cocaine use disorder (CUD) represents a significant public health challenge in the United States. The lack of effective pharmacotherapeutics to suppress relapse vulnerability is an unmet need in the treatment of CUD (Volkow and Skolnick, 2012). Impulsivity and cue reactivity are two key behavioral phenotypes that engender relapse vulnerability (O'Brien et al., 1998; Drummond, 2001; Moeller et al., 2001a; Koob and Volkow, 2010; Saunders et al., 2013). Impulsivity has been defined clinically as rapid, unplanned reactions to stimuli, reduced sensitivity to negative consequences, and a disregard for long-term consequences (Moeller et al., 2001a). Rapid-response impulsivity (impulsive action; difficulty withholding a prepotent response) and impulsive choice (decision-making; delayed reward measures) are dimensions of impulsivity that have been associated with CUD in humans (Moeller et al., 2001a,b; Patkar et al., 2004) and in rodents (Perry et al., 2005; Belin et al., 2008; Anastasio et al., 2014a). Cue reactivity refers to the attentional orienting response to drug-related stimuli that predict reward (Maas et al., 1998; Carter and Tiffany, 1999; Garavan et al., 2000; Field and Cox, 2008). We demonstrated that levels of impulsive action positively correlated with the attentional bias toward cocaine-associated cues in cocaine-dependent humans (Liu et al., 2011) and rodents (Anastasio et al., 2014b); two meta-analyses recently corroborated this link (Coskunpinar and Cyders, 2013; Leung et al., 2017). High baseline levels of self-reported impulsivity (Barrett Impulsivity Scale-11) (Moeller et al., 2001b, 2007) or attentional bias for cocaine-associated cues (Cocaine Word Stroop Task) (Carpenter et al., 2006) predicted poorer retention of

ABBREVIATIONS: 5-HT, serotonin; 5-HT$_{2A}$R, 5-HT$_{2A}$ receptor; ANCOVA, analysis of covariance; ANOVA, analysis of variance; CSRT, choice serial reaction time; CUD, cocaine use disorder; DOI, 2,5-dimethoxy-4-iodoamphetamine; FA, forced abstinence; FR, fixed ratio; ITI, intertrial interval; VEH, vehicle.

cocaine-dependent participants in outpatient treatment trials. These findings suggest that pharmacotherapeutic strategies that effectively diminish impulsive action and cocaine cue reactivity may reduce relapse during abstinence in CUD patients.

Serotonin (5-hydroxytryptamine; 5-HT) neurotransmission regulates the limbic-corticostriatal circuitry associated with the development and maintenance of addiction, as well as vulnerability to relapse (for reviews, see Cunningham and Anastasio, 2014; Koob and Volkow, 2016). Serotonin actions are transduced by 14 receptor subtypes, and the G-protein–coupled 5-HT$_{2A}$ receptor (5-HT$_{2A}$R) has been implicated in the neural mechanisms underlying impulsive action, defined as premature responses in the choice serial reaction time (CSRT) tasks (for review, see Cunningham and Anastasio, 2014). After systemic administration, the preferential 5-HT$_{2A}$R agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) (Titeler et al., 1988; Wischhof et al., 2011) elevated impulsive action measured in the 5-CSRT task (Koskinen et al., 2000a,b; Blokland et al., 2005). The selective 5-HT$_{2A}$R antagonist/inverse agonist M100907 (volinanserin) administered systemically suppressed premature responding assessed in the 1 or 5-CSRT task (Winstanley et al., 2004; Fletcher et al., 2007, 2011; Anastasio et al., 2011, 2015; Cunningham et al., 2013; Fink et al., 2015) and also suppressed cocaine- and cue-evoked reinstatement after extinction training from cocaine self-administration (Lacosta and Roberts, 1993; Fletcher et al., 2002; Filip, 2005; Nic Dhonnchadha et al., 2009). A time-sensitive increase in attentional bias toward cocaine-associated cues in rats (Neisewander et al., 2000; Grimm et al., 2001; Lu et al., 2004; Swinford-Jackson et al., 2016) has been observed during extended abstinence and is referred to as "incubation" (for review, see Pickens et al., 2011). In rodents, escalation of cue reactivity (lever presses that are reinforced by the discrete drug-paired cue complex) occurs up to 6 months after termination of cocaine self-administration (Neisewander et al., 2000; Grimm et al., 2001; Lu et al., 2004; Swinford-Jackson et al., 2016); however, the relationship between levels of impulsive action and incubated cue reactivity, as well as the effectiveness of a 5-HT$_{2A}$R antagonist/inverse agonist to suppress cocaine cue reactivity during early versus late abstinence from cocaine self-administration, is unknown.

Preclinical and early clinical evaluations of selective 5-HT$_{2A}$R antagonists support their potential efficacy as therapeutics in sleep disorders (Ancoli-Israel et al., 2011), psychosis (Weiner et al., 2001), psychosis in Parkinson's disease (Meltzer et al., 2012), and other psychologic disorders (Roberts, 2006); however, the investigational compound M100907 (volinanserin), which exhibits >100-fold selectivity for the 5-HT$_{2A}$R over the homologous 5-HT$_{2B}$R and 5-HT$_{2C}$R and other monoamine receptors (Kehne et al., 1996; Knight et al., 2004), never achieved approval for a clinical indication. The selective 5-HT$_{2A}$R antagonist/inverse agonist pimavanserin (Nuplazid, Acadia Pharmaceuticals, San Diego, CA) is now clinically approved for the treatment of psychosis in Parkinson disease (Sahli and Tarazi, 2018) and exhibits >100-fold selectivity for the 5-HT$_{2A}$R over 5-HT$_{2B}$R, 5-HT$_{2C}$R, and other monoamine receptors (Vanover et al., 2006; Hacksell et al., 2014). Notably, both M100907 and pimavanserin are potent 5-HT$_{2A}$R antagonists in vivo; for example, both compounds suppressed head-twitch behaviors and prepulse inhibition deficits induced by DOI in rats (Sipes and Geyer,

1995; Fantegrossi et al., 2010; McFarland et al., 2011), whereas M100907 is well characterized to suppress the discriminative stimulus properties of DOI and other 5-HT$_{2A}$R agonists (Smith et al., 2003; Winter et al., 2007).

This preclinical study was designed to establish the potential for repurposing pimavanserin as a therapeutic to forestall relapse vulnerability in CUD. Given that previous studies support the utility of the rodent CSRT tasks for screening pharmacologic interventions on impulsive action (for review, see Winstanley, 2011), we used the 1-CSRT task to test the hypothesis that pimavanserin would suppress impulsive action and cocaine cue reactivity during abstinence from cocaine self-administration, similar to M100907. Given that baseline levels of impulsive action positively correlated with cocaine cue reactivity in humans (Liu et al., 2011) and rodents (Anastasio et al., 2014b), we evaluated whether baseline levels of impulsive action are related to the effectiveness of pimavanserin to control cocaine cue reactivity in early (day 1) versus late abstinence (day 30).

Materials and Methods

General Methods

Animals. Male Sprague-Dawley rats ($n = 160$; Harlan, Houston, TX) weighed 250–275 g at arrival and were housed in the colony room. Rats were housed two per cage under a 12-hour light/dark cycle with monitored and controlled temperature (21–23°C) and humidity levels (45%–50%). Rats were acclimated to the colony room for 7 days before handling and experimentation commenced. Rats were food-restricted to ~90% free-feeding weight (confirmed by daily weights) during 1-CSRT task training and had ad libitum access to water except during daily operant sessions. All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (2011) and with the University of Texas Medical Branch Institutional Animal Care and Use Committee approval.

Drugs. (-)-Cocaine (National Institute on Drug Abuse Drug Supply Program, Bethesda, MD) was dissolved in 0.9% NaCl. M100907 [(R)-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol] (synthesized by Kenner Rice, National Institute on Drug Abuse, Bethesda, MD) was dissolved in 1% Tween-80 in 0.9% NaCl [vehicle (VEH) used for comparison with M100907]. Pimavanserin [1-(4-fluorobenzyl)-3-(4-isobutoxybenzyl)-1-(1-methylpiperidin-4-yl)urea] (Trylead Chemical Technology Co., Ltd., Hangzhou, China) was dissolved in 0.9% NaCl brought to a pH ~6.0 using 1 M HCl (VEH used for comparison with pimavanserin). M100907 and pimavanserin were administered by the intraperitoneal or subcutaneous route, respectively.

1-Choice Serial Reaction Time Task. All sessions conducted in the 1-CSRT task occurred in five-hole, nose-poke operant chambers containing a house light, food tray, and external pellet dispenser that delivered 45-mg dustless precision food pellets (Bio-Serv, Frenchtown, NJ) housed within ventilated, sound-attenuated cubicles (MedAssociates, St Albans, VT). The 1-CSRT task method was previously reported in detail (Anastasio et al., 2011, 2013, 2014b; Cunningham et al., 2013; Fink et al., 2015). To summarize, rats were initially exposed to a pretraining stage during which they were habituated to the test chamber. A nose-poke response into the illuminated-center stimulus hole (i.e., a *target* response) resulted in simultaneous illumination of the magazine light on the opposite chamber wall and delivery of a 45-mg food pellet. Rats were progressed through a series of training stages after completion of the pretraining stage. Each stage consisted of daily 100-trial sessions to be completed in a maximum of 30 minutes. The stimulus duration was incrementally shortened throughout each training stage until a final stage of 0.5 second was achieved with a limited hold of 5 seconds and an intertrial interval (ITI) of 5 seconds (ITI5).

A maximum of 100 target responses in a session resulted in a maximum of 100 reinforcers delivered. Incorrect nontarget responses, premature responses, or omissions resulted in a timeout period (5 seconds) that reduced the potential number of reinforcers delivered. Before progressing through each training stage, rats were required to achieve acquisition criteria: ≥50 reinforcers earned, >80% accuracy (target responses/(target + nontarget responses) · 100) and <20% omissions (omitted responses/trials completed · 100) (Anastasio et al., 2011, 2013, 2014b; Cunningham et al., 2013; Fink et al., 2015).

The number of premature responses, omissions, and reinforcers earned, percent accuracy, latency to first response, and time to finish the 1-CSRT task were recorded. Premature responses, the primary output measure to assess impulsive action, were categorized into three types: target, nontarget, and total (i.e., target + nontarget). The number of reinforcers earned assessed task competency and provides an additional measure of impulsive action. The percent accuracy was a general indication of attentional capacity. Percent omissions indicated motivation to perform the task, and latency to first response in the 1-CSRT task provided a secondary measure of motivation and an indication of general motor impairment.

Cocaine Self-Administration and Cue Reactivity. Rats (n = 144) were anesthetized (8.6 mg/kg of xylazine, 1.5 mg/kg of acepromazine, 43 mg/kg of ketamine in bacteriostatic saline) before surgical implantation of indwelling jugular catheters with back mounts; rats were allotted 7 days to permit postoperative recovery (Cunningham et al., 2011, 2013; Anastasio et al., 2014a,b). Rats received a 0.1-ml infusion of a bacteriostatic saline solution that contained heparin sodium (10 U/ml; American Pharmaceutical Partners, East Schaumburg, IL), streptokinase (0.67 mg/ml; Sigma Chemical, St. Louis, MO), and ticarcillin disodium (66.67 mg/ml; Research Products International, Mt. Prospect, IL) into the catheter immediately after daily cocaine self-administration sessions to ensure catheter patency during experimentation.

The cocaine self-administration assay used standard operant conditioning chambers (Med Associates, Inc.) housed within sound-attenuated, ventilated cubicles equipped with fans (Med Associates, Inc.). Operant chambers were fitted with two retractable response levers, a stimulus light above each of the response levers, a houselight on the wall opposite the response levers, and an external pellet dispenser. Cocaine infusions were delivered through syringes that were loaded daily into infusion pumps (Med Associates, Inc.) located outside the cubicles. The infusion pumps were connected to liquid swivels (Instech, Plymouth Meeting, PA) fastened to catheters via polyethylene 20 tubing encased inside a metal spring leash (Plastics One, Roanoke, VA).

Cocaine self-administration training sessions were 180 minutes in duration and occurred daily. Rats were trained to perform a lever press response reinforced by a cocaine infusion (0.75 mg/kg/0.1 ml infusion) (Cunningham et al., 2011; Anastasio et al., 2014a,b; Swinford-Jackson et al., 2016). Schedule responses on the active lever resulted in delivery of a cocaine infusion over a 6-second period; each infusion was simultaneously paired with the illumination of the house and stimulus lights and activation of the infusion pump (i.e., discrete cue complex paired with cocaine delivery). Inactive lever presses were recorded but had no scheduled consequences. The stimulus light and infusion pump were inactivated following delivery of cocaine. The house light remained on to signal a timeout period (20 seconds); lever presses committed during the timeout period had no scheduled consequences. Rats were trained on a fixed ratio (FR)1 schedule of reinforcement and progressed to a FR5 schedule after achieving seven infusions/hour with less than 10% variability for 3 consecutive days. Once stable cocaine self-administration was acquired, rats were subjected to forced abstinence (FA) from cocaine self-administration for 1 (FA day 1) or 30 days (FA day 30). During the FA period, rats were returned to their home cages, weighed, and handled daily. After the assigned FA period, rats were evaluated in a cue reactivity test session (60 minutes) in which presses on the previously active lever were reinforced by the discrete cue complex (stimulus-light illuminated,

infusion-pump activated) on an FR1 schedule. Inactive lever presses were recorded but had no scheduled consequences.

Research Design

Cohort 1: Pimavanserin and M100907 Suppress Impulsive Action Measured in the 1-CSRT Task. Rats (n = 16) were required to meet acquisition criteria in the 1-CSRT task [≥50 target responses, >80% accuracy, and <20% omissions on the final training stage (0.5-second stimulus duration, 5-second limited hold, and ITI5)] for at least 3 consecutive days. Performance in the 1-CSRT task was assessed after systemic administration of M100907 or pimavanserin. Pretreatment with vehicle (1 ml/kg, i.p.) or M100907 (0.001, 0.01, 0.1 mg/kg, i.p) occurred 30 minutes before start of the 1-CSRT task session under an ITI5 schedule. Five rats failed to meet the acquisition criteria under an ITI5 schedule in the 1-CSRT task and were excluded from analysis of M100907 (n = 11 rats analyzed).

After pharmacologic evaluations with M100907, rats established stable 1-CSRT task training under an ITI5 schedule for a minimum of 30 days to permit drug washout. Pharmacological test sessions with pimavanserin commenced once rats met the 1-CSRT task acquisition criteria for at least 3 consecutive days. Pretreatment with VEH (1 ml/kg, s.c.) or pimavanserin (0.3, 1, 3 mg/kg, s.c.) occurred 30 minutes before start of 1-CSRT task sessions under an ITI5 schedule. Two rats failed to maintain stable performance under an ITI5 schedule in the 1-CSRT task and were excluded from analysis of pimavanserin (n = 9 rats analyzed). Rats were treated with VEH the day before drug pretreatments, which were separated by a minimum of 3 days. The order of M100907 and pimavanserin injections was randomized across rats in a within-subjects design.

Cohort 2: Baseline Levels of Impulsive Action Predict the Efficacy of Pimavanserin to Suppress Cue Reactivity. Rats (n = 144) were trained on the 1-CSRT task. Baseline levels of impulsive action were established once rats met the acquisition criteria of ≥50 target responses, >80% accuracy, and <20% omissions on the final training stage (0.5-second stimulus duration, 5-second limited hold, ITI5) for 3 consecutive days. Three rats failed to maintain stable performance and were excluded from analysis. After identification of baseline levels of impulsive action, 1-CSRT task sessions ceased. Rats were returned to their home cages and were freely fed for at least 5 days before surgical implantation of indwelling jugular catheters. Cocaine self-administration began after at least 5 days postcatheterization. After acquisition of stable cocaine self-administration, rats were assigned to either FA day 1 or FA day 30 and returned to their home cages for the assigned FA duration. Fifteen rats were excluded from analysis for technical issues in the cocaine self-administration assay (n = 126 rats analyzed).

Pharmacologic test sessions were used to evaluate the efficacy of pimavanserin to suppress cocaine cue reactivity on FA day 1 or FA day 30. Pretreatment with VEH (1 ml/kg, s.c.) or pimavanserin (0.3, 1, 3, 10 mg/kg, s.c.) occurred 30 minutes before the cue reactivity session on FA day 1 (n = 68; n = 12–15/group) or FA day 30 (n = 58; n = 11 or 12/group) in a between-subjects design.

Statistical analyses. A one-way repeated measures analysis of variance (ANOVA) was used to evaluate the effects of either M100907 (VEH, 0.001, 0.01, 0.1 mg/kg) or pimavanserin (VEH, 0.3, 1, 3 mg/kg) on 1-CSRT task measures (target, nontarget, or total premature responses, reinforcers earned, % omissions, % accuracy, latency to first response, time to finish the 1-CSRT task); Dunnett's procedure was employed to analyze planned comparisons (Keppel, 1973). The dose of M100907 or pimavanserin estimated to decrease total premature responses by 50% of the maximum suppression (ID$_{50}$) in the 1-CSRT task was quantified by a four-parameter logistic nonlinear regression (Ratkowsky and Reedy, 1986; Tallarida and Murray, 1987). Student's *t* test was used to assess total cocaine intake throughout acquisition and maintenance of cocaine self-administration. A two-way ANOVA with the factors of FA day (FA day 1, FA day 30) and medication pretreatment (VEH, 0.3, 1, 3, 10 mg/kg of pimavanserin) was

used to analyze 1-CSRT task performance to ensure equal distribution of rats across groups. A two-way ANOVA with the factors of FA day (FA day 1, FA day 30) and medication pretreatment (VEH, 0.3, 1, 3, 10 mg/kg of pimavanserin) was used to analyze previously active lever presses, inactive lever presses, and the latency to first response during the cue reactivity test session. A one-way ANOVA was used to analyze previously active lever presses at each FA day after medication pretreatment; planned comparisons were assessed using Dunnett's procedure or Student's t test, where appropriate (Keppel, 1973). A four-parameter logistic nonlinear regression was used to calculate the ID_{50} of pimavanserin to suppress previously active lever presses during the cue reactivity session on FA day 1 versus FA day 30 (Ratkowsky and Reedy, 1986; Tallarida and Murray, 1987). A one-way analysis of covariance (ANCOVA) was used to evaluate the relationship between target premature responses and previously active lever presses on FA day 1 versus FA day 30 after pretreatment with VEH. A one-way ANCOVA was used to evaluate the relationship between target premature responses and previously active lever presses at each FA day with five between-subject pretreatment conditions (VEH, 0.3, 1, 3, 10 mg/kg of pimavanserin) and the covariate (target premature responses in the 1-CSRT task).

Results

M100907 and Pimavanserin Suppress Impulsive Action. We tested the hypotheses that M100907 or pimavanserin would suppress impulsive action measured in the 1-CSRT task relative to VEH. Figure 1A displays the mean ($\pm$S.E.M.) number of target premature responses after pretreatment with VEH or M100907 ($n = 11$). There was a main effect of M100907 on target premature responses ($F_{(3,30)} = 4.17$; $P < 0.05$); planned comparisons with Dunnett's procedure showed that 0.1 mg/kg of M100907 decreased target premature responses versus VEH ($P < 0.05$; Fig. 1A). The ID_{50} of M100907 to suppress target premature responses was 0.007 mg/kg. In addition to target premature responses, there was a main effect of M100907 on total (target + nontarget) premature responses ($F_{(3,30)} = 4.88$; $P < 0.05$), % omissions ($F_{(3,30)} = 8.69$; $P < 0.05$), and time to finish the 1-CSRT task ($F_{(3,30)} = 7.21$; $P < 0.05$); planned comparisons with Dunnett's procedure showed that 0.1 mg/kg of M100907 decreased total premature responses, increased % omissions, and increased the time to finish the 1-CSRT task versus VEH ($P < 0.05$; Table 1). There was no main effect of M100907 on nontarget premature responses ($F_{(3,30)} = 1.55$; n.s.], reinforcers earned [$F_{(3,30)} = 1.13$; n.s.], % accuracy [$F_{(3,30)} = 0.422$; n.s.], or latency to first response in the 1-CSRT task ($F_{(3,30)} = 1.04$; n.s.) versus VEH (Table 1).

Figure 1B displays the mean ($\pm$S.E.M.) number of target premature responses after pretreatment with pimavanserin ($n = 9$). There was a main effect of pimavanserin on target premature responses ($F_{(3,24)} = 17.3$; $P < 0.05$); planned comparisons with Dunnett's procedure showed that 0.3, 1, 3 mg/kg of pimavanserin decreased target premature responses versus VEH ($P < 0.05$; Fig. 1B). The ID_{50} of pimavanserin to suppress target premature responses was 0.05 mg/kg. In addition to target premature responses, there was a main effect of pimavanserin on total premature responses ($F_{(3,24)} = 15.8$; $P < 0.05$), % omissions ($F_{(3,24)} = 5.29$; $P < 0.05$), and time to finish the 1-CSRT task ($F_{(3,24)} = 7.79$; $P < 0.05$); planned comparisons with Dunnett's procedure showed that 0.3, 1, and 3 mg/kg of pimavanserin decreased total premature responses, whereas 1 and 3 mg/kg of pimavanserin increased % omissions, as well as the time to finish the 1-CSRT task versus VEH ($P < 0.05$; Table 1). There was no main effect of pimavanserin on nontarget premature responses ($F_{(3,24)} = 1.15$; n.s.], reinforcers earned [$F_{(3,24)} = 1.25$; n.s.], % accuracy [$F_{(3,24)} = 0.713$; n.s.], or latency to first response in the 1-CSRT task [$F_{(3,24)} = 2.54$; n.s.] versus VEH (Table 1).

Pimavanserin Suppresses Cue Reactivity on FA Day 30, but Not FA Day 1. Rats ($n = 126$) were trained in the cocaine self-administration assay after screening on the 1-CSRT task. Rats stably acquired cocaine self-administration (i.e., FR5 schedule; seven infusions/hour for a minimum of three sessions) and displayed <10% variation in the number of cocaine infusions earned during maintenance (Fig. 2A). Rats were assigned to specific FA day and medication pretreatment group based on counterbalancing across target premature responses (Table 2), total cocaine intake across acquisition, and maintenance of self-administration. The results of a two-way ANOVA indicated no main effect of FA day, pretreatment, or FA day X pretreatment interaction for premature responses (target, nontarget, total), reinforcers earned, % accuracy, % omissions, latency to first response, and time to finish the 1-CSRT task between rats assigned to FA day 1 or FA day 30 and pimavanserin pretreatment groups (Table 2), indicating that rats were appropriately counterbalanced before training on cocaine self-administration. Total cocaine intake across self-administration sessions was not different in rats assigned to FA day 1 (361.8 $\pm$ 7.68 mg/kg; $n = 68$) or FA day 30 (364.3 $\pm$ 7.85 mg/kg; $n = 58$) [$t(124) = 0.234$; n.s.].

We tested the hypothesis that pimavanserin would suppress cue reactivity (previously active lever presses for the discrete cue complex) during FA from cocaine self-administration.
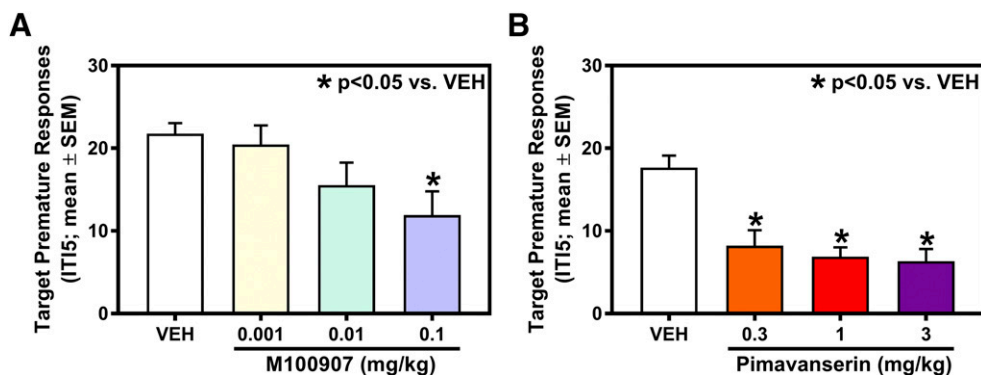

Fig. 1. M100907 and pimavanserin suppress target premature responses in the 1-CSRT task. The effects of M100907 (0.001, 0.01, and 0.1 mg/kg; $n = 11$) or pimavanserin (0.3, 1, 3 mg/kg; $n = 9$) were each evaluated under an ITI5 schedule in the 1-CSRT task. (A) M100907 significantly decreased target premature responses at 0.1 mg/kg (*$P < 0.05$ vs. VEH). (B) Pimavanserin significantly decreased target premature responses at 0.3, 1, and 3 mg/kg (*$P < 0.05$ vs. VEH).

TABLE 1
1-CSRT task descriptive statistics for M100907 and pimavanserin pretreatment (mean ± S.E.M.)

Pretreatment	Dose	Premature Responses			Reinforcers Earned	% Accuracy	% Omissions	Latency to First Response (s)	Time to Finish 1-CSRT Task (s)
		Target	Nontarget	Total					
M100907	Vehicle	21.8 ± 1.27	1.61 ± 0.265	23.4 ± 1.43	68.4 ± 1.33	97.0 ± 0.433	6.15 ± 0.817	s 3.38 ± 1.85	872 ± 15.9
	0.001 mg/kg	20.5 ± 2.30	1.91 ± 0.719	22.4 ± 2.32	69.5 ± 1.06	97.1 ± 0.674	6.00 ± 1.95	1.00 ± 0.185	874 ± 23.7
	0.01 mg/kg	15.5 ± 2.71	0.818 ± 0.325	16.4 ± 2.88	71.2 ± 2.60	97.5 ± 0.483	10.6 ± 2.83	2.88 ± 1.49	938 ± 40.6
	0.1 mg/kg	11.9 ± 2.87*	1.36 ± 0.338	13.3 ± 2.86*	66.8 ± 2.12	97.7 ± 0.750	18.3 ± 2.70*	2.17 ± 0.862	1022 ± 35.8*
Pimavanserin	Vehicle	17.7 ± 1.44	0.963 ± 0.286	18.6 ± 1.50	73.6 ± 1.40	97.5 ± 0.704	5.89 ± 0.957	1.44 ± 0.258	859 ± 11.5
	0.3 mg/kg	8.22 ± 1.88*	1.00 ± 0.441	9.22 ± 2.09*	79.6 ± 2.06	98.4 ± 0.631	9.89 ± 1.96	0.613 ± 0.161	898 ± 25.9
	1 mg/kg	6.89 ± 1.11*	0.333 ± 0.167	7.22 ± 1.16*	77.2 ± 3.36	98.3 ± 0.674	14.1 ± 3.85*	1.16 ± 0.378	948 ± 41.9*
	3 mg/kg	6.33 ± 1.47*	0.667 ± 0.236	7.00 ± 1.53*	74.4 ± 4.11	98.3 ± 0.657	17.1 ± 4.74*	1.51 ± 0.236	984 ± 46.0*

*$P < 0.05$ vs. Vehicle in each respective group.

A main effect of FA day ($F_{(1,116)} = 34.1$; $P < 0.05$), pretreatment ($F_{(4,116)} = 2.75$; $P < 0.05$), and a FA day X pretreatment interaction ($F_{(4,116)} = 3.29$; $P < 0.05$) was observed for previously active lever presses. Planned comparisons indicated that previously active lever presses were significantly elevated in rats treated with VEH on FA day 30 versus FA day 1 from cocaine self-administration [$t(23) = 5.546$; $P < 0.05$] (Fig. 2B). Further, planned comparisons revealed that pimavanserin (1, 3, 10 mg/kg) did not alter previously active lever presses on FA day 1 (Fig. 2C) but did suppress previously active lever presses on FA day 30 (Fig. 2D) relative to VEH ($P < 0.05$). The

ID$_{50}$ of pimavanserin to suppress previously active lever presses was 0.22 mg/kg on FA day 30; however, the four-parameter logistic nonlinear regression was unable to model the data for FA day 1, and an ID$_{50}$ value was not determinable. There was no main effect of FA day ($F_{(1,116)} = 0.038$; n.s.), pretreatment ($F_{(4,116)} = 1.154$; n.s.), or a FA day X pretreatment interaction ($F_{(4,116)} = 1.003$; n.s.) for inactive lever presses in the cue reactivity session. Further, there was no main effect of FA day ($F_{(1,116)} = 0.603$; n.s.), pimavanserin pretreatment ($F_{(4,116)} = 0.465$; n.s.), or a FA day X pretreatment interaction ($F_{(4,116)} = 1.227$; n.s.) for latency to first response in the cue reactivity session.
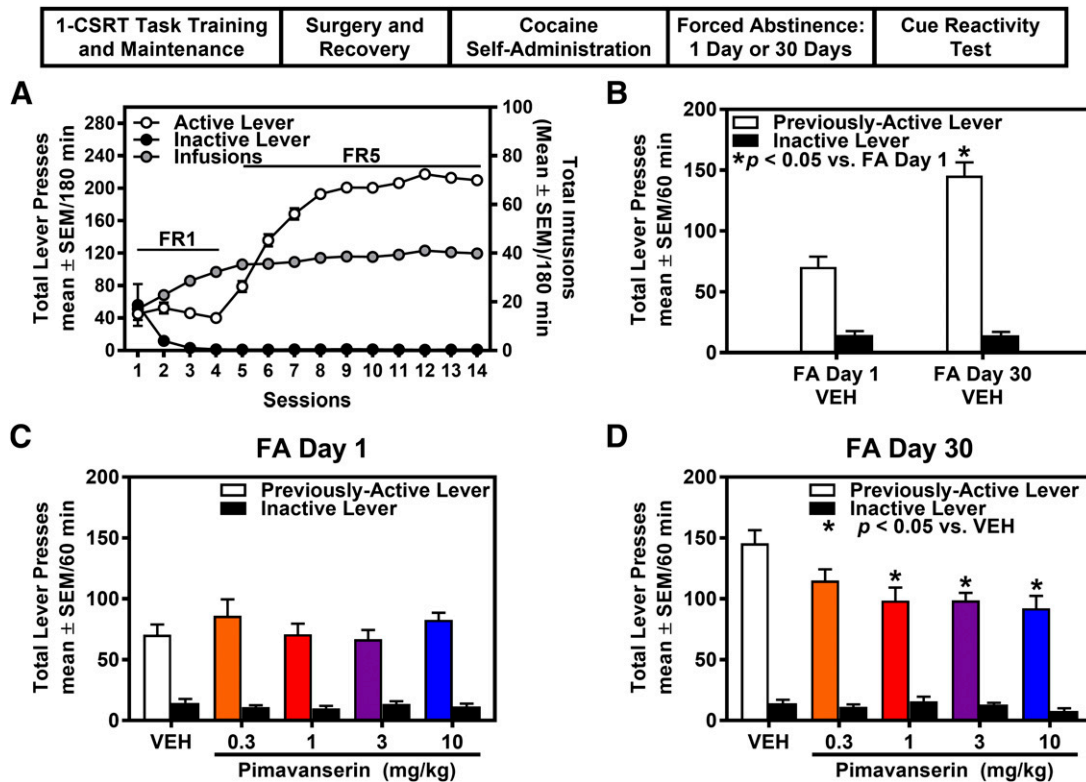

Fig. 2. Pimavanserin suppresses cue reactivity on FA day 30, but not FA day 1, from cocaine self-administration. (A) Total presses (mean ± S.E.M.) on the active (white circles) or inactive lever (black circles; left y-axis), and total number of cocaine infusions (mean ± S.E.M.) obtained (gray circles; right y-axis) are presented for the acquisition and maintenance phase of cocaine self-administration. (B) Previously active and inactive lever presses (mean ± S.E.M.) are presented for the cue reactivity test session in VEH-treated rats on FA day 1 and FA day 30 from cocaine self-administration. Cue reactivity is significantly elevated on FA day 30 vs. FA day 1 from cocaine self-administration (*$P < 0.05$; $n = 11$ or 14/group). The effects of pimavanserin (0.3, 1, 3, 10 mg/kg) on previously active and inactive lever presses (mean ± S.E.M.) on (C) FA day 1 ($n = 12$–15/group) or (D) FA day 30 ($n = 11$–12/group) from the last cocaine self-administration session are presented. Pimavanserin suppressed previously active, but not inactive, lever presses on FA day 30 from cocaine self-administration (*$P < 0.05$ vs. VEH).

TABLE 2
1-CSRT task descriptive statistics and results of two-way analysis of variance (ANOVA)

Forced Abstinence (FA) Day	Pimavanserin Pretreatment	Premature Responses			Reinforcers Earned	% Accuracy	% Omissions	Latency to First Response (s)	Time to Finish 1-CSRT Task (s)
		Target	Nontarget	Total					
Descriptive statistics									
FA day 1	Vehicle	25.7 ± 2.33	1.43 ± 0.327	27.1 ± 2.26	62.7 ± 1.91	98.6 ± 0.377	9.29 ± 1.98	1.16 ± 0.370	981 ± 49.4
	0.3 mg/kg	25.5 ± 2.00	2.00 ± 0.467	27.5 ± 2.21	61.6 ± 2.36	98.3 ± 0.530	9.77 ± 1.86	1.02 ± 0.221	953 ± 24.4
	1 mg/kg	21.1 ± 2.25	1.40 ± 0.306	22.5 ± 2.19	65.2 ± 1.89	98.1 ± 0.371	11.1 ± 1.47	1.17 ± 0.347	948 ± 32.4
	3 mg/kg	24.4 ± 2.05	1.21 ± 0.318	25.6 ± 2.11	65.9 ± 2.64	98.5 ± 0.597	7.57 ± 1.34	1.03 ± 0.171	912 ± 22.6
	10 mg/kg	27.7 ± 1.76	2.00 ± 0.357	29.7 ± 1.76	61.9 ± 1.85	96.8 ± 0.895	6.58 ± 0.839	1.32 ± 0.241	948 ± 31.8
FA day 30	Vehicle	26.2 ± 2.28	2.27 ± 0.384	28.5 ± 2.33	60.4 ± 1.91	97.8 ± 0.702	9.91 ± 1.09	1.15 ± 0.299	984 ± 42.6
	0.3 mg/kg	26.9 ± 2.33	1.67 ± 0.541	28.6 ± 2.06	63.4 ± 2.09	98.7 ± 0.568	7.17 ± 1.24	1.57 ± 0.307	935 ± 26.5
	1 mg/kg	28.5 ± 2.51	1.36 ± 0.432	29.9 ± 2.71	56.9 ± 2.09	97.5 ± 0.601	11.8 ± 2.42	1.37 ± 0.432	977 ± 44.2
	3 mg/kg	20.5 ± 2.28	1.17 ± 0.322	21.7 ± 2.51	66.5 ± 3.29	98.4 ± 0.783	10.9 ± 2.25	1.55 ± 0.469	972 ± 37.2
	10 mg/kg	28.3 ± 2.63	1.00 ± 0.275	29.3 ± 2.80	64.1 ± 1.96	99.2 ± 0.323	6.08 ± 1.52	14.6 ± 10.8	943 ± 42.2
Results of two-way ANOVA									
	FA day	$F_{(1,116)} = 0.716$	$F_{(1,116)} = 0.208$	$F_{(1,116)} = 0.563$	$F_{(1,116)} = 0.671$	$F_{(1,116)} = 0.509$	$F_{(1,116)} = 0.087$	$F_{(1,116)} = 1.96$	$F_{(1,116)} = 0.358$
	Pretreatment	$F_{(4,116)} = 1.63$	$F_{(4,116)} = 1.06$	$F_{(4,116)} = 1.87$	$F_{(4,116)} = 1.53$	$F_{(4,116)} = 0.513$	$F_{(4,116)} = 2.26$	$F_{(4,116)} = 1.63$	$F_{(4,116)} = 0.451$
	FA day x pretreatment	$F_{(4,116)} = 1.66$	$F_{(4,116)} = 1.36$	$F_{(4,116)} = 1.63$	$F_{(4,116)} = 1.75$	$F_{(4,116)} = 2.18$	$F_{(4,116)} = 0.787$	$F_{(4,116)} = 1.52$	$F_{(4,116)} = 0.373$

Baseline Levels of Impulsive Action Predict the Efficacy of Pimavanserin in Suppressing Cocaine Cue Reactivity on FA Day 30. We tested the hypothesis that baseline levels of impulsive action would predict previously active lever presses during the cue reactivity test on FA day 1 or FA day 30 in VEH-treated rats (Fig. 3A). We found that the covariate target premature responses predicted the number of previously active lever presses exhibited on FA day 1 and on FA day 30 in VEH-treated rats ($F_{(1,22)} = 5.081$, $P < 0.05$) (Fig. 3A). We also tested the hypothesis that baseline levels of impulsive action would predict the efficacy of pimavanserin to suppress previously active lever presses during the cue reactivity test on FA day 1 or FA day 30. No relationship between target premature responses and the number of previously active lever presses exhibited on FA day 1 after pretreatment with VEH or pimavanserin ($F_{(4,62)} = 0.405$; n.s.) (Fig. 3B). Target premature responses predicted the number of previously active lever presses exhibited on FA day 30 after pretreatment with VEH or pimavanserin ($F_{(4,52)} = 4.04$, $P = 0.05$) (Fig. 3C).

Discussion

We demonstrated that pimavanserin suppressed impulsive action measured in the 1-CSRT task, similarly to M100907 (Winstanley et al., 2004; Fletcher et al., 2007, 2011; Anastasio et al., 2011, 2015; Cunningham et al., 2013; Fink et al., 2015). Moreover, baseline levels of impulsive action predicted incubated levels of cocaine cue reactivity on FA day 30, replicating our previous observations that baseline impulsive action predicted cocaine cue reactivity on FA day 14 (Anastasio et al., 2014b). We also found that baseline impulsive action predicted the effectiveness of pimavanserin to suppress incubated cue reactivity in late abstinence from cocaine self-administration at doses that were ineffective in early abstinence. Taken together with the extinction-reinstatement studies with M100907 (Lacosta and Roberts, 1993; Fletcher et al., 2002; Filip, 2005; Nic Dhonnchadha et al., 2009), these data suggest that the length of abstinence and associated neuroadapations that drive incubated cocaine cue reactivity are interlocked with 5-HT$_{2A}$R mechanisms that underlie rapid response impulsivity.

Our findings suggest that identification of baseline levels of impulsive action could be useful in guiding pharmacotherapeutic intervention in CUD. Baseline levels of impulsive action can be defined clinically through self-report questionnaire measures (e.g., Barrett Impulsiveness Scale; Eysenck Impulsiveness Questionnaire) or behavioral laboratory tasks (e.g., Go/No Go Task; Continuous Performance Test; Stop Signal Task; CSRT tasks) (for reviews, see Moeller et al., 2001a; Hamilton et al., 2015). The integration of clinical measures of impulsive action may inform refined, personalized pharmacotherapeutic intervention for the treatment of relapse vulnerability in CUD and improve patient care for afflicted populations.

Pimavanserin is marketed as a 5-HT$_{2A}$R inverse agonist to treat Parkinson disease psychosis (Vanover et al., 2008; Meltzer et al., 2010; Sahli and Tarazi, 2018). Both pimavanserin and M100907 act as 5-HT$_{2A}$R inverse agonists to attenuate basal constitutive 5-HT$_{2A}$R signaling in cells designed with over-expression of the native 5-HT$_{2A}$R or transfection with a 5-HT$_{2A}$R mutation targeted to increase constitutive activity
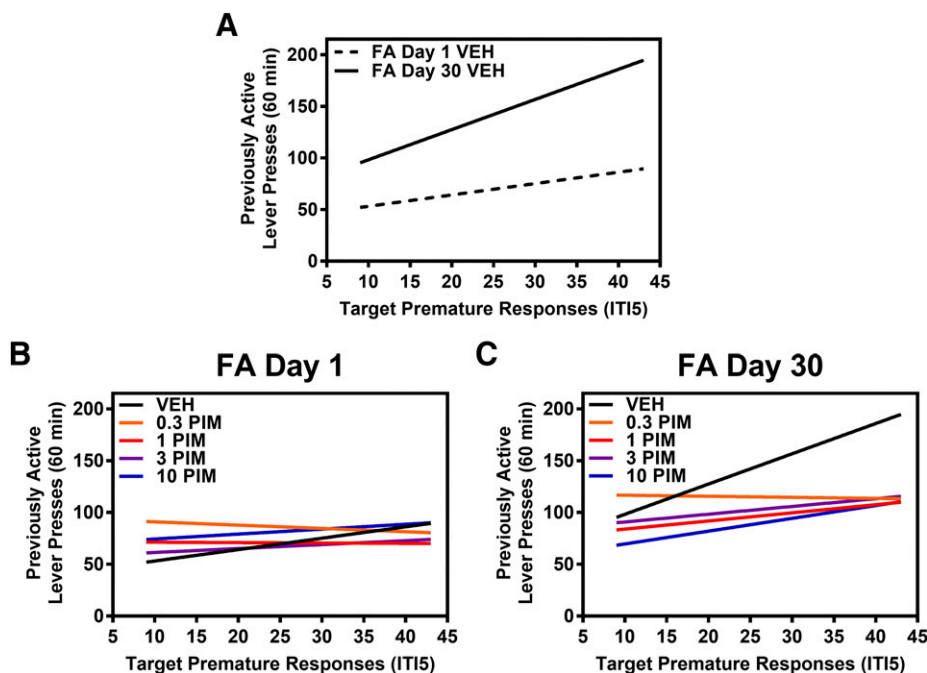
Fig. 3. Baseline levels of impulsive action predict the efficacy of pimavanserin to suppress cocaine cue reactivity on FA day 30 from cocaine self-administration. Target premature responses under an ITI5 schedule in the 1-CSRT task (mean ± S.E.M.) are presented on the x-axis, and previously active lever presses (mean ± S.E.M.) on the cue reactivity test session are presented on the y-axis. The relationship between target premature responses and previously active lever presses after pretreatment with VEH or pimavanserin (PIM; 0.3, 1, 3, 10 mg/kg) is represented by a linear regression line for each pretreatment condition. (A) Target premature responses predicted the number of previously active lever presses exhibited on FA day 1 and on FA day 30 in VEH-treated rats ($F_{(1,22)} = 5.081$, $P < 0.05$). (B) No relationship was found between target premature responses and the number of previously active lever presses exhibited on FA day 1 after pretreatment with vehicle or pimavanserin ($F_{(4,62)} = 0.405$; n.s.). (C) Target premature responses predicted the number of previously active lever presses exhibited on FA day 30 after pretreatment with vehicle or pimavanserin ($F_{(4,52)} = 4.04$, $P = 0.05$).

(Weiner et al., 2001; Vanover et al., 2004; Muntasir et al., 2006; Vanover et al., 2006). A definitive role for negative 5-HT$_{2A}$R efficacy in the control of behavior is suggested by a limited literature in conditioned behaviors (Welsh et al., 1998; Romano et al., 2006). For example, reduced constitutive 5-HT$_{2A}$R activity is proposed to account for the impairment of conditioned learning evoked by the 5-HT$_{2A}$R antagonist/inverse agonist MDL11,939 (Welsh et al., 1998). Presently, we are unable to attribute definitively the effects of M100907 or pimavanserin to their 5-HT$_{2A}$R antagonist versus inverse agonist properties.

Pimavanserin and M100907 promote the maintenance of sleep in humans (Rosenberg et al., 2008; Ancoli-Israel et al., 2011). Notably, sleep disorders (Morgan et al., 2010), as well as psychosis (Caton et al., 2000; Peer et al., 2009), commonly co-occur with CUD, and dopamine replacement therapy in Parkinson's disease elicits side effects that include increased risk-taking and impulsive behaviors (Park and Stacy, 2011). Our present findings demonstrate that pimavanserin suppressed impulsive action, as well as cocaine cue reactivity. Thus, a compelling case can be made that pimavanserin or other 5-HT$_{2A}$R antagonists/inverse agonists that may be ultimately available for clinical use will be efficacious in suppressing relapse vulnerability and potentially improve concomitant sleep and psychiatric disorders seen in CUD. The marketing of pimavanserin provides the opportunity to assess the efficacy of pimavanserin to extend abstinence and improve the health status of CUD patients.

Authorship Contributions

Participated in research design: Sholler, Boone, Wang, Moeller, Anastasio, Cunningham.

Conducted experiments: Sholler, Stutz, Fox.

Contributed reagents or analytical tools: Rice.

Performed data analysis: Sholler, Anastasio.

Wrote or contributed to the writing of the manuscript: Sholler, Stutz, Fox, Boone, Wang, Moeller, Anastasio, Cunningham.

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